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UNDERWATER PHYSIOLOGY VI

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PREFACE

This volume marks the sixth in a continuing series of Symposia initiated 27 years ago by the University of Pennsylvania and the Office of Naval Research. These sponsors invited the newly formed National Oceanic and Atmospheric Administration and the Undersea Medical Society to join as co-sponsors from the Fourth Symposium on. This *Proceedings* also represents the transfer of direct responsibility for the series from the University of Pennsylania to the Undersea Medical Society. The sponsors have asked the Undersea Medical Society to assume responsibility for the planning and publication of the Seventh Symposium, with the assistance of the Underwater Symposium Governing Board. It is the hope and expectation of all who have profited from this series that future symposia will provide even greater stimulus to undersea physiology than have the six volumes so far.

Previously published symposia in this series include (sponsored by the University of Pennsylvania and the Office of Naval Research): *Proceedings of the Underwater Physiology Symposium*, National Academy of Sciences—National Research Council, Washington, D.C., 1955; *Proceedings of the Second Symposium on Underwater Physiology*, National Academy of Sciences—National Research Council, Washington, D.C., 1963; *Underwater Physiology: Proceedings of the Third Symposium on Underwater Physiology*, Williams & Wilkins, Baltimore, Maryland, 1976; *Underwater Physiology: Proceedings of the Fourth Symposium on Underwater Physiology*, Academic Press, New York, 1971. Sponsorship of the Fourth Symposium was joined by the National Oceanic and Atmospheric Administration and the Undersea Medical Society. *Underwater Physiology V: Proceedings of the Fifth Symposium on Underwater Physiology*, FASEB, Bethesda, Maryland, 1976, was sponsored by the University of Pennsylvania, the Office of Naval Research, the Undersea Medical Society, and the National Oceanic and Atmospheric Administration.

FOREWORD

Exposure to the high-pressure underwater environment causes what is probably the most severe combination of physiological stresses encountered in purposeful human activity. Despite this, it has become possible for man, using saturation techniques, to carry out practical work in the open sea at pressures equivalent to 1000 feet of seawater or greater, and to work in laboratory chambers at pressures equivalent to 1600 feet and greater. It is now evident that not only the deep extremes of the continental shelves, but an additional thousand feet of the slopes beyond the shelves are available for manned undersea work. Advances have included improvement in diving effectiveness in the important shallower regions and the application of engineering principles to extend diving depth and duration. The subsurface area now open to specific exploration is equivalent in size to the area of the African Continent, the combination of South America and Europe, or the entire lunar surface. Almost all of the scientific and practical advances responsible for access to this area have occurred in the period covered by this symposium series, most of them during the past ten years alone.

An opportunity would be lost if it were not here recalled that keenness and enthusiasm to improve diving through physiological research have not always existed. Just prior to the first symposium in this series, shortly after World War II, physiologists generally were unaware of or uninterested in the effects of the high-pressure environment. The First Symposium was actually generated as part of a planned attempt to arouse civilian and naval scientists to the exceptional scientific opportunities presented by undersea physiology. A continuing series of expanding scope was not then anticipated. In fact, it was exceedingly difficult at that time and for a number of years thereafter for undersea investigative work to be conducted within either university or naval laboratories, and industry evidenced no interest in research at all. Despite the dedicated efforts of individual naval and civilian workers, naval pressure laboratories in several countries had become essentially inactive, government science and health agencies did not engage in research into the effects of the undersea environment, and some universities dismantled even the rudimentary pressure or altitude chamber systems they controlled.

About ten years ago, a series of large steps and events began to influence undersea research and, eventually, operations at sea. Hyperbaric oxygen therapy was introduced into clinical medicine, largely in support of cardiac surgery, in ancillary therapy of gas gangrene, and in the treatment of carbon monoxide poisoning. Many civilian hospitals, generally unaware of the limitations imposed by oxygen toxicity, developed large pressure facilities for clinical purposes. Most of these facilities have since been put aside, and only a few continue to be active in their original purpose. However, out of the broadened interest in oxygen came a general awakening to the importance of research in pressure and undersea medicine. Studies of intermittent oxygen exposure and the development of oxygen treatment tables for decompression sickness

allowed more rational and convenient methods to be substituted for the earlier heroic air therapy of serious "bends." Helium diving re-emerged and expanded. With the proposal of the ingenious idea of saturation diving and its successful demonstration in chambers and at sea, opportunities for extended undersea physiological research and diving applications emerged. The Undersea Medical Society was established to act as an international focus for undersea biomedical scientific communication.

The National Institutes of Health added their important support to basic investigative programs. The National Oceanic and Atmospheric Administration, interested in civilian diving, was formed. The Air Force and NASA installed positive pressure chambers to treat decompression sickness arising from aerospace procedures. Major advances in diving research were generated by scientists from Switzerland, France, England, Sweden, and Italy.

In detailed and sometimes unrelated studies, laboratories probing tolerance to high ambient pressure, narcosis, respiratory gas density, decompression, and isolation have progressively extended both the pressure and duration limits of useful human undersea work. The possible chronic effects of repeated exposure have been cause for concern, and in the course of taking these large investigative steps, the effects of hydrostatic pressure itself—new to the diver's experience but already known to the general biologist—have emerged as perhaps the most prominent limitation of all.

Through the accelerating gains derived from these research endeavors, the biomedical sciences were able to pave the way for essentially all of the major operational advances required for offshore oil resource development and naval diving functions to date. Both civilian and naval interests in many countries have been stimulated to the extent that new scientific observations on human responses to the undersea environment tend to be used almost immediately. This is desirable when interpretations are sound, but there is at present a large requirement for research to consolidate recent scientific progress; for the first time in many years, the momentum of application threatens to generate an information gap in both shallow and deep undersea activities. To prevent this, the laboratory research systems responsible for recent advances must be held together to pay continued attention to basic and applied underwater investigations. Improvements in technical communication must be provided and will strengthen both research and operations; progress has come from communication as well as from research. Now is the time to strengthen operational capability by investing in research designed to lead to a real understanding of the basic phenomena encountered at high pressures, during high rates of compression, and in adaptation to the pressure environment at all tolerable levels. This is not in any way a time for satisfaction in laboratory or at sea.

The most serious concern at present, and a handicap to further progress, is the surprising failure of the national leadership to establish a positive policy for the support of long-term research in manned undersea activity, for industry to avoid investment even into research for its own long-term needs, and for many investigators to move away from technical areas with uncertain futures or potential hazards. The reemergence of this combination threatens to cripple the still young field of undersea biomedical research and to limit its capacity for further applications. The field is now well advanced, but it is not stable. It is therefore to the advantage of all concerned to seek to strengthen this exceptionally difficult form of research—in breadth, in quality, and in continuity.

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Part I. DISRUPTION OF NERVOUS SYSTEM AND PERFORMANCE AT INCREASED PRESSURE

HPNS: A CLINICAL STUDY OF 30 CASES

X. Fructus and J. C. Rostain

In 1969, Brauer et al. (3) and Fructus et al. (9) observed manifestations different from those known as inert gas narcosis during a series of helium-oxygen dives to between 300 and 365 meters. They gave the name High Pressure Nervous Syndrome (HPNS) to these symptoms.

Various dives since then have confirmed and documented the characteristics of HPNS (1, 6, 7, 12).

Methods

HPNS was studied during 12 dives to depths between 300 and 610 meters. These dives were carried out by a total of 24 subjects, some of whom made 2 dives. The dives can be classified as follows: (Table I) (1) Physalie I to VI, exploration dives with a short stay at bottom depth, 2 subjects each; (2) Sagittaire I to IV, saturation dives, 4 subjects in Sagittaire I and III, and 2 subjects in Sagittaire II and IV; (3) Janus III, A and B, working dives, each with 3 subjects.

These dives had different profiles, especially as far as compression was concerned (Table II).

The elevated Po_2 during the first dives (375.0-451.1 mmHg for Physalie I to IV) was lowered to 300 mmHg during later dives. Temperature varied between 31 °C and 33 °C, according to depth. Humidity, elevated during the first dives (>70%), was maintained at between 40 and 60% during later dives.

During these 12 dives, the subjects underwent a series of tests which allowed the clinical and electrophysiologic symptoms of HPNS to be studied. Table III shows the number of times men were exposed to various depths during these experiments.

Results

In practice, HPNS is divided into 2 groups of symptoms: (1) clinical symptoms, which include tremor, dysmetry, fasciculations and muscular jerks, and drowsiness (microsleep); (2) electrophysiologic symptoms, consisting of EEG modifications, accentuation of theta fre-

TABLE I

CLASSIFICATION OF DIFFERENT DIVES PERFORMED BETWEEN 300 AND 610 METERS

TYPE OF DIVES	NAME	DEPTHS IN METRES
EXPLORATION DIVES. SHORT STAY AT BOTTOM	PHYSALIE	I 335 · II 360 · III 365 · IV 300 · V 520 · VI 610
SATURATION DIVES	SAGITTAIRE	I 300 · II 500 · III 300 · IV 610
WORK DIVES	JANUS	III A 390-415-460 · III B 390-415

quency activity, depression of α and β frequency activity, transformation of the waking EEG trace into that of Stage I sleep, and disturbed organization of sleep.

Results which have relevance only to the clinical signs are presented here.

TABLE II

ANALYSIS OF DIFFERENT COMPRESSION PROFILES

COMPRESSION	DIVES AND BOTTOM DEPTHS			
FAST	PHYSALIE I 335 m	PHYSALIE II 360 m	PHYSALIE III 365 m	
FAST. STAGES AT INTERMEDIATE DEPTH	PHYSALIE IV 300 m	SAGITTAIRE I 300 m	SAGITTAIRE III 300 m	
SLOW. DECREASING WITH DEPTH	JANUS III A 460 m	SAGITTAIRE II 500 m		
SLOW. DECREASING WITH DEPTH AND STAGES AT INTERMEDIATE DEPTH	JANUS III B 415 m	PHYSALIE V 520 m	PHYSALIE VI 610 m	SAGITTAIRE IV 610 m

TABLE III

NUMBER OF TIMES THAT MAN HAS REACHED
300-METER DEPTH AND MORE (24 SUBJECTS)

300 METERS	=	30
335	=	20
360	=	18
365	=	16
415	=	14
460	=	11
500	=	8
520	=	6
610	=	4

TREMOR

Tremor is first visible at the extremity of the limbs. It accentuates with depth, and progresses up the length of the limbs. Measurements of tremor were carried out with a geophone placed on the middle finger of the right hand, with the right arm extended horizontally from the body. The results indicated a rapid frequency (8 to 12 Hz), which appeared between 200 and 300 meters. It can appear below 300 meters, even if the compression is very slow. The degree of tremor is a function of the compression rate.

For example, Physalie V had a compression to 520 meters in a total of 75 hours, including two 12-hour stages at 350 and 460 meters, and rapid compression between 350–400 meters and 460–490 meters. Between 490 and 520 meters tremor reached very high levels, on the order of 700% of normal. During Physalie VI, when the compression to 610 meters was made in 177 hours with 3 stages (46 hours at 350 meters, 14 hours at 535 and 565 meters) but without rapid compression, the tremor did not rise above 250% of normal.

If the subject is kept at constant depth, the tremor persists. This was the case during dives with long stays at 300 meters (Sagittaire I and III) and certainly at 500 meters (Sagittaire II). During the 100 hours passed at 500 meters, the tremor remained notable on the last day. This tremor disappeared during the decompression, more rapidly if it was not very serious at bottom depth. The effect of speed and mode of compression is apparent in Fig. 1, which recapitulates the clinical symptoms observed during the 12 dives. Tremor was very intense during shallower dives where the compression was generally rapid, and was much less intense for the deepest dives accomplished with slow compression.

DYSMETRY

Dysmetry was observed during classical neurologic tests, and was inconstant and more or less visible from subject to subject. It can appear beginning at 300 meters during rapid compressions, but is generally observed below 400 meters, and disappears during the decompression.

FASCICULATIONS AND MYOCLONIA

Fasciculations and myoclonia are superimposed on the tremor when the latter attains a relatively high intensity. Myoclonia generally follows the appearance of fasciculations. They

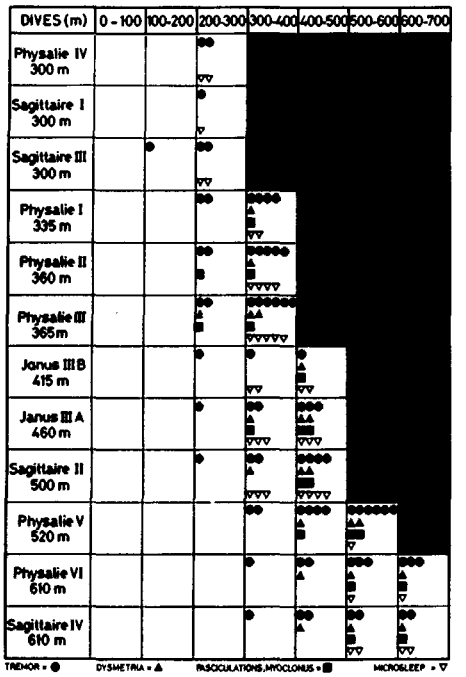

Fig. 1. Analysis of clinical symptoms of HPNS during the 12 heliox dives with 24 subjects.

occur predominately in the upper limbs and the extremities. At high pressures, they have a tendency to ascend the length of the limbs, and sometimes occur in the neck and face muscles. These symptoms occur between 200 and 300 meters for rapid compressions and below 500 meters for slow compressions (Physalie VI and Sagittaire IV). They regress slightly during a prolonged stay at constant depth. Their disappearance occurs during decompression in the depth region where they appeared during compression.

Drowsiness

Somnolence (microsleep) was not found in every subject. It appeared in a subject left at rest, but was easily vanquished by exterior stimulation of either affective or intellectual type. It was predominant in dives with rapid and/or continuous compression. It was less constant and afflicted fewer subjects during slow compressions which included stages at intermediate depth. Drowsiness persisted during extended stays at constant depth. It generally disappeared during the decompression (in the 200-meter zone) but in some cases persisted until after leaving the pressure chamber.

Other Symptoms

In addition to diverse symptoms of neurologic disorder, it is necessary to cite another manifestation which occurs below 400 meters. It consists of nasal congestion, and exists

regardless of the humidity, be it high (>70%) or low (<70%). This could result from edema of the pituitary mucosa.

Conclusions

The results concerning the clinical symptoms observed during these 12 dives lead to the following conclusions:

(1) The effect of the rate and mode of compression on the appearance and intensity of diverse neurologic disturbances (tremor, fasciculations and myoclonia, and drowsiness) is irrefutable. The symptoms are less intense and less numerous if the compression is slower with more stages. The utilization of slow compression allowed the 365-meter mark to be passed (8, 9) and pushed the experimental "depth limit" ~~(1, 6, 7, 12)~~ back progressively.

(2) The intervention of other hyperbaric parameters is apparently responsible for the persistence of certain neurologic symptoms (tremor and drowsiness) during prolonged stay at constant depth. Pressure itself might intervene, or perhaps breathing gas mixtures at high pressures plays a role. These hypotheses are equally supported by different authors with experiments carried out in humans or animals (2, 4, 5, 10, 11).

(3) The nervous origin of these disturbances and their mechanisms is still unknown. With the present state of knowledge for depths between 300 and 610 meters, it is necessary to use slow compression and to intersperse stages at intermediate depths. Only such a schema will permit the diver to arrive at the bottom in a physiologically acceptable state, able to accomplish effective work.

Finally, it is necessary to be very prudent, since the observed symptoms are the first manifestations of a prodromic phase and it is not known how these symptoms evolve into the syndromic phase.

Acknowledgments

This work was supported in part by the CNEXO and the DRME.

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HUMAN NEUROPHYSIOLOGICAL DATA OBTAINED FROM TWO SIMULATED HELIOX DIVES TO A DEPTH OF 610 METERS

J. C. Rostain and R. Naquet

Since the publication of reports (3, 9) of the High Pressure Nervous Syndrome (HPNS) during helium-oxygen dives to depths between 300–365 meters, several experiments have shown that HPNS can be modified by experimental conditions. Depths thought to be limited by HPNS can be extended still further. In this way, 457 meters was attained (1) and 500 meters has been surpassed (6, 8, 16). The results obtained, particularly those from the COMEX laboratory, suggest that improving some of the experimental conditions, especially the method of compression, would permit man to descend to 600 meters and to stay for several hours with relatively insignificant HPNS.

To test this hypothesis, two simulated dives in an atmosphere of helium-oxygen were made to a depth of 610 meters and the major manifestations of HPNS (tremor and EEG modifications) were analyzed.

I. The Dives: Technical Aspects

In the first dive (Physalie VI) two subjects were taken to a depth of 610 meters in 177 hours. Compression was carried out at decreasing speeds, depending on the depth, and was interrupted by a 46-hour stage at 350 meters and two 14-hour stages at 535 and 565 meters. The subjects stayed at the bottom for 80 minutes and the decompression was accomplished over 233 hours (Fig. 1).

The second dive (Sagittaire IV) took two subjects to a depth of 610 meters in 261 hours. The compression curve was interrupted by a primary resting stage of 17 hours at 200 meters, another of 45 hours at 400 meters and two further stages lasting 46 hours at 550 and 580 meters. The speed decreased in relation to the depth down to 400 meters, was constant at 3 meters/hour between 400 and 550 meters, and was maintained at 10 meters/hour between 550 and 610 meters.

The subjects stayed at the bottom for 50 hours, and decompression was accomplished over 231 hours. This second dive was undertaken to study 1) the effects on HPNS of compression slower than that used in Physalie VI between the surface and 550 meters; and 2) the effects of fast compression at great depth (between 550 and 610 meters).

The different parameters of the two dives are shown in Table I.

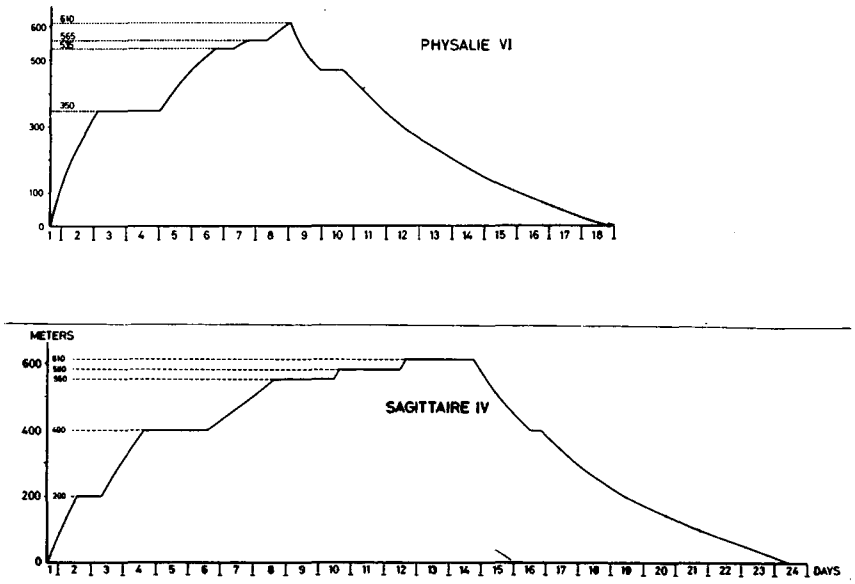

Fig. 1. Profiles of the two dives to 610 meters.

TABLE I

PARAMETERS OF THE TWO DIVES

	Physalie VI	Sagittaire IV
Depth, meters	610	610
P_{IO_2}, ATA	0.40	0.42
P_{IN_2}, ATA	0.13	0.13
P_{ICO_2}, ATA	0.003	0.003
H_2O, % of saturation	30–50	40–50
Temperature	32° ± 1	33° ± 0.5
Specific gravity at 31 °C, gm/liter	10.51	10.11

The P_{IO_2} was raised to 0.50 ATA during decompression. The divers were confined for 4 to 5 days in an atmosphere of helium-oxygen at atmospheric pressure before each dive.

II. Tremor

METHODS

Tremor was recorded with a geophone (Geo-space HS-J) placed on the middle finger of the right hand. Measurements were made several times a day at fixed intervals during the helium-oxygen confinement at atmospheric pressure and during the dives. The signals were recorded on analog magnetic tape and were later analyzed by computer (PDP-12, Digital Equipment Corporation) to obtain the mean amplitude and the power spectrum using programs written

in the laboratory (STREMOR and MSAMBR). These analyses consisted of three successive periods each lasting 17 seconds. Results were obtained by averaging the data for the three periods.

Results

Physalie VI

At the surface both subjects showed barely perceptible physiological tremor (Fig. 2). During compression they both manifested tremor between 200 and 300 meters. This had increased by 100% at 350 meters; it tended to decrease toward the end of the stage at 350 meters. It rose again during compression from 350 to 610 meters. It did not decrease during the 14-hour stages at 535 and 565 meters and it was more marked in the morning at the end of the stage than it was at the beginning of the evening before.

The maximal increase in tremor was recorded between 565 and 610 meters for both subjects; it did not exceed 250%. During decompression, tremor decreased rapidly for *subject A*. It disappeared more slowly for *subject B*.

Sagittaire IV

At the surface, the degree of tremor differed for the two subjects; it was more marked and more variable for *subject B*; *subject A* showed almost no tremor. There was no increase in tremor due to the time spent in confinement (Fig. 3). During the dive, the level of tremor expressed in percentage change from control values differed from the mean value recorded at

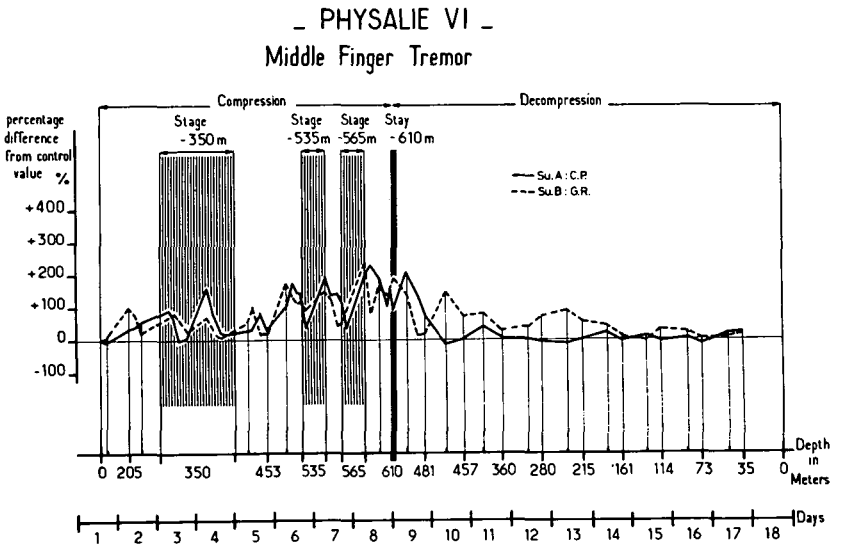

Fig. 2. Evolution of tremor during Physalie VI dive. *Ordinate* shows increase expressed as percentage difference from control values obtained at the surface. *Abscissa* shows depth in meters and days. Hatched areas represent stages at intermediate depths and stay at bottom.

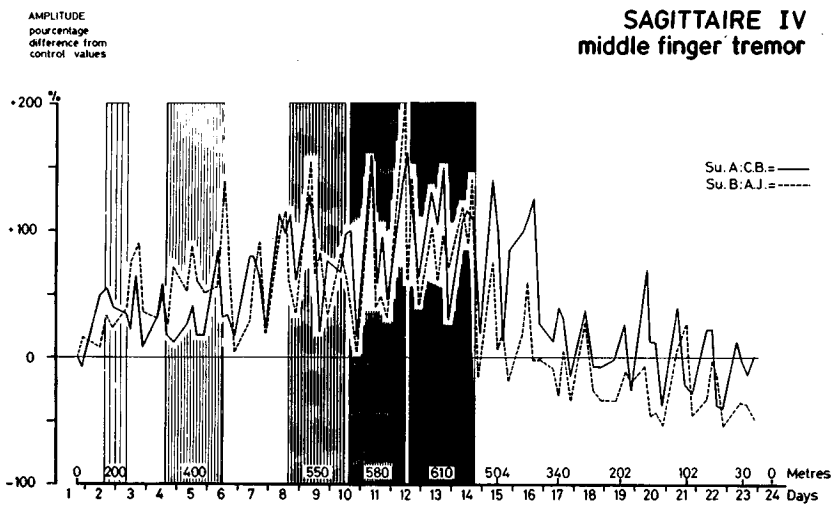

Fig. 3. Evolution of tremor during Sagittaire IV. *Ordinate* shows increase expressed as percentage difference from control values obtained at surface. *Abscissa* shows depth in meters and days. Hatched areas represent stages at intermediate depths and stay at bottom.

the surface; it increased when the subjects reached 200 meters. This rise was slight (less than 50%) and tended to disappear by the end of the stage.

During compression from 200 to 400 meters the tremor hardly varied; at 400 meters the increase was on the order of 50-100%; it did not increase during the stage. Tremor showed a further increase from 400 to 500 meters; a 100-120% increase was noted when 550 meters was reached, and this rose to 150% by the morning of the second day at this depth. It started to decrease in the evening of that day; after this, the trembling was observed to be more marked in the morning than in the evening.

The rapid compression from 550 to 580 meters and from 580 to 610 meters did not cause important modifications. On the other hand, tremor did increase during the stage at 580 meters and it reached its highest level on the final day at this depth, with a 160% increase for *subject A* and 195% increase for *subject B*.

Frequency of the tremor was relatively high; the power spectra showed an activity ranging from 7 to 11 Hz, with a peak at 8 Hz. This frequency was the same at the surface as during compression to 610 meters. Furthermore, despite the daily variations in amplitude, the frequency remained stable throughout each day.

III. Modifications in EEG

Method

The EEG activity was recorded with "hameçon ECEM" electrodes implanted in the scalp and held in place with gauze and collodion throughout the dive (this method has been used since 1971 and is satisfactory for experiments of long duration). The electrodes were placed in the frontopolar, central, midtemporal and occipital regions of one hemisphere.

EEG recordings were made daily at fixed times both during the confinement and the actual

dives. EEG activity, recorded by twin bipolar electrodes (Fp-C, C-Tm, Tm-O) was reproduced on an Alvar polygraph and stored on analog magnetic tape. The recorded sequences, retrieved by an analog magnetic tape counter (SEVME Informatique) were later analyzed to obtain the power spectra corresponding to each successive 7.5-second analysis period. The distribution of spectral densities in each frequency band (1–4 Hz, 8–13 Hz, 14–22 Hz), and the frequency of the highest density and their values were printed out.

Results

Physalie VI

Examination of the waking EEG trace reveals a certain number of changes. The modifications for *subject A* (Fig. 4), starting at 270 meters, consisted of a strengthening of theta activity in the frontocentral and centrotemporal regions, and a decrease in amplitude of posterior alpha activity.

Furthermore, from 565 meters downward, a very slight instability was noted in the EEG trace during waking after the subject closed his eyes; this trace tended to be replaced by a trace similar to that of stage one sleep. The frequency of the alpha rhythm slowed after 350 meters and reached its slowest level (2 Hz) at 610 meters. The power spectra of the EEG activity meant that the modifications taking place throughout the dive could be followed exactly (Fig. 5).

There was a slight increase in theta activity in the anterior and middle regions starting at 270 meters. When 350 meters had been reached, there was also an increase in delta rhythm in the anterior region. These modifications were accompanied by a decrease in rapid alpha and beta rhythms. During the stage at 350 meters these modifications stabilized and sometimes even subsided. They reappeared during compression from 350–610 meters and they did not subside during the 14-hour stages at 535 and 565 meters. Theta rhythm appeared predominantly in the middle region, and the increase observed between 535 and 610 meters was on the order of 500%. These modifications subsided during decompression and returned to nor-

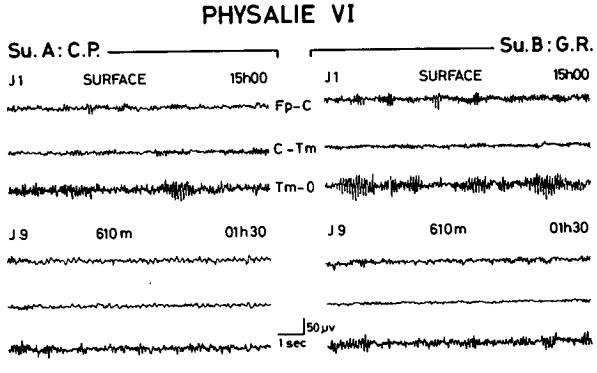

Fig. 4. Electroencephalogram (EEG) trace, with eyes closed, for 2 subjects of Physalie VI. Left: *subject A, CP*; right: *subject B, GR*. The following regions are shown for such subject, top to bottom, surface to 610 m: Fp-C; frontopolar-central; C-Tm: central-midtemporal; Tm-O: midtemporal-occipital.

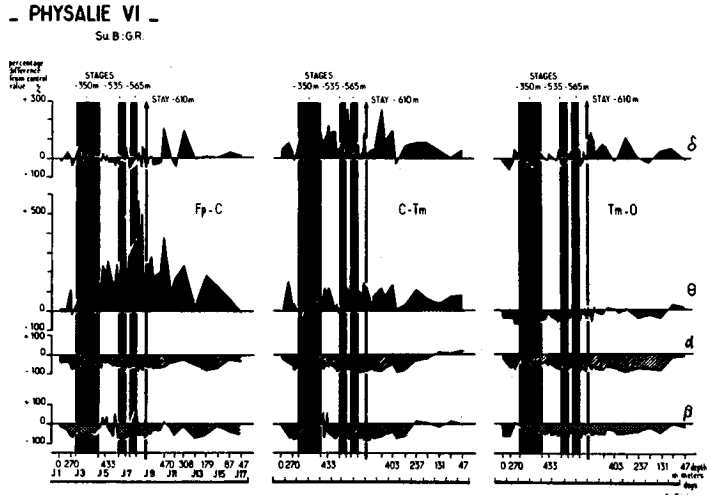

Fig. 5. Changes in power spectra of EEG activity as function of depth for *subject B, GR,* from Physalie VI. *Ordinate* shows increase expressed as percentage difference from control values obtained at surface. *Abscissa* shows depth in meters and days. EEG's were made from the following regions: left: Fp-C (frontopolar-central); center: C-Tm (central-midtemporal); right: Tm-O (midtemporal-occipital). Four frequency bands are shown for each trace; from top to bottom, delta (1–4 Hz); theta (4–7 Hz); alpha (8–13 Hz); beta (14–22 Hz). Vertical black areas represent stages at intermediate depths, or stay at bottom.

mal between 200 and 100 meters. The alpha rhythm regained its initial frequency when the subjects left the chamber.

The modifications for *subject B* (Fig. 4) consisted of a reduced amplitude in the alpha from the occipital region at 300 meters, followed by the appearance of theta activity in the frontocentral region at about 400 meters. The alpha started to slow from 350 meters on. The power spectra show that theta activity strengthened in the anterior region after 300 meters, and there was also an increase in delta rhythm activity in the middle region after 350 meters. These modifications coincided with a decrease of alpha and beta frequency bands over all recordings.

During the stage at 350 meters some of these modifications stabilized and even subsided. They redeveloped during compression from 350 to 610 meters. The theta activity reached its maximum between 535 and 610 meters (500%). The delta rhythm did not exceed 300% at the same depths. These modifications started to recede during decompression between 400 and 300 meters and reached a normal level between 200 and 100 meters. The alpha, which had slowed to 1–2 Hz, recovered its original frequency after the subjects had left the chamber.

Sagittaire IV

The two subjects showed differing modifications in EEG during the dives. The modifications for *subject A* consisted of a display of theta activity in the anterior regions and a decrease in amplitude of the alpha. These modifications, which appeared at about 300 meters, remained slight until 400 meters and throughout the stage at this depth (Fig. 6).

The changes increased between 400 and 550 meters, and by 550 meters, theta activity oc-

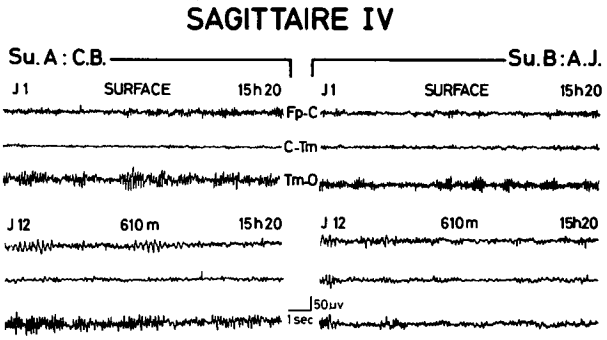

Fig. 6. Electroencephalogram (EEG) trace, with eyes closed, for two subjects of Sagittaire IV. Left: *subject A, CB*; right: *subject B, AJ*; from top to bottom, surface to 610 meters. For each subject the following regions are shown: Fp-C: frontopolar-central; C-Tm: central-midtemporal; Tm-O: midtemporal-occipital.

curring in relatively large bursts of 5–6 Hz was observed in the anterior and middle regions of the hemisphere, predominating in the anterior region. The alpha rhythm stayed regular.

There was a further increase in such irregularity while descending to 580 meters, and again when descending to 610 meters. On arrival at 610 meters and during the stay at the bottom, theta activity appeared in bursts, sometimes at high amplitude (5 Hz) in the same regions. The posterior alpha was more or less stable, though a slowing down of 1–2 Hz was recorded.

Instability in vigilance, revealed by the transformation of the waking trace into one of stage one sleep, was rare for this subject. This condition was nevertheless more frequent when compression was over, at the beginning of the resting stage, and at the beginning of the stay at bottom.

The power spectra (Fig. 7) show that the theta activity started to get stronger at about 400 meters and grew particularly strong between 550 and 610 meters. It showed no marked increase during the stages or during the stay at bottom. Theta activity increased at 610 meters from 2000–4000% in the anterior regions and from 500–1000% in the middle regions. Delta rhythm also increased, particularly beyond 500 meters in the middle region, but it did not exceed 300%. There was a slight decrease of the more rapid rhythms.

These modifications began to subside during decompression after 400 meters, but did not return to normal until several days after the subjects had left the chamber. Alpha activity, which slowed down sharply during decompression (2 to 3 Hz), did not regain its original value until after the subjects had left the chamber.

The modifications manifested by *subject B* consisted of a very feeble appearance of theta in the middle region, and more particularly by the transformation of a waking trace into a stage one sleep trace several seconds after the subject shut his eyes during a resting period. These modifications appeared at 300 meters and remained slight until 400 meters. They rose between 400 and 550 meters while the alpha slowed down to 1 to 2 Hz. A tendency to sleep occurred frequently between 550 and 610 meters; at the same time the bursts of theta became greater in the anterior and middle regions.

The power spectra of EEG activity for this subject show an increase in theta after 350 meters, which predominated initially in the centrotemporal regions. There was a simultaneous weakening of rapid rhythms in all regions. Theta grew stronger again during compression from 400 to 550 meters, but this was particularly marked between 580 and 610 meters: 1000–

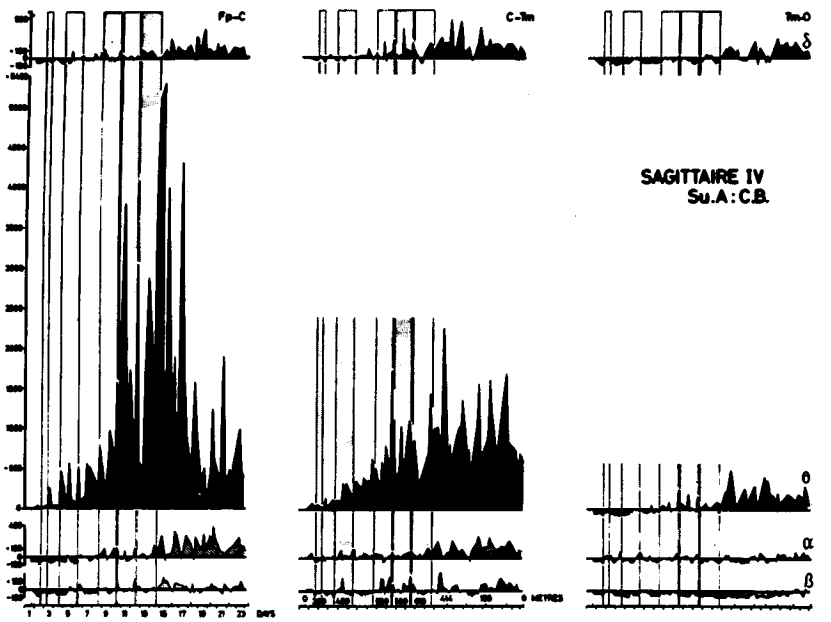

Fig. 7. Changes in power spectra of EEG activity as a function of depth for *subject A, CB. Ordinate* shows increase expressed as percentage difference from control values obtained at surface. *Abscissa* shows depth in meters and days. EEG's were made from the following regions: Left: Fp-C (frontopolar-central); Center: (central midtemporal); Right: (midtemporal-occipital). Four frequency bands are shown for each trace. From top to bottom, delta (1–4 Hz); theta (4–7 Hz); alpha (8–13 Hz); beta (14–22 Hz). Vertical grey areas represent stages at 200, 400, 550 and 580 meters, and stay at 610 meters.

2000% in the anterior and middle regions, and 500–1000% in the posterior. The delta rhythm in the anterior and middle region increased up to 200%.

All the various modifications began to decrease during decompression from 300 meters, but they continued at significant intensity until the subjects left the chamber.

IV. Other Symptoms of HPNS

Waking activity during intellectual tasks. During certain intellectual tasks (number ordering, symbol recognition) the theta increased.

Disturbances in the organization of sleep. The EEG recordings reveal disturbances in the organization of sleep after 300 meters: (1) stage II was more frequent and lasted longer; (2) stage III diminished; (3) stage IV also diminished and sometimes disappeared; and (4) paradoxical phases became irregular.

V. Conclusions

If the development of clinical and electrophysiological symptoms during both the dives are compared, the following facts are observed:

(1) Confinement in an atmosphere of helium-oxygen at atmospheric pressure for 5 days does not lead to modifications; thus it is hyperbarometric conditions that bring about the various manifestations of HPNS.

(2) Lengthening the duration of compression by decreasing speed at certain depths and by adding resting stages at intermediate depths did not preclude the development of HPNS, tremor and EEG modifications.

(a) Tremor appeared in both cases at about 300 meters but the level was much below that observed in preceding experimental dives (8, 14, 16) (400–700% at 500 and 520 meters for Sagittaire II and Physalie V, as opposed to 200 to 250% during the last dives). This seems to be the result of the ameliorating effect of slow compression and stages; the more numerous and longer the stages, the more effect they have, because the lowest level of tremor occurred in Sagittaire IV. It must be noted, however, that the tremor did not decrease systematically during the resting stage; the effect of the stages seemed to be to stabilize the tremor and moderate its increase during compression. Rapid tremor, as already reported (14), corresponds to an increased physiological or normal resting tremor caused by compression or pressure in a helium-oxygen atmosphere. The mechanisms of this increase remain to be discovered.

(b) The EEG modifications developed differently, depending on the dive. During Physalie VI, the increase in theta activity, which appeared at about 300 meters, is relatively slight in comparison with Sagittaire II and Physalie V (1800 and 2000%, respectively). On the other hand, during Sagittaire IV, although the modifications were extremely slight down to 400 meters, they became significant after 550 meters despite a rest of 45 hours at this depth. The speed of compression, 10 meters/hour, which is rapid by the standards of preceding work (1, 6, 7, 8, 16), evidently cancelled out the "stabilizing effects" of the various long-duration rests.

The method of compression (speed, absence or presence of stages, number and depth of stages) evidently plays an essential role in the intensity of HPNS symptoms.

(c) EEG modifications differ for different subjects, even during the same dive. This difference is related to the individual's susceptibility to hyperbarometric conditions and confirms preceding observations (1, 3, 6, 7, 8, 16). Why a lowering of the level of vigilance predominates in some subjects and an increase of either middle or anterior region theta rhythm predominates in others needs further study.

(d) The persistence of clinical and electrophysiological disturbances during stages poses certain problems. Is a longer time necessary before the symptoms can clearly be seen to abate? Are these disturbances due rather to the simultaneous effects of pressure and compression, or are they a mixed effect of breathing under pressure? Results obtained for the dives of long duration at 300, 400, and 500 meters (13, 16) favor the last two of these hypotheses. Results obtained by numerous authors in human and animal studies also support these hypotheses (2, 4, 5, 10, 11, 12, 13, 15).

Furthermore, the persistence of EEG modifications during decompression in Sagittaire IV, which had already been observed during Sagittaire II (16), can be accounted for by the significant increase (above 1000%) occurring in these dives; the greater the disturbance, the longer it took to return to normal.

(e) There is an evident dissociation between tremor and EEG modifications. Compared to Physalie VI, there was less tremor during Sagittaire IV at greater depths, but the EEG modifications were more marked between 550 and 610 meters. It therefore seems that the mechanisms involved in the two types of symptoms are different.

(f) If results are compared for the two dives, Physalie VI and Sagittaire IV, it is clear that the slow compression between 0–550 meters in Sagittaire IV had a beneficial effect on HPNS,

but the fast compression between 550 and 610 meters increased HPNS; the results in Physalie VI at 610 meters are better.

In conclusion, results obtained for the two dives suggest that man can reach a depth of 610 meters in reasonable physiological and physical condition provided that certain measures are taken: rapid compression must be avoided, and there must be resting stages at intermediate depths during compression. Ignoring either one of these measures can cancel out the benefit obtained from the other. The length of the resting stage chosen (taking the physiological results into account) should not be less than 24 hours.

Finally, it is necessary to choose subjects who show no significant HPNS when diving to great depths. The criteria for selection are yet to be defined.

ACKNOWLEDGMENT

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SPINAL REFLEX ACTIVITY IN MAN UNDER HYPERBARIC HELIOX CONDITIONS (31 AND 62 ATA)

J. P. Roll, M. Lacour, M. Hugon and M. Bonnet

The quantitative study of monosynaptic reflexes in normal man is a classic one (10, 14), and can be extended to monkeys (15, 16). This study supplies information relative to the spinal functioning of the proprioceptive reflex pathways and their central control. A series of neuromotor functioning indices were studied at 101 ATA in normal baboons using electromyographic recordings of spontaneous and reflex motor activities (3). Under these extreme dive conditions, the baboons did not demonstrate any peripheral neuromuscular electrophysiological disorders. Central nervous disorders did occur, as indicated by tonic hyperactivity, sensorimotor hyperreactivity, and tremor of central origin.

During the dives Sagittaire III (heliox, 31 ATA, 1973) and IV (heliox, 62 ATA, 1974) the study of spinal reflex activity in man was continued. The disorders observed were attributed to effects of supraspinal origin rather than to segmentary reflex mechanisms. Modifications of monosynaptic reflex properties should be related to motor disorders of the High Pressure Nervous Syndrome (HPNS). The observations of Fructus, Brauer and Naquet (9) and Chouteau et al. (6, 7, 8) on goats noted paroxysmic hypertonic manifestations during dives with heliox at very high pressures.

However, knowledge of reflex modification in a particular subject at rest does not allow us to predict clearly the eventual motor performances of the same subject. Physiological and psychological compensatory mechanisms can conceal disturbances affecting basic motor mechanisms during performance tasks.

Methods

Monosynaptic reflexes of proprioceptive origin are elicited in the soleus muscle either by mechanical stimulation of the Achilles heel (tendon reflexes (T)), or by electrical stimulation of the posterior tibial nerve (Hoffmann's reflex (H)). Tendon stimulation excites primary afferent fibers (Ia) through neuromuscular spindles. The afferent pool depends on spindle stretch velocity. Electrical stimulation directly excites primary afferent fibers in the nerve. The effects obtained depend on the intensity of the electrical stimulus, but not on the peripheral proprioceptor. A comparison of H and T reflexes enables conclusions to be drawn about fusi-motor system activity. Moderate stimulation of the soleus muscular nerve excites primary fibers without exciting alpha motor fibers; a more intense stimulus elicits alpha fiber activity, a direct motor response (M) and motor axon antidromic activity up to alpha motoneurons, which have a depressive effect on the H response. The significant indicator is the

soleus muscle H, M, or T electromyographic response amplitude. The experiments to be described studied the following factors.

(1) Recruitment curves of M and H responses in relation to electrical stimulus intensity are shown in Fig. 1*A*. Pertinent components are the appearance threshold for each type of response, the maximal value of M and H, given by the ratio RRm = H_{max}/M_{max}. M_{max} indicates that all the muscle motor axons were activated by the electrical stimulus; RRm indicates what fraction of the motoneuron population was activated by the reflex volley: this is the "recruitment ratio." It indicates reflex pathway maximal excitability.

(2) The spontaneous variability of H or T responses was also investigated. Modifications in spontaneous variability provide information on the linkage of elements which make up the tested motoneuron population.

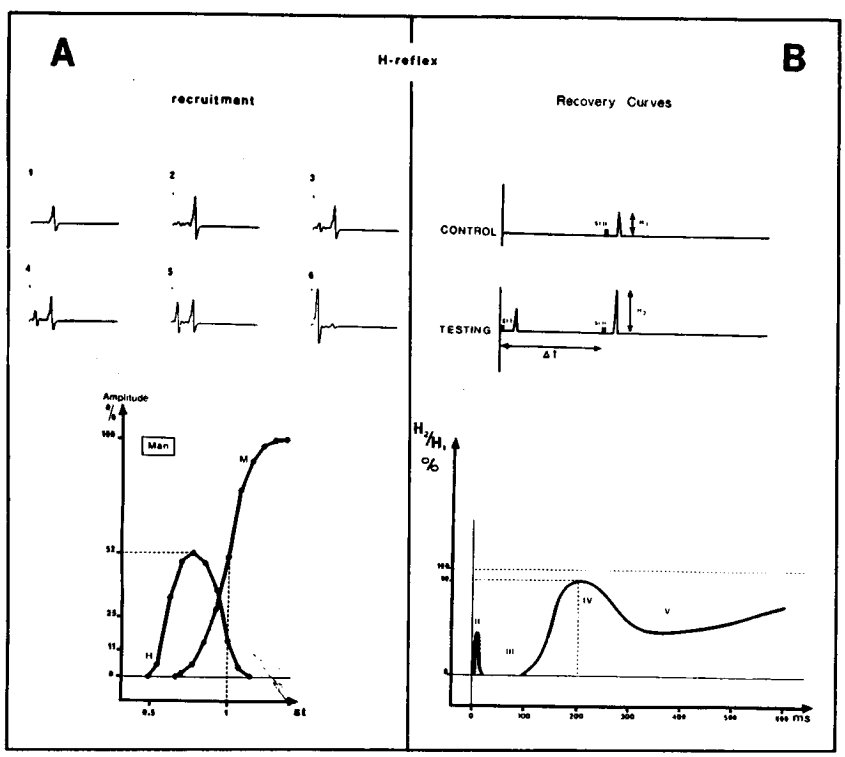

Fig. 1. Study concerning H reflex with single (*A*) and double (*B*) stimulation. *A, upper diagrams*: Hoffmann reflex recruitment. Effect of increasing stimulus intensity applied to tibial posterior nerve on H-reflex amplitude; maximal amplitude is attained in 2, decreases in 3, 4, 5. H decreases and disappears during 4, 5, 6; *A, lower diagram*: recruitment curves. *Ordinate*: H-reflex amplitude (H) and motor response (M) in percentage of normalized M max response. *Abscissa*: electrical stimulus intensity represents stimulation value necessary to obtain semimaximal M response. *B, upper diagrams*: schema of experimental procedure; *B, lower diagram*: recovery cycle. *Ordinate*: H-reflex amplitude elicited by test stimulus (H_2) in percentage of conditioning response (H_1). *Abscissa*: interstimulus delay between conditioning and test stimuli.

(3) Postreactional recovery cycles of monosynaptic reflexes using double stimulation are shown in Fig. 1*B*. Transient modifications of the excitability of the H-response pathway can be demonstrated by the ability of a second stimulus to induce a second H response. According to Paillard (14), 5 phases can be described in man. Phase I is a phase of total inexcitability of the system; its duration does not exceed 2 msec. Phase II lasts 1–10 msec and shows a short period of partial restoration of excitability. Phase III displays a total or subtotal inhibition of the test response, which lasts about 80–100 msec. Phase IV is a progressive return toward a normal or supernormal state of excitability, and lasts 120–300 msec. Phase V is a long phase of important subnormal excitability lasting several hundred milliseconds.

Each experimental session lasted about 2 hours. Measurements were taken on both legs, one after the other. The subject was comfortably seated in a specially designed armchair. He was told to remain calm and still. Reflex stimulations and their resulting contractions, however, are never painful. Data stimulation, detection and representation techniques were those defined by Paillard (14) and by Hugon (10). Soleus muscle electromyographic activities were recorded by surface electrodes.

In the experimental Sagittaire IV dive, with exposure to 62 ATA, 2 divers were studied. The results obtained during this dive were compared to those recorded during the Sagittaire III (31 ATA) dive (Fig. 2).

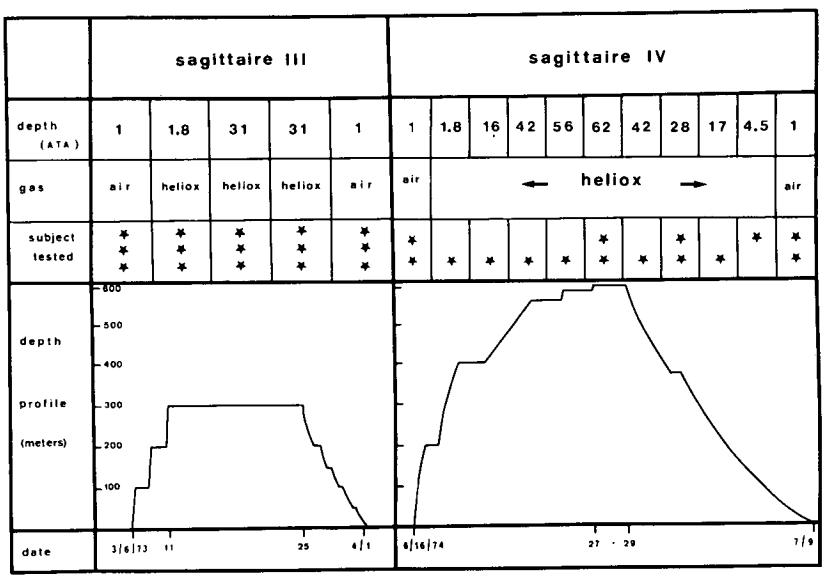

Fig. 2. General dive parameters in Sagittaire III (31 ATA) and Sagittaire IV (62 ATA) simulated dives. First horizontal column indicates habitat pressure measured in ATA; second relates to inspired inert gas composition (air before and after dive; heliox during dive); and third to number of tested subjects (H and T) at these simulated depths. General dive profiles are drawn at bottom with references to depth in meters and time in days.

Results

H, T, or M electromyographic response latency, form, and duration did not vary during

the Sagittaire IV dive. We can confirm, therefore, that nervous fiber conduction velocity and neuromuscular or central synaptic transmission delays are not modified under hyperbaric conditions (13).

Hoffmann reflexes are slightly facilitated at depth. RRm does not significantly increase at depth at pressures below 41 ATA (Sagittaire III and the beginning of Sagittaire IV); however, at 62 ATA, and afterward during decompression and at surfacing, one subject exhibited a considerably facilitated RRm.

Tendon reflex amplitude was not modified below pressures of 41 ATA (Sagittaire III and the beginning of Sagittaire IV). At the end of compression one subject exhibited hyperreflexivity (+50%) which was reduced at saturation and then reappeared during decompression (Table I).

TABLE I

SUMMARY OF EVOLUTION OF DIFFERENT TESTS STUDIED DURING SAGITTAIRE IV (62 ATA, 1 SUBJECT)

		Base-line Control	Compression	Saturation, 61 ATA	Decompression	Normobar
Recruiting ratio	H	100	88	75	80	80
$\frac{\text{Reflex, max}}{\text{Motor, max}} \cdot 100$	T	41	47	35.5	61	46
Variation coeff.	H	20	24	25	31	17
Variation coeff.	T	19	17.5	27	17	23
Facilitation test	T	143	114	108	98	129
Polyphasic EMG responses			XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX			

Recruitment ratio = H or T max/M max × 100; variation coefficient = m × 100 = standard deviation/mean × 100; facilitation test T results given in percentage of T control values (Jendrassik's maneuver). XXX = presence of abnormal EMG polyphasic responses.

Proprioceptive stimulation elicits abnormal, asynchronized polyphasic discharges beginning at 56 ATA. These responses appear with a latency of 200 msec. Observed in monkeys under hyperbaric conditions, they corresponded to motor startle reactions. In man these motor startle reactions remain subclinical.

H and T reflex variability, indicated by the variation coefficient, increases at depth for both legs (Table I). This phenomenon occurs at 31 ATA (Sagittaire III) and is accentuated at 62 ATA, during saturation, and increases further during decompression.

Postreaction recovery cycles (H + H, Fig. 3) are significantly modified in phases III, IV and V. Phase III (10–150 msec) is increased at saturation and decreased during decompression. Phase IV (150–300 msec), which indicates a progressive restoration of spinal excitability, is very slight during compression and at saturation. Phase V (300–1000 msec) was considerably enhanced during Sagittaire III. This modification was not seen during Sagittaire IV.

Postreaction recovery cycles (T + T) are characterized by a quite marked attenuation of Phase IV, starting as early as 16 ATA, which increases with compression and slightly decreases during decompression. The facilitation of T reflexes ordinarily obtained through fist clenching (Jendrassik's maneuver) progressively decreases during compression, becomes very slight at saturation, nonexistent during decompression, and returns to normal on surfacing (Table I).

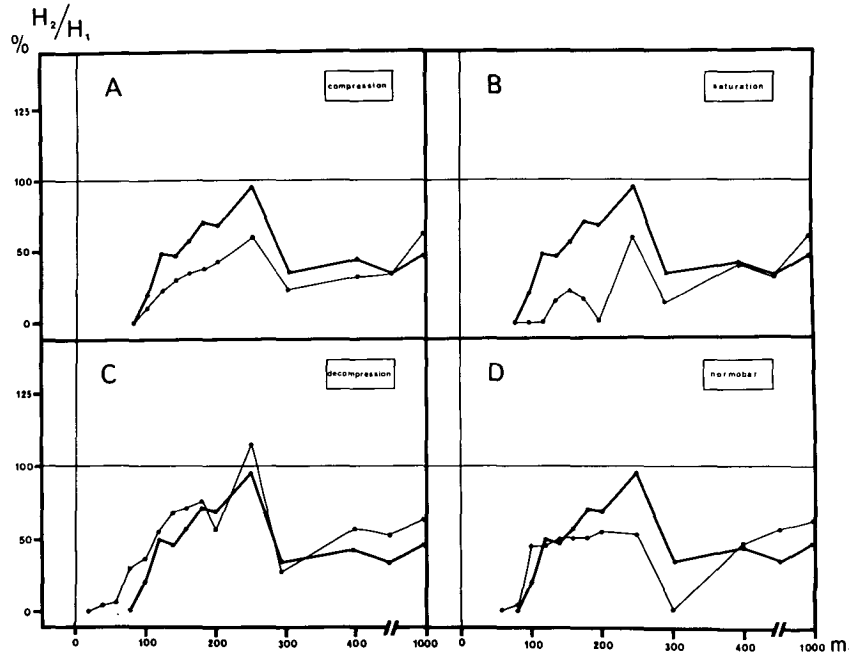

Fig. 3. Hoffmann reflex, recovery cycle. *Abscissa*: time period in msec between reflex test stimulus and reflex conditioning stimulus. *Ordinate*: amplitude of conditioned reflex responses in percentage of reflex test responses. Reflex stimuli have same intensity and are regulated to produce semimaximal reflex response. For all curves values obtained were averaged for right and left legs; for each experimental session, $n = 20$. *A-B-C-D lines*: control curve obtained in laboratory predive. *A*: compression average of curves obtained at 42 and 56 ATA; *B*: saturation average of curves obtained at 62 ATA; *C*: decompression average of curves obtained at 42, 22, 11, 3.5 ATA; *D*: return to surface.

Discussion

The results emphasize a significant variation in reflex indices. Taking into account the stability of the peripheral components of the nerve motor-muscle system, the modifications observed should be related to spinal, supraspinal, or central nervous structure damage.

The reflex data collected during Sagittaire IV confirm the effects noted in previous experiments. These modifications are distinct, appearing most clearly at about 40-50 ATA and persisting until surfacing. The disorders observed are very close to those we described in baboons during heliox dives to 100 ATA carried out in 1971-1972 (Fig. 4). It seems that these modifications are located in the supraspinal central structures, especially at the cortical level, which is indicated by the electroencephalographic modifications and fatigue. Deep dives seem to induce a subwakeful state in subjects, with numerous microsleep phases (1, 4, 9), sleep stages I and II (17), a decrease in critical flicker frequency (19), and an increase in theta waves (2) as signs of drowsiness.

Another interpretation is that some reticular disorder caused by hyperbaric conditions may be responsible for the EEG alterations and behavioral or sensorimotor impairments. To date, the evidence is not weighted in favor of either the cortical or the reticular hypothesis.

Paillard (14) has demonstrated that subwakeful states are compatible with a slight increase

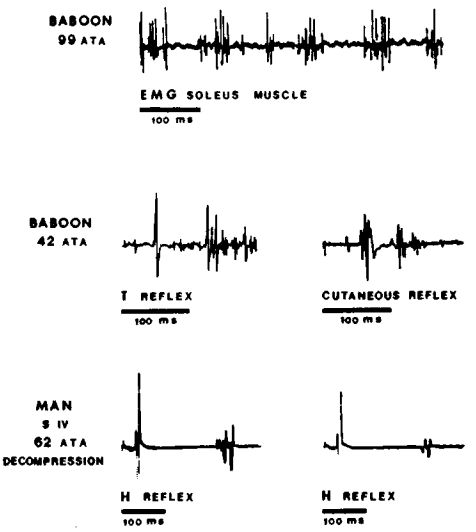

Fig. 4. Either spontaneous or elicited electromyographic responses, obtained during dives with baboons, Papiola III and IV (111 ATA) and with humans, Sagittaire IV (62 ATA). *Upper graph*: electromyogram of spontaneous tremor recorded in baboon's muscle (99 ATA); frequency is about 8 per second. *Middle graphs, left diagram*: note presence after T reflex of abnormal electromyographic polyphasic response; *right diagram*: polysynaptic reflex of cutaneous origin (first response) is followed by abnormal electromyographic polyphasic response. *Lower graph*; note presence of abnormal electromyographic polyphasic response after H reflex in man as in baboons.

in monosynaptic reflex activity (T) and a depression of the general recovery curve. A depression of corticocerebral activity may cause the inhibitory control of spinal motor structures to be released. However, this central structure motoneuron release controlling proprioceptive input is not directly confirmed by data obtained through double stimulation. Indeed, if a refractory phase (III) decrease occurs during decompression, the rebound phase (IV) always exhibits depression, regardless of reflex modality (H or T). Therefore it must be supposed that the release of the inhibitory mechanism of central origin affects both the peripheral inhibitory spinal pathways (Ib and Renshaw systems); thus, the increased excitability of the reflex pathway is explained.

If the hypothesis of a spinal motor pool release of inhibition is accepted, the increase in spontaneous variability of monosynaptic reflex response indicates the intervention of presynaptic inhibition. The results of Rudomin and Dutton (18) show that presynaptic inhibition at the spinal level which does not directly affect motoneuron excitability but does regulate afferent input causes a decrease in monosynaptic reflex variability. A presynaptic inhibition decrease of central origin acting on afferents results in, on one hand, an increase of H and T response variability and, on the other, of motor pool access. In this way motoneuron access to afferents of proprioceptive origin (H–T) and to reticulospinal influences (startle reaction) would be easier.

Electromyographic startle reaction phenomena seem to be specific to hyperbaric heliox conditions. Reflex stimulations activate a spino-bulbo-spinal pathway whose influence is extended, due to the reticular and spinal motor structure release of inhibitory control. This phenomenon is clearly developed in monkeys at extreme depths (100 ATA). A generalized

startle reaction follows the activation of different afferent modalities (cutaneous, auditory, proprioceptive). These motor phenomena have a proximodistal electromyographic development, indicating supraspinal origin (3). This global motor response, contrary to the classic startle reaction, does not undergo habituation. This lack of habituation implicates a specifically hyperbaric disorder.

The decrease of facilitation accompanying Jendrassik's maneuver could be attributed to an "Initial Value Law" effect (11). This result appears, then, as a new expression of motor-pool proprioceptive reflex reactivity modification. The balance between facilitory and inhibitory influences located at a different level which favor the former cannot be displaced by "fusimotor system activation."

The exaggeration of the vestibulo-spinal effects described elsewhere (12), during the same dives, could originate from the same cortical or reticular release mechanisms. Other experiments on animals are necessary to confirm this hypothesis. Nevertheless, it seems that the spinal mechanisms are not directly concerned with reflex disorders nor with HPNS. The disorders observed are caused by suprasegmentary dysfunction rather than by peripheral reflex mechanisms.

ACKNOWLEDGMENTS

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HUMAN VESTIBULOSPINAL REACTIVITY IN HYPERBARIC HELIOX ENVIRONMENTS (31 AND 62 ATA)

M. Lacour, J. P. Roll, M. Bonnet and M. Hugon

Labyrinthine disturbances occurring in deep dives can result from various factors, such as mechanical stimulation, bubble formation during decompression, and inspired inert gas composition. Labyrinthine function injuries can be caused by lesions in the end organ, the vestibular nerve, the brainstem vestibular nuclei, or in the supraspinal structures involved in vestibular output.

Such inner ear function disorders can be of permanent or transitory nature. Classical labyrinthine nystagmus, vertigo, unsteadiness and disequilibrium, nausea and occasional vomiting can indicate vestibular dysfunction. Results of hyperbaric research are sometimes inconsistent; they must be related to the appearance or absence of these vestibular symptoms, to the compression and decompression profiles, and the inspired gas composition. However, labyrinthine disturbances occurring in connection with the High Pressure Nervous Syndrome (HPNS) are incompletely understood.

Braithwaite et al. (6), in a simulated dive at 49.5 ATA, described measurements of vestibular function within normal limits. Appaix and Demard (2) and Hugon et al. (15) indicated a bilateral vestibular hyperexcitability in simulated dives at 25 and 31 ATA. Gauthier (10) found that the increase in the vestibulo-ocular reflex rose intermittently above normal values in a 62-ATA simulated dive. Other studies (1, 6) showed important deteriorations of the postural equilibrium.

In most cases, hyperbaric research on vestibular function relates to the oculomotor output of the vestibular apparatus. This particular study was designed to quantify the vestibulospinal response in deep dives.

Brodal et al. (8) indicate that spinal motoneurons are controlled by the vestibular system via the vestibulospinal and reticulospinal tracts. Kato and Tanji (16) have shown that vestibulospinal input facilitates α and γ motoneuron activity of extensor muscles. Kots and Martyanov (17), Bonnet et al. (4), and Lacour et al. (18), using electrical labyrinthine stimulation, demonstrated a maximally facilitating vestibulospinal effect with a time lag of about 100 msec after the beginning of the stimulation. Bonnet et al. (unpublished observations) noted a sinusoidal modulation of monosynaptic reflex amplitude when subjects were exposed to sinusoidal labyrinthine stimulation.

We have studied labyrinthine function using electrical and rotational tests during two simulated dives at 31 ATA (Sagittaire III) and 62 ATA (Sagittaire IV). Results showed an increase in vestibulospinal output during compression, saturation, and decompression, and particular modifications which were related to decompression.

Methods and Techniques

Vestibular function in deep dives was studied in 3 divers using electrical labyrinthine stimulation (Sagittaire III, 31 ATA) and in 2 other divers using a sinusoidal rotational labyrinthine stimulation (Sagittaire IV, 62 ATA). Spinal reactivity was checked by the proprioceptive Hoffmann's reflex amplitude (H reflex); we studied the spinal effects of these two types of labyrinthine stimulation through the analysis of the modulation of H-monosynaptic reflex amplitude.

Tests done before the dives at the surface (1 ATA, air) and during confinement (1.8 ATA, heliox) served as references. The tests were repeated during dives at different depths (Fig. 1). The modifications observed in these dives demonstrate the influence of diving conditions on vestibulospinal system functioning.

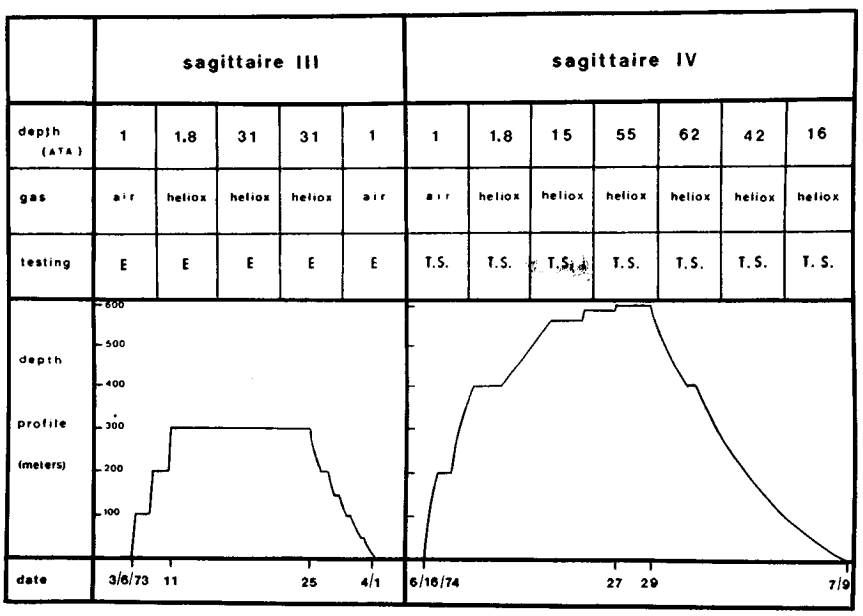

Fig. 1. General dive parameters in two simulated dives. Third row shows tests of labyrinthine function used at simulated depths; E = electrical stimulation; T.S. = torsion swing. General dive profiles are drawn at bottom, with depth in meters and time in days.

Hoffmann's Reflex Stimulation

To study the spinal effects of electrical or rotational labyrinthine stimulation we recorded the H reflex of an extensor foot muscle, the soleus. The subject was seated in a special armchair.

H stimulation was an electrical percutaneous stimulation of the posterior tibial nerve applied according to the method defined by Paillard (19); it was simultaneously applied to the two legs. The H-reflex response was recorded by skin surface electrodes and was measured by a digital signal amplitude detector. H stimulation was regulated to elicit a semi-maximal H reflex.

Electrical Labyrinthine Stimulation (ELS)

This was accomplished by an electrical percutaneous stimulation of the external ear area (see (18)). ELS was performed by means of a rectangular electric current (200-msec duration) through two silver electrodes; the anode (2-cm diameter) was fixed above the tragus of the ear and the cathode (1-cm diameter) on the mastoid on the same side of the head.

In a seated subject, ELS causes lateral rotation of the head away from the stimulated ear; under experimental conditions, ELS intensity was kept just below body and head displacement threshold (mean, 2.5 mA).

Subjects participated in 4 experimental series, 2 with left and 2 with right labyrinthine stimulation. Each series (Fig. 2) was composed of 72 H stimulations, 48 of which were conditioned by ELS and applied randomly with variable delays (10, 30, 50, 100, 150 and 350 msec). Twenty-four other H stimulations that were not conditioned made up the control level. The interval between stimuli also varied randomly, from 8–15 seconds.

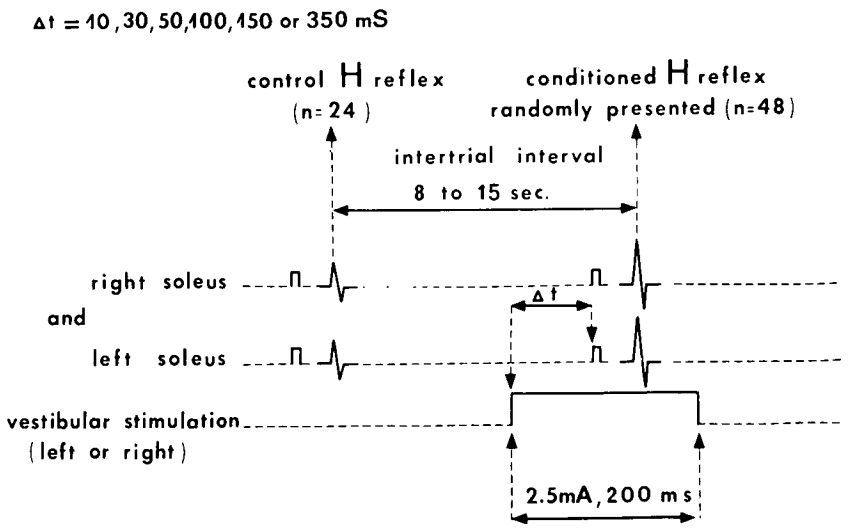

Fig. 2. Experimental procedure using electrical labyrinthine stimulation. H reflexes elicited simultaneously in both legs (right and left soleus). Electrical labyrinthine stimulation (200 msec, 2.5 mA) is applied either on left or right side of head. Each series is composed of 72 H stimulations; 48 of these 72 trials are randomly applied with variable Δt delays after beginning of labyrinthine stimulation. Remaining 24 trials not conditioned by labyrinthine stimulation represent control level. Intertrial interval is randomly varied, from 8–15 seconds.

Rotational Labyrinthine Stimulation

The subject was seated in an armchair which rotated in a yaw axis. An apparatus maintained the head so that the plane of the horizontal semicircular canals remained horizontal. The sinusoidal rotation period was 2.5 seconds, with a maximal amplitude of 24°; maximal velocity and acceleration were 60°/sec and 150°/sec^2, respectively. Rotation was maintained constant.

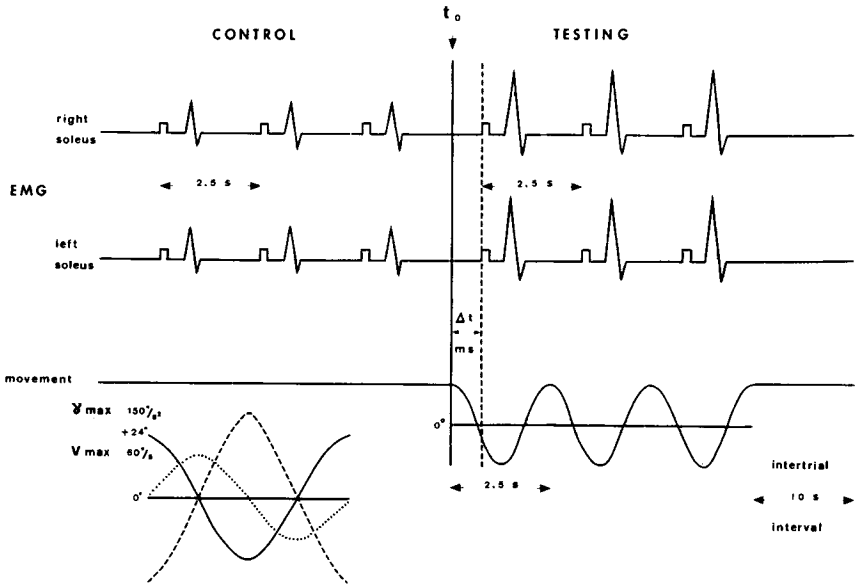

Fig. 3. Experimental procedure using a sinusoidal rotational labyrinthine stimulation (torsion swing). H reflexes are elicited simultaneously in both legs (right and left soleus) during sinusoidal rotation (test conditions) or 10 seconds after end of rotation (control). Each control or test series is composed of 3 bilateral H stimulations elicited at fixed intervals (2.5 seconds). H stimulations occurring during rotation are given after a Δt delay measured from beginning of rotation. Δt delays are progressively increased by 200 msec from 1 trial to next. *Lower left*: curves of movement (*solid line*), velocity (*dotted line*) and acceleration (*dashed line*) are drawn with reference to maximal displacement (24°), velocity (60°/sec) and acceleration (150°/sec²). Period of rotation is 2.5 seconds.

Each trial (Fig. 3) consisted of: (1) a reflex stimulation elicited simultaneously for the two legs 10 seconds after the armchair stopped rotating; this stimulation was repeated 3 times at set intervals of 2.5 seconds; the mean value of H-reflex responses recorded under these conditions made up the control level; (2) an identical series of 3 bilateral reflex stimulations distributed at different Δt moments of the sinusoidal rotation.

Thirteen similar trials were repeated; Δt, calculated from the beginning (t_0) of the rotation, was increased by 200 msec each time. The stop position of the rotating chair was arbitrarily set at +24°. The first half-period always took place in counterclockwise direction. The H-reflex modulation curves obtained before and during a dive were analyzed in relationship to the velocity and acceleration curves.

Data Analysis

Data were analyzed to give average and standard deviation of control values. The amplitude of H reflexes conditioned by electrical or rotational labyrinthine stimulation was expressed in percentage or Z score (i.e., by standard deviation of the control value distribution). For each subject, calculations as a function of the electromyographic reception side, of the ELS side, and of Δt delays were made.

Results

Spinal Effects of ELS (Sagittaire III)

Before the Dive (1 ATA, Air)

Classical ELS effects can be divided into aspecific effects due primarily to electrical skin stimulation (tingling) and specific effects that result from labyrinthine receptor excitation.

At 1 ATA (air), aspecific effects observed in the 3 divers were comparable to those observed in nondivers. Lateral rotation threshold (2.5 mA) was, as a rule, identical for all subjects. On the other hand, the concomitant inhibitory or facilitory vestibulospinal effects were considerably increased in the 3 divers (Fig. 4). For example, the ipsilateral facilitation resulting

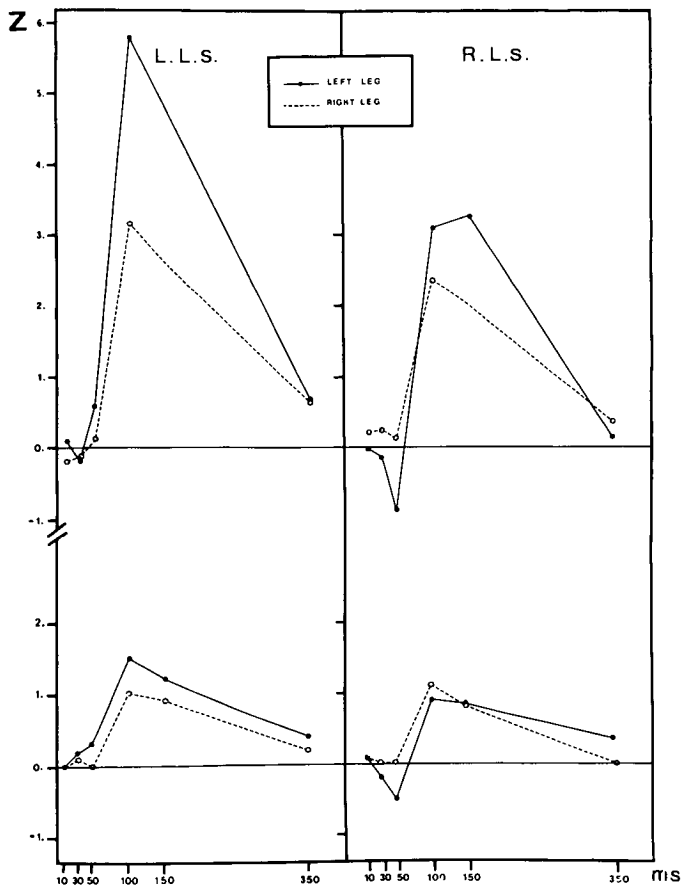

Fig. 4. Vestibulospinal effects of electrical labyrinthine stimulation at 1 ATA (air). Comparison of vestibulospinal effects recorded in divers (*upper graphs*: $n = 3$; each point is average of 36 samples) and normal subjects (*lower graphs*: $n = 25$; each point is average of 300 samples). Electrical labyrinthine stimulation is applied to left side of head (L.L.S. column) or to right side (R.L.S. column). H reflexes are recorded on left leg (*solid line*) and right leg (*dashed line*). Curves represent average of vestibulospinal effects recorded at variable delays for a 2.5-mA stimulation. *Ordinate*: amplitude of conditioned H reflexes plotted as Z score of control H reflexes. *Abscissa*: interstimulus delay in msec. Results show greater vestibulospinal effects in divers than in normal subjects.

from left labyrinthine stimulation corresponded in divers to 6 SD at Δt = 100 msec, whereas in nondivers it corresponded to 1.5 SD. Similar differences are found at every delay and imply that divers are a specific category of subjects.

During the Dive (31 ATA, Heliox)

The electrical stimulus was more efficient at depth than at the surface. If the aspecific effects remained unchanged, the lateral-rotation stimulation threshold was reduced from 2.5 mA to 1.5 mA.

The facilitory vestibulospinal effects recorded for this new stimulation intensity (1.5 mA) were increased for both legs (Fig. 5). As the spinal reflexivity was not significantly modified, the increase of vestibulospinal effects in the dive must be attributed to a bilateral vestibular hyperexcitability.

This increased vestibulospinal facilitation was almost the same at the beginning and at the end of saturation (no adaptation); however, a tendency toward an even greater facilitation was observed at the end of saturation. After surfacing, control values were once again noted.

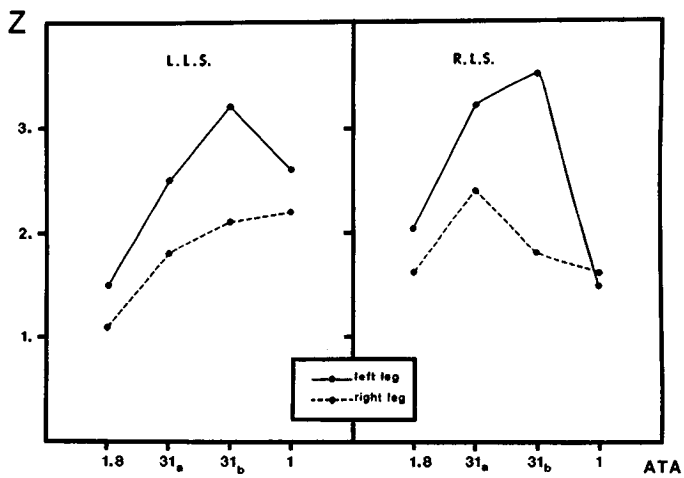

Fig. 5. Vestibulospinal effects of electrical labyrinthine stimulation in Sagittaire III. H reflexes are recorded on left leg (*solid line*) and right leg (*dashed line*); electrical labyrinthine stimulation is applied to left side of head (L.L.S.) or to right (R.L.S.). These curves represent average of vestibulospinal effects obtained for a 1.5-mA stimulation at an interstimulus delay of 100 msec. Each point is average of 48 samples. *Ordinate*: amplitude of conditioned H reflexes, plotted as a Z score of control H reflexes. *Abscissa*: chamber pressure in ATA; 1.8 = confinement before dive; 31_a = beginning of saturation; 31_b = end of saturation; 1 = test after dive (air).

Spinal Effects of Rotational Labyrinthine Stimulation (Sagittaire IV)

The vestibulospinal effects of rotational stimulation did not significantly differ from surface (1 ATA, air) to confinement (1.8 ATA, heliox). H-reflex amplitude modulation in relationship to rotating velocity was observed. This modulation was bilateral. The mean time-course of vestibulospinal effects is shown in Fig. 6*A*; it indicates moderate facilitation and inhibition (±20%).

During compression and at steady-state levels large modifications occurred. A bilateral increase in H-reflex facilitation occurred around 15 ATA; this phenomenon continues and increases up to 62 ATA. Facilitation peaks are related to maximal velocity, notwithstanding rotating direction. The inhibitory vestibulospinal effects remain unchanged. Figure 6*B* shows the mean time-course of vestibulospinal effects recorded during compression and saturation. A mean maximal facilitation of 150% and a phase lag of 25° (150–200 msec) could be noted.

A decrease of the two facilitation peaks described above occurs during decompression. Furthermore, the H-reflex modulation curve appears to be related to acceleration; a facilitation peak with a phase lag of 15° (100 msec) develops in relation to maximal positive acceleration. Figure 6*C* illustrates the mean time-course of vestibulospinal effects recorded during decompression. Maximal facilitation represented 165% of control values; inhibitory effects were also increased.

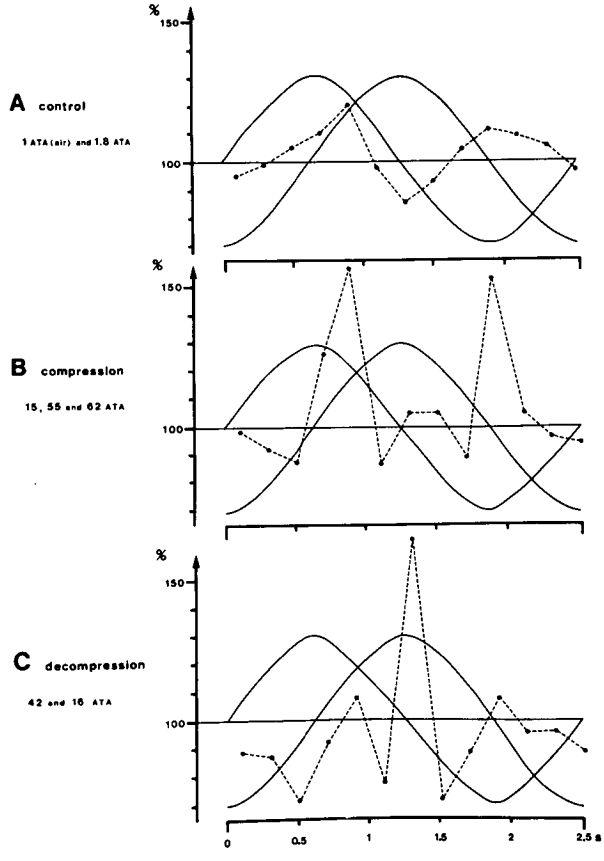

Fig. 6. Vestibulospinal effects of sinusoidal rotational labyrinthine stimulation in Sagittaire IV. Results are average of H reflexes recorded from both legs plotted on each graph with a dashed line. Velocity and acceleration curves are indicated by solid lines. Mean modulation of H-reflex amplitude obtained predive (1 ATA, air, and 1.8 ATA, heliox) is plotted in *A*. *B* shows mean modulation recorded during compression (15, 55, and 62 ATA) and *C* shows modulation during decompression (42 and 16 ATA). Each point is average of 18 samples. *Ordinate*: H-reflex amplitude plotted as percentage of reference. *Abscissa*: Δt delay between beginning of sinusoidal rotation and H stimulation.

Discussion

Vestibulospinal Reactivity in Divers Before the Dive

The effects of unilateral vestibular neurectomy have been analyzed in man and monkey (18); this study clearly showed that electrical labyrinthine stimulation as previously described excited vestibular system sensory receptors. Results obtained at the surface showed that the spinal reflex of divers does not significantly differ from that of nondivers. On the other hand, divers have a distinctly greater spinal reactivity to electrical labyrinthine stimulation. Increase in vestibular system spinal output in divers can be of labyrinthine (sensory receptors) or of vestibular (vestibular nuclei, archeocerebellum) origin. Similar observations made by Gauthier (10) demonstrated that the vestibular system oculomotor output increased intermittently in divers under normal atmospheric pressure.

Acute labyrinthine injuries as well as progressive cochleovestibular damage that appears gradually in divers undergoing repeated barotrauma at the middle ear level (9) could create a subclinical modification of their vestibular apparatus; this could explain the increase in vestibular system spinal and oculomotor output. However, Gauthier (11) showed that normal subjects who wore magnifying lenses for a certain length of time showed a lasting increase in vestibulo-ocular reflex gain. Thus, it seems appropriate to consider the vestibular system as a structure capable of adaptating its gain to environmental conditions. Ito (unpublished observation) indicates that the archeocerebellum, where vestibular and visual information converge, could assume the role of a vestibular system gain regulator.

Vestibulospinal Reactivity in Deep Dives

Results recorded during the Sagittaire III and IV dives point to a large and bilateral increase in vestibulospinal effects. This phenomenon can be observed during compression, saturation, and decompression, without any major monosynaptic spinal reflex modifications (14, 20). If the spinal cord is not influenced by physicochemical dive factors, it seems that vestibulospinal hyperexcitability originates at the level of the labyrinthine and/or the vestibular system.

This could be due to an osmotic disorder occurring in hydrophobic tissues (13) during compression. However, the persistence and accentuation of signs and symptoms after prolonged saturation, which should allow complete tissue equilibration, make this hypothesis insufficient. Furthermore, the absence of spontaneous vestibular symptoms (vertigo, nausea, nystagmus) makes the hypothesis of an acute labyrinthine injury highly improbable.

It is therefore concluded that an increase in vestibular system spinal output gain is of labyrinthine or vestibular origin. Efferent inhibitory fibers have been shown at the sensory receptor level; their function is probably to regulate receptor sensitivity (12). Deep dives could produce a decrease of these inhibitory influences originating at the cortical or subcortical level. Vestibular system spinal output gain could be due to nuclear hyperexcitability or to a greater primary and secondary vestibular afferent relay structure activity (reticular formation, flocculo-nodular lobe, fastigial nucleus). Indeed, only vestibular influences which are able to reach the lumbar spinal cord run through vestibulo-reticulo-spinal or lateral vestibulospinal paths after cerebellar relay (7). Under these conditions the increase in vestibulospinal effects at depth could result from cortical control modification over these subcortical struc-

tures. The hypothesis of an alteration in supraspinal structure function was suggested by Bonnet et al. (5); this interpretation may be related to the observations of Rostain et al. (21), and Bennett and Towse (3), who pointed out important modifications in electroencephalographic recordings. However, it is impossible to say whether this involves the release of inhibitory or facilitory mechanisms.

Notwithstanding the vestibulospinal hyperexcitability observed during all dive phases, a particular modification occurs during decompression. It consists of a change in the relationship between the H-reflex modulation curve and the velocity-acceleration curves. This phenomenon is probably due to a dysfunction at the end organ level. It may also reflect an increase in perilymphatic and endolymphatic system viscosity related to bubble formation.

In conclusion, our results suggest that the increase in vestibular system spinal output observed during compression, saturation, and decompression is one of the factors causing the appearance of HPNS symptoms. The particular modification observed during decompression seems to be related to the general decompression sickness syndrome.

ACKNOWLEDGMENT

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EFFECTS OF TEMPERATURE ON THE HIGH PRESSURE NERVOUS SYNDROME IN RATS

J. A. Cromer, W. L. Hunter, Jr. and P. B. Bennett

Signs and symptoms of what is today called the High Pressure Nervous Syndrome (HPNS) were first described by Zaltsman in humans and have since been demonstrated by other investigators in a variety of other species. For example, Zaltsman reported these signs and symptoms in dogs and mice (21), Lever et al. noted them in mice and newts (12), Albano et al. investigated HPNS in rats (1), and Brauer and his co-workers have studied this syndrome in some twenty different species (5,8,9). All of these investigations show that the syndrome includes tremors, myoclonic jerks, and tonic or tonic-clonic generalized convulsions.

A parameter often used in these studies to detect the influence of variables on HPNS is the convulsion threshold pressure (P_c). However, the absence of an accepted definition of convulsion threshold pressure has made comparison of the results of these studies difficult. Many studies rely on visual observation, which varies from observer to observer, for determining P_c. There is also disagreement about the effect of certain environmental factors on HPNS, specifically ambient temperature. As early as 1961, Russian workers stated that increases in ambient temperature lowered P_c, while other laboratories have reported that variations in ambient temperature have no effect (7, 21). Still other studies in hydrostatically compressed liquid-breathing animals have suggested that increasing ambient temperature increases P_c (11,13).

This study was designed with two objectives: to devise a clear-cut, objective method of determining HPNS convulsion threshold pressure, and to clarify the effects of temperature on HPNS.

Methods

Electrode Preparation and Implantation

Thirty-two adult male Wistar rats with an average weight of 325–350 grams were anesthetized with an intraperitoneal injection of sodium pentobarbital. The animals were positioned in a Kopf stereotaxic unit (Model 1404), and a midline incision exposing the surface of the skull was made. Using Pellegrino's *A Stereotaxic Atlas of the Rat Brain* (14), three areas were identified. With extreme care, five separate burr holes were made above the following locations: right frontal cortex, left frontal cortex, left hippocampus, left red nucleus, and right red nucleus. A sixth hole was made in the posterior occipital region into which an anchor screw was inserted.

Two types of electrodes were fabricated. Cortical electrodes consisted of 1-72 stainless steel screws with attached stainless steel wire leads. During surgery, this first type of electrode was manually screwed into the proper burr holes and positioned so that it was touching the dura. Subcortical electrodes consisted of 0.01-inch diameter stainless steel wire cut to a length of 3.0 cm. Insulation was removed from the tips for a distance of 1 mm. Using the stereotaxic carrier, this second type of electrode was positioned through the appropriate burr hole into the desired subcortical area. Affixed to each electrode was an Amphenol "Rely-Tac" microminiature connector. In addition, an extracranial silver ground wire with its female connector was positioned. Dental acrylic was used to affix the electrodes and ground wire permanently to the skull. All female connectors were inserted into an Amphenol strip connector of appropriate length and additional acrylic was applied to stabilize and attach the plug unit permanently to the animal's skull. Verification of electrode placement was accomplished by serial sectioning of brains from randomly selected animals after completion of the experiments.

Electroencephalogram (EEG) Recordings

All EEG's were recorded on a Brush recorder (Model 440) using Brush EEG medical preamplifiers and couplers. Three bipolar channels were simultaneously recorded, consisting of *a*) right frontal cortex-left frontal cortex, *b*) right red nucleus-left red nucleus, and *c*) left hippocampus-left red nucleus.

In addition, the circuit of each channel employed a light-weight dual-channel field effect transistor (FET) (16). These FET's were located in the detachable plug of the recording circuit. Signals from channels *a*) and *c*) were passed through a Nihon-Khoden EEG frequency analyzer and printed on the EEG tracing (3). Integrity of the electrical circuitry was checked within 48 hours of surgery in each animal.

Dive Procedures

Seven days or more after surgery, the animal was weighed and placed in a restraint system that allowed freedom of head and limb movement. EEG circuits were connected, the colonic thermistor probe was inserted, and the animal was positioned in an 8.3-liter steel hyperbaric chamber. The chamber was sealed, and an exterior light source placed in front of the port. Both chamber and deep colonic temperatures were recorded. Surface control EEG's were obtained at this time with the animal breathing air.

The chamber was then flushed with 100% oxygen for 15 minutes. Helium and oxygen gases were added to establish a mixture containing at least 60% oxygen. Gas mixing was accomplished by means of a venturi inlet. The chamber atmosphere was sampled and analyzed to confirm the mixture. A second surface control EEG was obtained at this point while the animal breathed the new gas mixture. Compression from this point was accomplished using 100% helium at a rate of 40 atm/hr to a maximum depth of 4500 fsw (137 ATA). Throughout compression, EEG recordings and temperature readings were obtained at 5-minute intervals. Visible changes in the animals' behavior were recorded as they occurred. Animals which survived to maximum depth were held there for one hour, during which periodic recordings were obtained. At the termination of the experiments, animals were killed by rapidly decompressing them to the surface.

Temperature Alterations and Recordings

Three different ambient temperature environments were used. By utilizing the high thermal conductivity of helium and manipulating chamber ambient temperature, the animal's colonic temperature was either lowered, raised, or maintained at control levels. Control colonic temperature was designated as that reading obtained with the animal breathing room air five minutes after insertion of the thermistor probe. In each animal the probe was inserted to a depth of 6.5 cm proximal to the anal opening (20).

The high thermal conductivity of helium has been shown to decrease core temperature in rats if chamber temperatures are not raised above room levels (17). In the first series of animals, this characteristic of helium was used to lower colonic temperatures to hypothermic levels (26.0-29.2°C, mean 26.0°C). By making no effort to control chamber temperatures (which remained substantially at room temperatures of 21-24°C), colonic temperatures showed marked decreases as compression proceeded.

In the other two groups of animals, a heat tape surrounding the exterior of the chamber was used to increase chamber ambient temperature. By maintaining chamber temperatures of 33–35°C with the heat tape, colonic temperatures for any one animal were kept to within ± 0.5°C of control readings in the second euthermic group. The control readings for all animals were 37.2 ± 1.0°C. Again using the heat tape to raise chamber temperatures to 38–41°C, a third group of animals was maintained at hyperthermic levels with colonic temperatures of 40° ± 0.5°C.

All temperatures were measured using Yellow Springs thermistors and Yellow Springs telethermometers.

Control Procedures

Other variables which might have interfered were controlled. To eliminate effects of the diurnal cycle and variable lighting, all dives were performed at the same time of day, and a constant level of lighting was maintained throughout the dive (7). Weights were recorded at the time of surgery and immediately prior to the dive, to guarantee healthy specimens and to determine any age differences.

To observe any time- or method-induced EEG changes, randomly selected animals scheduled to be used in subsequent dives were run as controls. These control experiments used the same experimental apparatus and procedures used in an actual dive, except for the absence of pressure. Both 1-ATA air and 1-ATA helium-oxygen experiments were made on successive days immediately prior to an actual dive. These control experiments lasted the same amount of time as the actual experiment.

Immediately after the helium-oxygen atmosphere reached 1 ATA, periodically during compression, and immediately prior to decompression, chamber atmosphere was sampled and measured on a Perkin-Elmer mass spectrometer. Chamber oxygen levels were maintained between 0.4 and 0.6 ATA; the lowest reading obtained was 0.42 ATA (320 mmHg). Carbon dioxide levels were controlled by using Baralyme in the chamber, and the level was kept below 1% surface equivalent at all times. The maximum reading obtained was 7 mmHg.

Results

The techniques and procedures used in this study yielded EEG recordings which were free of artifact in all channels despite random head and limb movement by the animal during re-

cording periods. Figure 1 represents a 1-ATA air control recording obtained from an animal immediately prior to a dive. Due to minor differences in frequency and amplitude of EEG's in different animals, each animal's air control EEG was used as its standard of comparison. In the group of animals for which EEG's were recorded for simulated dive durations in both 1-ATA air and 1-ATA helium-oxygen, there were no significant differences between EEG patterns under these conditions and EEG patterns of air controls immediately preceding a dive. There also were no significant differences between recordings obtained at 1-ATA air and 1-ATA helium-oxygen in the same animal. The recording in Fig. 1 is typical of control recordings obtained from all animals in this study. The amplitude and frequency characteristics of this recording are compatible with those described in the literature for these locations (19,20).

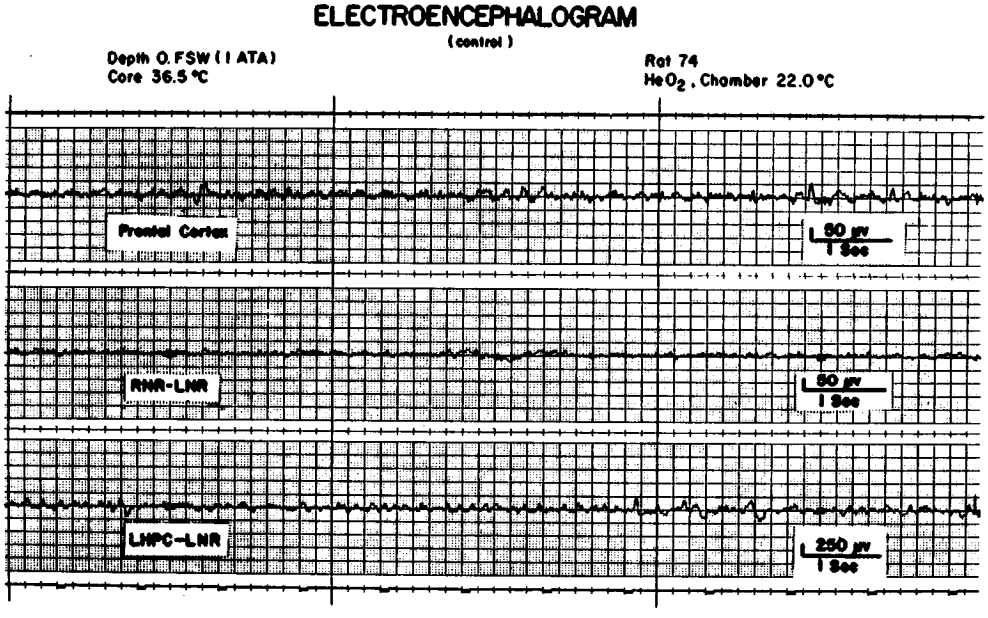

Fig. 1. Sample EEG control recording from animal breathing air at 1 ATA from frontal cortex, right red nucleus–left red nucleus (RNR–LNR), and left hippocampus–left red nucleus (LHPC–LNR).

Figure 2 is a recording of EEG seizure activity in the same animal whose control recording is shown in Fig. 1. The depth at the time of this recording was 3800 fsw (116 ATA) and the animal's colonic temperature was at control levels. All three channels simultaneously show repetitive, synchronous spike and wave discharges of 250–500 μv amplitude with a frequency of 2–5 Hz. These discharges are typical of EEG seizure activity seen during generalized seizures (10). Occurring simultaneously with these EEG patterns was an observable, sustained tonic-clonic generalized convulsion.

The EEG recording in Fig. 3 is from an animal in a hyperthermic series. This recording also shows typical seizure patterns in all channels concurrently with an observable generalized convulsion. Figures 2 and 3 are representative of seizure patterns seen during all convulsive episodes.

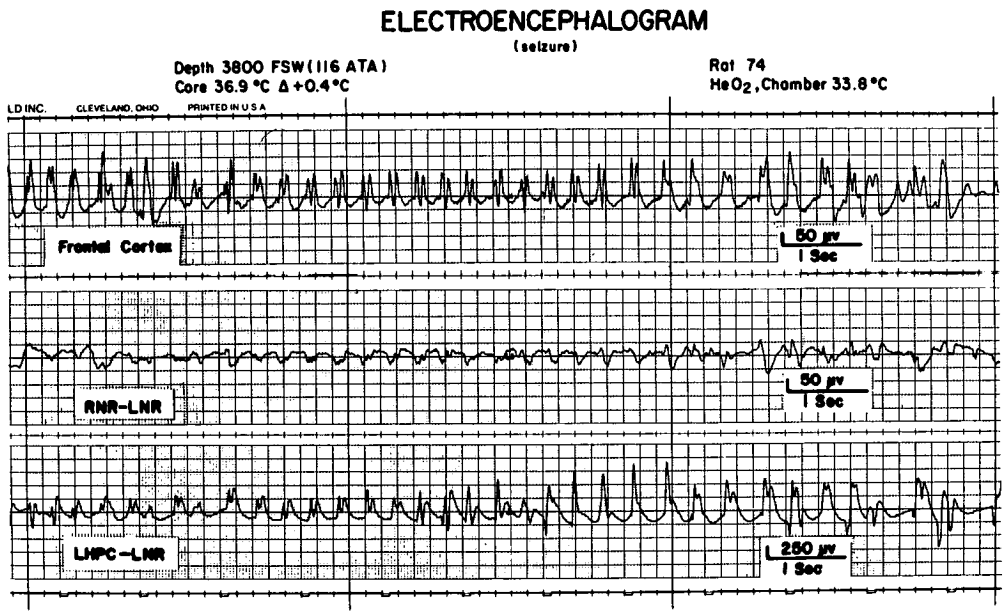

Fig. 2. Sample EEG seizure recording from animal in euthermic series breathing helium-oxygen at 116 ATA. (Same areas as for Fig. 1.)

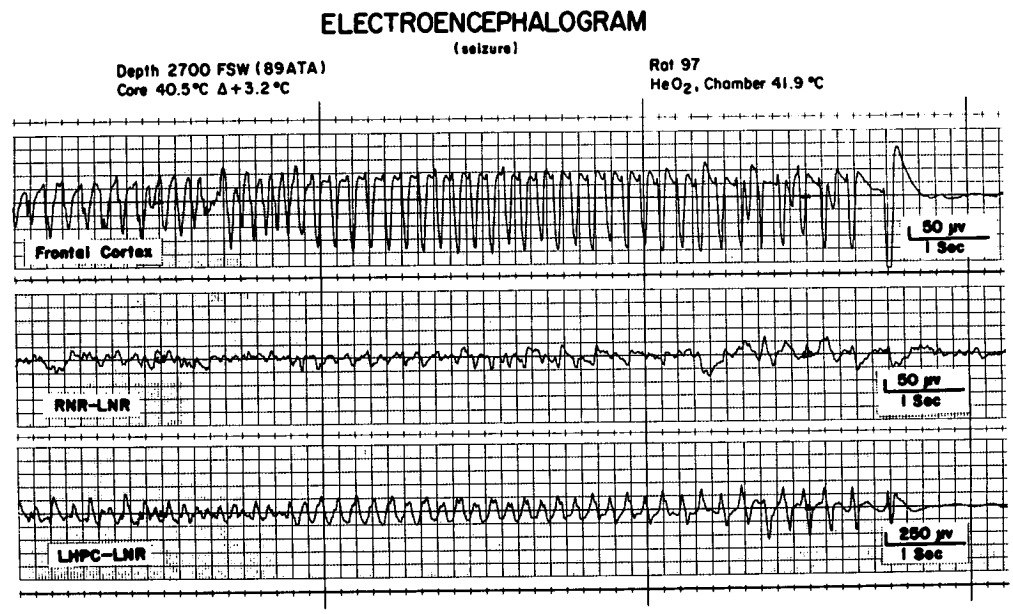

Fig. 3. Sample EEG seizure recording from animal in hyperthermic series breathing helium-oxygen at 89 ATA. (Same areas as for Fig. 1.)

Observations showed that all animals in the study had both tremors and myoclonic jerks of varying severity prior to convulsive activity. However, no EEG manifestations that correlated with tremors and myoclonic jerks could be detected. In several animals, typical seizure patterns were seen in a single channel that were unaccompanied by any observable convulsive activity. However, observed generalized convulsive activity was in every case accompanied by EEG seizure patterns in *all three channels.* Therefore, the initial occurrence in each animal of seizure patterns in all channels and observable convulsion was selected as the definition of convulsion threshold pressure (P_c) in this study.

The effects of temperature on HPNS were demonstrated by using this definition of P_c in the three different temperature conditions described previously. All 10 animals in the euthermic series displayed convulsive activity and EEG patterns similar to those shown in Figs. 2 and 3. The 10 euthermic animals had a mean P_c of 3700 fsw (113 ATA) as shown in Table I. The eight animals in the hyperthermic series all displayed convulsive activity and EEG seizure patterns similar to those shown in Figs. 2 and 3, but at much lower pressures. The hyperthermic animals had a mean P_c of 2450 fsw (78 ATA).

TABLE I

CONVULSION THRESHOLD PRESSURES

	Range, fsw	Mean, fsw	SD†
Hyperthermic Animals, $n = 8$	2100–2700	2450 78 ATA	200.0
Euthermic Animals, $n = 10$	3300–4200	3700 113 ATA	298.1
Hypothermic Animals, $n = 10$	*	* (>4500)	*

Comparison of mean HPNS convulsion threshold pressures in 3 temperature conditions. Hyperthermic group = colonic temperature 40 ± 0.5°C; euthermic = 37.2 ± 1.0°C; hypothermic = 27.4°C (26.0–29.2°C). †P = <0.0005. *No hypothermic animal convulsed within limits of available chamber capacity.

Figure 4 is a recording from an animal in the hypothermic series. The recording was obtained at 4500 fsw (137 ATA), the maximum capability of the chamber. Colonic temperature at the time of the recording was 24.2°C. All three channels show a marked decrease in frequency and amplitude when compared to control recordings. This recording is typical of those obtained from all 10 animals in the hypothermic series. Neither EEG seizure patterns nor observable convulsive activity occurred in any animal in the hypothermic series at any time (Table I).

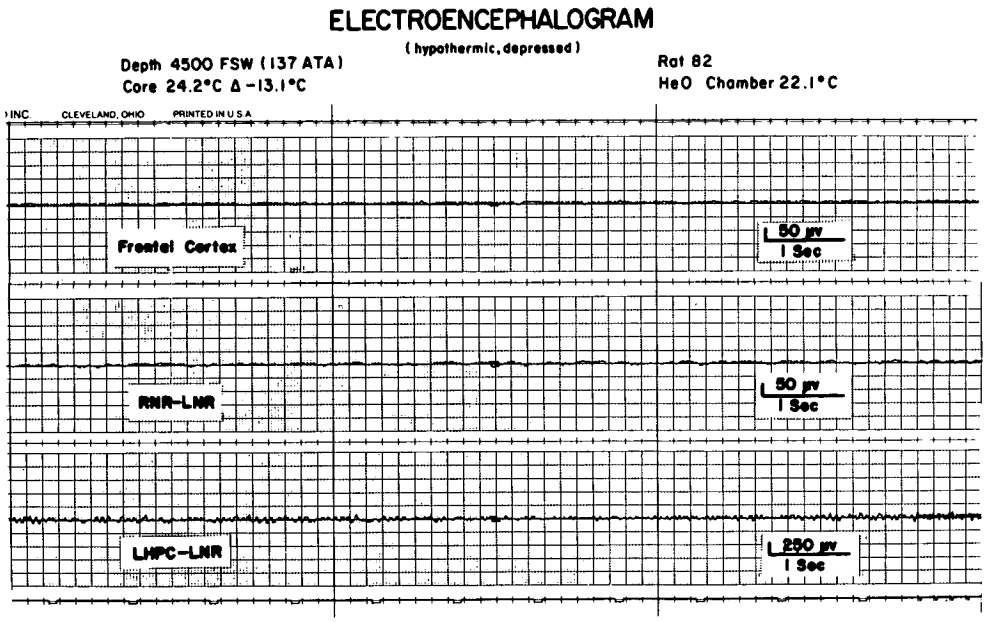

Fig. 4. Sample EEG recording from animal in hypothermic series breathing helium-oxygen at 137 ATA. (Same areas as for Fig. 1.)

Table I shows a comparison of the results of changes in colonic temperature on the convulsion threshold pressure in each of the three groups of animals. *T*-test analysis of the data reveals highly significant differences between the three groups.

Discussion

Numerous laboratories have investigated a variety of different variables which could possibly influence HPNS (3,6,9). By necessity, these studies have used changes in threshold pressures of the various components of the syndrome as the indicator of the effect of such variables. Visual observation of sometimes subtle behavioral changes in animals, such as onset of tremor or loss of normal posture, are examples of indicators that have been used. In our laboratory, it was found that observer differences in noting behavioral changes made such procedures unreliable. Therefore, an objective method of determining threshold pressures was sought. A system was developed that would consistently yield artifact-free EEG recordings from both cortical and subcortical areas simultaneously (Fig. 1). In all animals, whenever typical seizure patterns appeared simultaneously in all channels, generalized sustained convulsive activity was observed (Figs. 2 and 3). This technique was used only for investigating convulsion threshold pressure (P_c).

No EEG changes could be detected that correlated with tremor or myoclonic jerks. It is possible that other subcortical areas might yield such information if this technique was used. Threshold pressures for tremor and myoclonic jerks were noted by visual observation for all animals, but such observations are inherently less accurate. Even though statistical

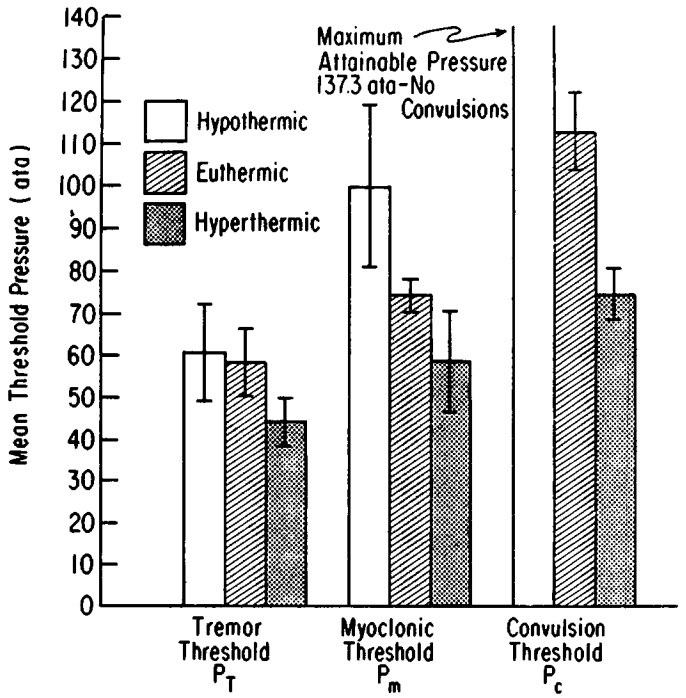

Fig. 5. Comparison of effects of alterations in colonic temperature on threshold pressures for HPNS tremor, myoclonic jerks, and convulsions.

differences between the three groups were noted for these parameters (Fig. 5), it is felt that such differences are unreliable.

In hyperbaric studies using visual observation only, the effects of temperature on HPNS have been unclear (3,5,6,7,8,12,21). Investigators who have reported lack of dependence of P_c on temperature have monitored only ambient temperatures. However, Stetzner and De Boer have documented significant decreases in core temperature in rats in room temperature hyperbaric helium-oxygen environments (17). Using colonic temperature rather than chamber temperature as the variable, we observed a marked dependence of HPNS convulsion threshold pressure on temperature (Figs. 4, 5).

It was not possible to generate HPNS seizures in the hypothermic animals within the available chamber pressure capability. Perhaps compression to depths in excess of 4500 fsw (137 ATA) or compression at a more rapid rate would have accomplished this. Alternatively, decreasing the magnitude of the hypothermic stress might also have led to seizures. It can only be stated that the data indicate that at a compression rate of 40 atm/hr, P_c in the hypothermic group is greater than 4500 fsw (137 ATA).

In the hyperthermic series, only 8 of the 12 dived animals survived to the convulsive states. Although a limit for colonic temperature of 40°C was maintained in all animals in this series, the heat stress appears to have caused early death in 4 animals.

Numerous investigators have reported temperature-dependent changes in EEG activity (2,4,18,19,20). Uniformly, hypothermia has been shown to decrease overall EEG activity. In hyperbaric studies, such overall EEG depression seen during dives has been attributed to hydrostatic pressure effects (3,15). However, since this pattern of depression was seen only in the hypothermic animal series, the data suggest that the overall EEG depression seen during hyperbaric helium-oxygen exposures may well be a result of decreased core temperature rather than increased hydrostatic pressure.

Although the conclusions of this study pertain directly only to the rat, the significance of the effects of temperature on the High Pressure Nervous Syndrome in humans must be considered. This is especially true in dives with very rapid compression rates in which high temperatures could become a problem.

ACKNOWLEDGMENT

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ROLE OF MONOAMINE NEUROTRANSMITTERS IN THE COMPRESSION-RATE DEPENDENCE OF HPNS CONVULSIONS

R. W. Brauer, R. W. Beaver and M. E. Sheehan

Previous work has shown that the High Pressure Nervous Syndrome can be seen to develop in three stages (4). These stages are respectively characterized by tremors and other minor central motor disturbances, isolated nonvolitional myoclonic jerks which are frequently associated with spiking in the electrocorticogram, and the development of generalized motor and electroencephalographic convulsions. From the beginning there have been certain indications that the syndrome might appear sooner or more markedly at rapid than at slow compression rates (2, 6). A more detailed analysis of the relationship between compression rate, time at pressure, and the onset of the several stages of HPNS in the squirrel monkey and in baby and adult mice has recently been published (5). In the adults of these species, convulsion threshold pressures were found to decrease with the logarithm of compression rate over a 200-fold range of the latter. The threshold for the myoclonic jerk stage, designated as the coarse tremor stage in the squirrel monkey, showed a similar compression-rate dependence, while the onset of fine tremors was nearly independent of the speed of compression. Of particular theoretical interest was the finding that mice less than 10 days old, which show a well-developed HPNS with a clearly marked convulsion stage, have convulsion thresholds which do not vary with compression rate. This observation suggested that the primary phenomenon underlying HPNS may be elicited by high hydrostatic pressure per se, and may thus be independent of compression rate. Based on this hypothesis, the onset of the actual convulsive episodes in the adult monkey and mouse would be modified by a compression-rate-dependent mechanism. It would follow from this hypothesis that one of the events associated with maturation of the newborn mouse is the progressive emergence of this secondary mechanism, beginning shortly after the age of 10 days.

This paper will report on the nature of this compression-rate-dependent component of HPNS, using pharmacological and comparative physiological methods as investigative tools.

Compression-Rate Effects in Different Species

The effect of compression rate on HPNS convulsion thresholds was studied in 12 species, including two amphibians, two reptiles, two birds, four species and nine strains of rodents, one carnivore and two primates. The results are incorporated in Table I and illustrated in Fig. 1. In each case the experimental results could be represented adequately over the range

of compression rates tested (20-fold or greater, as indicated by the solid line segments in Fig. 1) by the equation

$$ {}^{a}P_c - {}^{b}P_c = K \cdot \log \frac{b}{a} \qquad (1) $$

where ${}^{a}P_c$ and ${}^{b}P_c$ are the convulsion threshold pressures at compression rates ${}^{a}\dot{P} = a$ and ${}^{b}\dot{P} = b$. Values of K and of the correlation coefficients were computed by least square methods from the actual data for each species; ${}^{1000}P_c$ was in most cases determined directly while ${}^{1}P_c$ (for $\dot{P}$ = 1 atm/hr) was derived by extrapolation from *Eq.* (*1*) for the majority of animal models. Estimated magnitude of the variance for each P_c value is shown in Table I by the appropriate reference index; the computed variance for K, σ_K, is equal to or smaller than 0.1 throughout.

Figure 1 illustrates the complexity of these data. Both the slope and position of the lines representing the several species vary widely, and with little apparent regularity. In particular, the values of K range from near 0 for newts, frogs, lizards, quail, and hamsters, to −25 for the rhesus monkey. These results do show, however, that HPNS convulsion thresholds are substantially independent of compression rate for at least one species in each of the four vertebrate classes tested, thus confirming and extending our previous conclusions (5).

Phylogenetic relations between P_c values are discernible in ${}^{1000}P_c$, but can barely be seen in ${}^{1}P_c$: if the species are ranked and then identified as amphibians (A), reptiles (R), birds (B), rodents (Ro), carnivores (C), or primates (P), the descending rank order for ${}^{1000}P_c$ is: A, A,

TABLE I
COMPRESSION-RATE EFFECT ON HPNS CONVULSIONS IN VARIOUS SPECIES

#	Order or Class	Species and Strain	${}^{1}P_c$, atm	${}^{1000}P_c$, atm	K, atm	r	$K/{}^{1}P_c$	$K/{}^{1000}P_c$
1	Amphibia	Diemictylus viridescens	203†	195*	2.3	0.01	0.01	0.01
2		Rana pipiens	186†	132*	17.9	0.6	0.10	0.11
3	Reptilia	Anolis carolinensis	105†	100*	1.6	0.02	0.01	0.02
4		Kinosternon scorpiodes	177†	154*	8.0	0.11	0.045	0.05
5	Aves	Colinus virginianus	98†	98*	0.0	0.00	0.00	0.00
6		Melopsittacus undulatus	126†	79*	16.8	0.93	0.13	0.21
7	Mammalia Rodentia	Rattus norvegicus Fischer	140†	85*	19.9	0.90	0.14	0.23
8		Long-Evans	120†	76*	14.6	0.74	0.12	0.19
9		Wistar	120†	90*	8.3	0.49	0.07	0.09
10		Sprague Dawley	116†	92*	9.6	0.59	0.08	0.10
		Mus musculus						
11		A/J	106†	63*	13.4	0.74	0.13	0.21
12		129/J	122†	73*	17.3	0.92	0.15	0.24
13		CD-1	119*	65*	16.3	0.94	0.14	0.25
14		Mesocricetus auratus	89†	93*	−1.3	0.01	−0.01	−0.01
15		Meriones unguiculatus	133†	66*	27.0	0.75	0.17	0.34
16	Carnivora	Mustelo furo	100†	66*	11.2	0.79	0.11	0.17
17	Primates	Saimiri sciureus	75*	43†	9.8	0.45	0.13	0.23
18		Macaca mulatta	109†	36††	25	0.9	0.23	0.69

* = SD ± 5%; † = SD ± 3%; †† = extrapolated values.

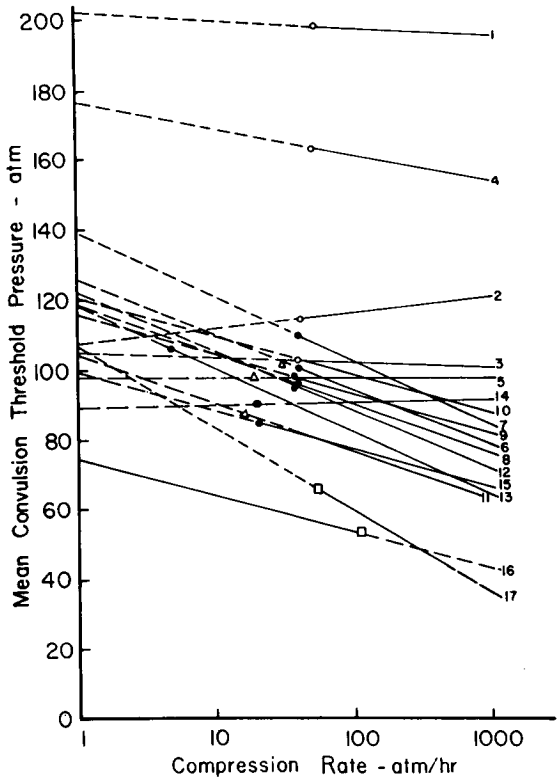

Fig. 1. Comparison of effect of compression rate on HPNS convulsion threshold in 17 animal models. Numbers identifying each line correspond to those in Tables I and II. Solid parts of each line designate range of compression rates actually tested for species or strain designated; dashed parts represent extrapolation based upon *Eq. 1* of text.

R, R, B, Ro, Ro, Ro, Ro, B, Ro, Ro, C, Ro, Ro, Ro, P, P; for $^{1}P_c$ the corresponding sequence is: A, R, Ro, Ro, B, A, Ro, Ro, Ro, Ro, Ro, P, Ro, R, C, B, Ro, P. If one hypothesizes that on phylogenetic grounds an ideal sequence might be A, R, B, Ro, C, P, one can assess the degree of order or disorder in the experimentally determined sequences in terms of the number of inversions (movements) required to make the experimental sequence conform to the hypothetical one. For the $^{1000}P_c$ sequence, the observed number of such inversions is 7; for the $^{1}P_c$ sequence the corresponding value is 30. Thus, $^{1000}P_c$ emerges as the parameter which appears to bear the closer relation to phylogenetic position, with the most complex central nervous systems most susceptible to HPNS convulsions.

Nature of the Convulsion-Delaying Compression-Rate-Dependent Effect in Vertebrates: Contributions from the Study of Reserpine Effects on HPNS

Effect of Reserpine on HPNS in Mice

Reserpine is an alkaloid whose most prominent effects involve blockage of monoamine

neurotransmitter storage and uptake in synaptic vesicles both centrally and peripherally (7). Reserpine has been found to facilitate the action of several (but not all) convulsants, and there is reason to associate these effects with altered vesicular monoamine uptake, presumably in monoaminergic nerve endings in the CNS (1).

The effect of reserpine on the development of HPNS has been studied most intensively in the CD-1 mouse. In this animal, administration of reserpine (Serpasil) in a dose of 5-10 mg/kg produces marked sedation. The animals can be aroused, and will then reveal no abnormality other than movements that appear somewhat stiffer than those of unmedicated animals. When subjected to a standard compression regime, partial reversal of the sedation is noted from about 20 atm upward, with alternating periods of sedation or immobility and relatively normal activity. Characteristic HPNS tremors associated with volitional activity are observable above 40 atm. From about 50 atm upward, these give way to preconvulsive, spontaneous myoclonic jerks and to coarse tremors not dependent upon volitional movements. Generalized convulsions in such reserpinized CD-1 mice occur at or near 70 atm, in contrast to the mean value of 98 atm for unmedicated control animals.

Time Course of Reserpine Effect on HPNS in CD-1 Mice

Intramuscular (i.m.) injections of 5 mg/kg of reserpine (Serpasil) were administered to mice at those times of the day or night which would cause any seizures elicited by compression to occur between 1400 and 1700 h. On the basis of pilot experiments compressions at 40 atm/hr were begun 1, 3, 12, 24, 30, 48, 60 and 70 hours after the reserpine injections. The mean values of the tremor and convulsion thresholds observed at these various times after reserpine injection are shown in Fig. 2. In the CD-1 mouse the tremor threshold is not significantly affected by 5 mg/kg of reserpine at any time during the first 72 hours after injection ($P > 0.2$ for control vs. 6- to 24-hr values). In contrast, the convulsion thresholds decrease rapidly during the first five hours after reserpine administration, and then continue to do so more slowly until a minimum is reached after about 24 hours. Recovery begins shortly after 24 hours and is essentially complete 72 hours after the reserpine administration.

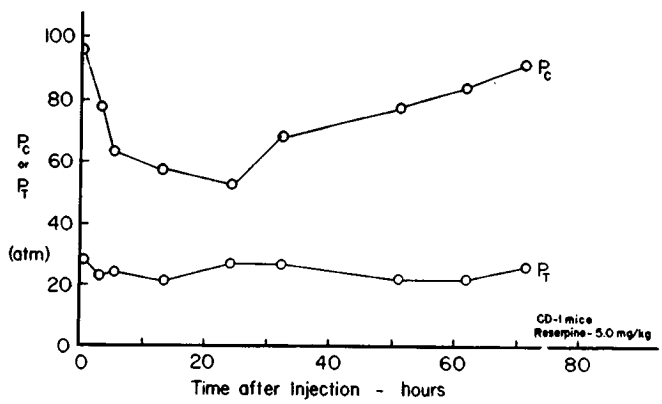

Fig. 2. Time course of reserpine effect on HPNS tremor (P_T) and convulsion (P_C) thresholds in CD-1 mice.

Dose-Response Relationship of the Reserpine Effect on HPNS Convulsions in Mice

HPNS convulsion thresholds were determined in CD-1 mice compressed at a rate of 40 atm/hr, 24 hours after intramuscular administration of reserpine in doses ranging from 2 to 50 mg/kg. The results shown in Fig. 3 indicate that the maximum attainable depression of HPNS convulsion thresholds is achieved by 4 mg/kg of reserpine. Increasing reserpine dosage up to 50 mg/kg fails to produce significantly greater effects on this reaction, though the degree of behavioral depression is recognizably deepened.

Effect of Changes in Compression Rate on HPNS Convulsion Thresholds in Reserpinized Mice

Control and reserpinized mice (5 mg/kg, i.m.) were subjected to compression in heliox atmospheres at compression rates from 5 to 1500 atm/hr. The start of each compression experiment was timed so that seizures would occur between 1400 and 1700 h, and between 22 and 26 hours after reserpine injection. The mean convulsion thresholds, shown in Fig. 4 as a function of the logarithm of compression rate, conform well to the form of *Eq. 1.* The regression equation for P_c on log $\dot{P}$ is $P_c = 63.4 + 1.54 \log \dot{P}$ ($r = 0.48$) for the reserpinized animals, and $P_c = 130.1 - 19.1 \log \dot{P}$ ($r = 0.79$) for the controls. The difference between the two slopes as determined by the regression coefficients is well-secured statistically ($P < 0.005$); the slope for the reserpinized group does not differ significantly from zero ($P = 0.2$). The reserpine and control regression lines meet near $\dot{P} = 1500$ atm/hr.

Antagonism or Reversal of Reserpine Effect on HPNS Convulsions

Three agents were employed which are known to antagonize specific reserpine effects: tryptophane, said to antagonize certain reserpine effects by increasing serotonin stores and

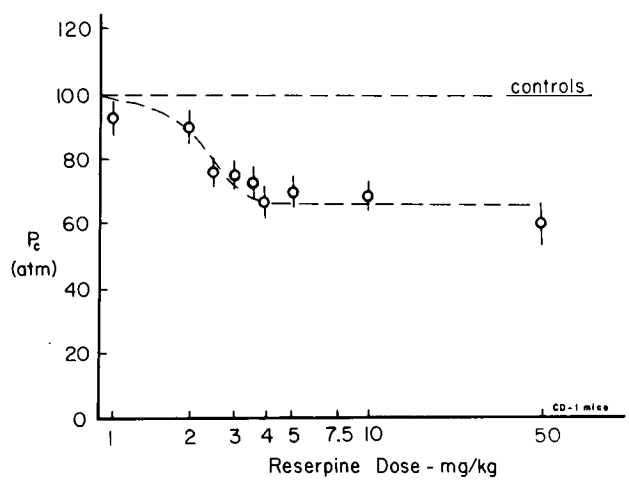

Fig. 3. Dose-response relations for effect of reserpine on HPNS convulsion thresholds in CD-1 mice 24 hrs after injection.

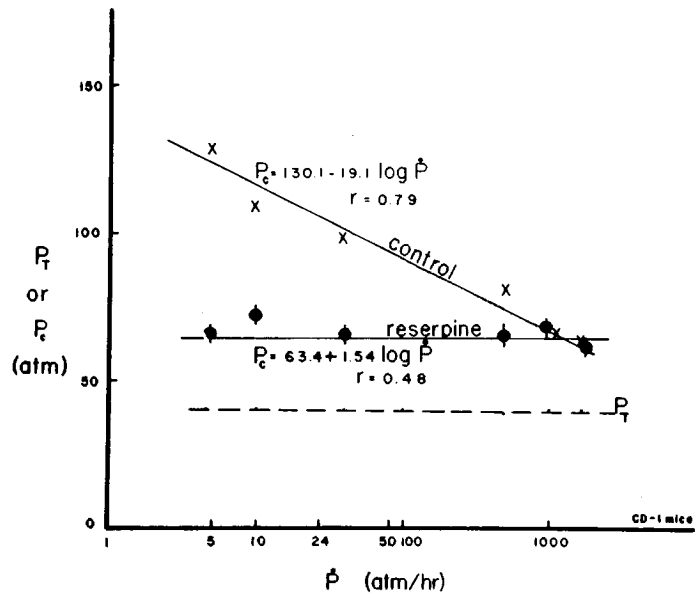

Fig. 4. Abolition of compression-rate effect on HPNS convulsion thresholds in CD-1 mice 24 hrs after administration of 5 mg/kg reserpine.

thus countering the effect of reserpine upon serotonergic terminals (3); tranylcypromine, a monoamine oxidase inhibitor which, if given before reserpine, protects catecholaminergic presynaptic terminals (8); and amphetamine, which reverses certain reserpine effects, again primarily by acting at the level of catecholaminergic nerve endings (9).

L-tryptophan was administered in a series of graded doses to establish the maximal anti-reserpine effect attainable. As shown in Fig. 5 (*upper panel*) this amounts to a decrease of the reserpine (R) effect by about 40%, attained at a level of tryptophan which by itself does not modify the HPNS convulsion threshold of mice.

Tranylcypromine given prior to reserpine antagonized the effect of reserpine on HPNS by at least 75% (Fig. 5, *lower left*).

Finally, amphetamine reversed at least 60% of the reserpine effect (Fig. 5, *lower right*); however this dosage of amphetamine itself produces a moderate lowering of HPNS convulsion thresholds, and its action on the reserpine effect may thus be underestimated. It may produce a reversal closer to 80%, if calculated using the amphetamine-treated animals as the base line.

Taken together these data indicate that the effect of reserpine on HPNS convulsion thresholds of mice involves monoaminergic nerve terminals in the CNS, and may reflect blockade of both catecholaminergic and serotonergic nerve endings.

Comparison of Reserpine Effects in Different Strains and Species

The effect of reserpine on HPNS tremor and convulsion thresholds was tested in 17 of the animal models represented in Table I; the rhesus monkey was omitted. In each case

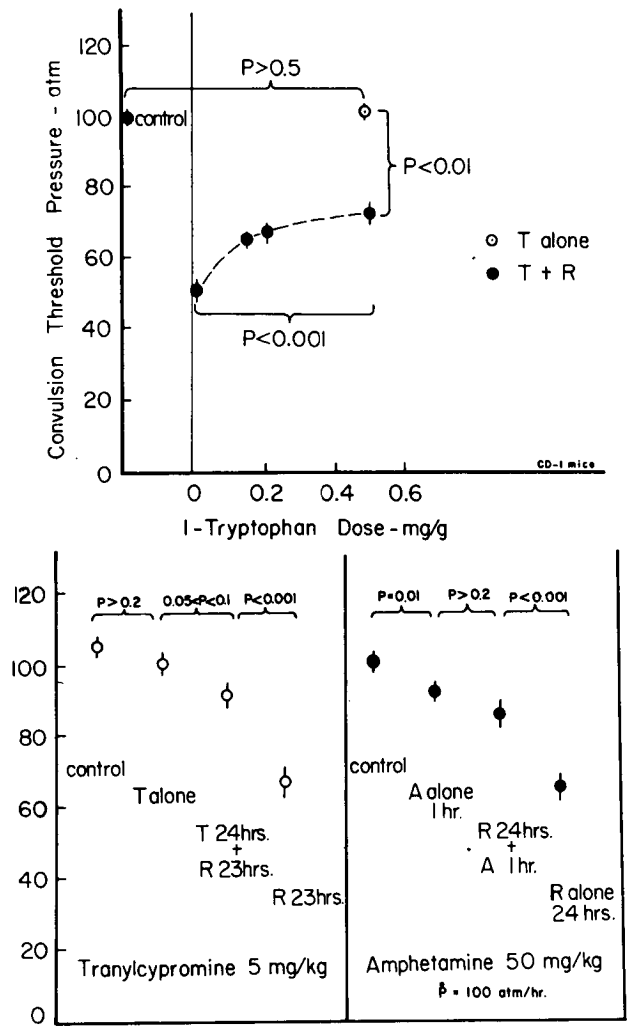

Fig. 5. Antagonism between effect of reserpine on HPNS convulsion thresholds and drugs affecting monoaminergic neurotransmission. *Upper panel:* L-tryptophan (dose-response relation); *lower left:* tranylcypromine 1 hr before reserpine; *lower right:* amphetamine sulfate 1 hr before beginning of compression.

a suitable dose level was determined by preliminary tests; a dose which would give the maximal effect on HPNS thresholds attainable in the particular species without undue behavioral changes was chosen. Column 3 of Table II shows both the range of doses tested and the dose level chosen. Compression rates throughout were 40 atm/hr, except for the squirrel monkey, where the most extensive control data are available at 24 atm/hr. Each P_c value is based upon eight or more animals. Standard deviations of the means throughout are ±4 atm or less. Control and reserpine animals were alternated as before to eliminate secular effects, and the precautions described above were taken to eliminate possible effects attributable to diurnal fluctuations in convulsion susceptibility. Reserpine was administered 20-24 hours

TABLE II
COMPARISON OF RESERPINE EFFECT ON HPNS CONVULSIONS IN VARIOUS SPECIES

#	Species	Range of Reserpine Dose Tested, mg/kg	$^{40}R_c$	$^{1000}R_c$
1	Newt	15 (5-15)	−0.015	—
2	Frog	10 (5-15)	0.15	—
3	Lizard	15 (5-15)	0.03	—
4	Turtle	15 (5-15)	0.24	—
5	Quail	10 (5-15)	0.05	—
6	Parakeet	0.2 (0.1-0.5)	0.14	—
7	Rat-Fischer	10 (2.5-10)	0.17	−0.03
8	Long-Evans	5 (2.5-10)	0.17	—
9	Wistar	5 (2.5-10)	0.23	0.13
10	Sprague Dawley	5 (1-10)	0.19	—
11	Mouse-A/J	5 (2.5-5)	0.53	—
12	129/J	5 (2.5-5)	0.52	0.36
13	CD-1	5 (1-50)	0.45	−0.03
14	Hamster	5 (2.5-10)	−0.18	0.12
15	Gerbil	5 (2.5-10)	0.91	0.15
16	Ferret	5 (5-10)	0.35	0.05
17	Squirrel Monkey	5 (1-10)	0.35	—

prior to the anticipated HPNS seizures. For comparison among species, the effect of reserpine on the HPNS convulsion threshold is expressed as the R effect (R), calculated as the depression of P_c under the effect of the drug, divided by $^{1000}P_c$ as previously determined for each animal model (column 9 of Table I). The values for R computed in this fashion are shown in column 4 of Table II. Mathematical analysis shows that the variances for these derived statistics range from 0.01 to 0.03. Thus, differences of less than 0.06 between R values should be seen as having questionable significance.

The effects of the reserpine doses employed for the studies summarized in Table II generally resembled those described for the CD-1 mouse. The one striking exception was the parakeet, which was found to be excessively sensitive to the peripheral effects of reserpine. In the parakeet, dose levels of 0.3 mg/kg or greater produced an unacceptably high mortality rate, which limited the test dose to 0.2 mg/kg.

Perusal of Table II suggests that high values of R are usually restricted to mammals while low values predominate among the nonmammalian species. The one clear exception was the hamster, in which reserpine pretreatment produced an increase in the HPNS convulsion threshold at a compression rate of 40 atm/hr. The gerbil showed an R effect under the same conditions far greater than that for any other species, which may be related to this animal's predisposition to spontaneous seizures.

To determine whether the magnitude of the R effect for the different species is related to the magnitudes of the compression-rate effect previously determined (Table I), R was plotted against K, $K/^1P_c$, and $K/^{1000}P_c$. Applying the Fischer test to 2 × 2 contingency tables, highly significant correlations were found for R and K, and for R and $K/^{1000}P_c$

($P < 0.001$) but not for R and $K/^{1}P_c$ ($P \geq 0.2$). The regression equations for R and $K/^{1000}P_c$ are

$$R = 1.76 \cdot K/^{1000}P_c - 0.04 \quad (2)$$

and

$$R = 2.65 \cdot K/^{1000}P_c - 0.14 \quad (2a)$$

with a correlation coefficient of 0.82 (Fig. 6). Corresponding calculations for R and K yielded a correlation coefficient of 0.71.

Finally, in seven mammalian animal models the hypothesis that the mechanism blocked by reserpine accounts, to a significant extent, for the compression-rate dependence of HPNS convulsion thresholds was tested. The results are included in the last column of Table II. In every case the reserpine effect at a compression rate of 1000 atm/hr was significantly lower than the reserpine effect at a compression rate of 40 atm/hr. In three of the animal models tested, reserpine showed no measurable effect on HPNS convulsion thresholds at the high compression rate.

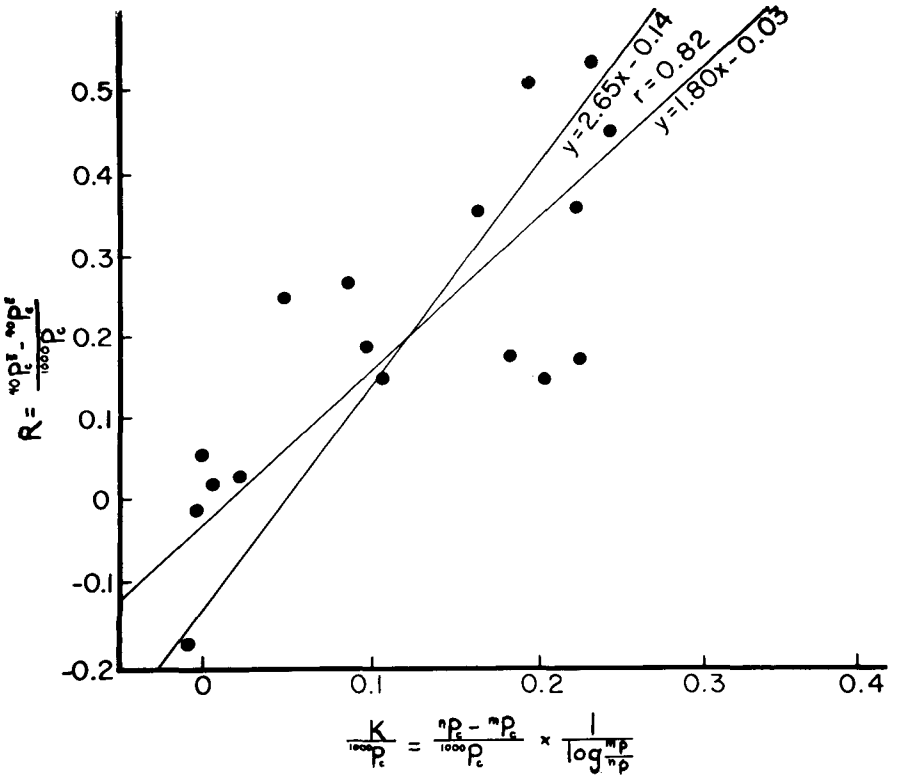

Fig. 6. Correlation between relative compression-rate effect (x axis, $K/^{1000}P_c$ from column 9, Table I), and reserpine effect (y axis, ^{40}R from column 4, Table II) for 17 animal models.

Discussion

The results indicate:

1) Compression-rate dependence of HPNS convulsion thresholds is not an invariable characteristic of this syndrome.
2) Analysis of the convulsion thresholds at high and at low compression rates suggests that, for the 17 species tested, the convulsion thresholds observed at high compression rates appear to reflect phylogenetic regularities to a greater degree than those observed at low compression rates.
3) The effect of reserpine on HPNS in the mouse has been described in some detail. Reserpinization lowers the HPNS convulsion thresholds in mice when they are compressed at slow or moderate rates; the time/course and dose-response relationships are compatible with the hypothesis that this effect is mediated by a blockade of monoaminergic synaptic vesicles.
4) These conclusions are strengthened by the results of experiments with agents known to block or reverse these effects of reserpine.
5) The effect of reserpine on HPNS convulsion thresholds decreases markedly as compression rates are increased, vanishing in the case of the CD-1 mouse at a compression rate of 1000 atm/hr.
6) Comparative studies indicate that there is a good correlation between the magnitudes of the reserpine effects and the compression rate effects among the 17 species tested.
7) In all animal models tested, the reserpine effect was markedly less at high than at low compression rates.

It is possible to infer from these data that the HPNS in a typical mammalian species consists of at least two components, a compression-rate-independent primary component, which may give rise to localized subcortical paroxysmal activity in the central nervous system, and a compression-rate-dependent secondary component which modifies the pressure at which this primary event is translated into generalized convulsions. Furthermore, the data indicate that monoaminergic pathways are important in connection with this secondary mechanism, although the data also suggest that other mechanisms (possibly mediated by neurotransmitters other than the monoamines) are also important, and that the relative importance of monoamine-dependent and monoamine-independent components of this convulsion-delaying effect vary from species to species.

ACKNOWLEDGMENT

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INFLUENCE OF INCREASED NITROGEN TENSIONS ON PROPERTIES OF IDENTIFIED NEURONS IN *APLYSIA CALIFORNICA*

J. E. Blankenship, R. Feinstein and B. Butler

Some of the passive membrane properties of identified neurons of the marine mollusc *Aplysia californica* have been studied under conditions of hyperbaric nitrogen (air) tensions up to 10 atmospheres absolute (ATA). The purpose of these studies was to gain insight into the cellular mechanisms which underlie neurological dysfunctions and maladaptive behaviors of higher organisms exposed to pressures in this range. Hyperbaric nitrogen has long been recognized as a narcotic or anesthetic-like agent which depresses nervous activity when administered to vertebrates (2, 3, 4, 15, 22), but neurophysiological studies have been comparatively general and have not been concentrated at the cellular level.

At pressures between 41 and 61 atmospheres, nitrogen blocks brain waves in the frog (20). Mice show depressant symptoms at pressures between 10 and 17 atmospheres of nitrogen (8, 20). Marshall (20) has shown that the threshold and action potentials of isolated frog sciatic nerve and the efficacy of junctional transmission in sciatic nerve-whole gastrocnemius muscle preparations were unimpaired by nitrogen pressures up to 96 and 82 atmospheres, respectively, but that spinal cord reflexes were blocked at 17 atmospheres.

Direct studies of neuronal activity at the cellular level provide an index of permeability states and conductance changes of a cell (6, 13, 16, 18, 19, 27). If changes in these states occur in hyperbaric nitrogen, evidence as to whether nitrogen narcosis and volatile anesthetics act in a predictable way to alter membrane permeability could be gathered (3, 4, 26). Previous work on nonneural systems has indicated that permeability changes occur because of interactions between gas and lipid components of the membrane. Whether such interactions lead to decreased (21, 24, 25) or increased (1, 4, 5, 9) permeability to cations is still not resolved.

Methods

Forty experiments were performed to study the effects of increased nitrogen tensions on certain of the passive electrophysiological properties of identified neurons in the abdominal ganglion of *Aplysia californica.* The ganglion was pinned to the paraffin base of a lucite recording chamber filled with seawater which was then secured to a platform in a hyperbaric chamber. A plastic-coated copper coil, through which constant temperature liquid was circulated, was placed around the ganglion in the bathing solution. Adequate temperature control and rapid equilibrium of gases was obtained by using bathing solution volumes of about

25 ml in the chamber well which was approximately 7 × 8 × 0.5 cm (volume partially displaced by cooling coil). A single identified neuron, usually cell R2 (the giant cell) or cell R14 (large neurosecretory neuron), was impaled with two independent microelectrodes (cf. (12) for terminology applicable to ganglion cells). A third microelectrode was placed in the bath as a neutral reference for voltage measurements. All electrodes were filled with 2.7 M KCl and matched initially for similar impedance values. The hyperbaric chamber was sealed and control values of resting potential, membrane resistance, time constant, action potential amplitude and duration, and temperature were obtained. Spontaneous firing pattern and the occurrence of synaptic potentials were also noted.

Data were acquired as follows. The computer sampled the differential recording of membrane voltage for one minute, taking 300 data points, and calculated and plotted the mean resting potential and standard deviation. A current (I)-voltage (V) curve for membrane resistance was then obtained by passing 5-sec pulses of DC current through the second intracellular electrode and measuring the amount of current given with each stimulation and the induced voltage change. The computer plotted this information on its graphic display as an I-V relationship, using the resting potential as the zero current point. The computer also sampled the rising phase of some of the induced voltage steps and calculated the time to achieve 1/e of the final voltage as the time constant. Spike height and duration were measured on an oscilloscope, and temperature was measured by a thermistor probe in the seawater bath near the ganglion. The initial temperature was between 14° and 17°C and was maintained to within ± 1 °C throughout an experiment.

After completion of control measurements, the pressure in the chamber was increased in 1-atm pressure steps; the measurements above were taken at each step through 10 ATA and back to 1 ATA. The pressure was changed over a 2- to 5-min period to minimize temperature changes and mechanical disturbances. After a new pressure level was reached, there was a 5–10 minute delay before data were taken, to allow gas and temperature equilibration with the seawater bath. Data acquisition time at each pressure took 5–10 minutes, and a complete experiment normally required a total of 5–6 hours.

After the completion of an experiment, the electrodes were withdrawn from the cell, the DC potential was recorded, and the resting potential was corrected for drift or offset in the measurement system. In addition, in most experiments the pressure was again taken to 10 ATA and back with the electrodes in the bath to observe any fluctuations in electrode impedance or differential DC level that might have occurred due to the influence of pressure on the recording system. These types of changes were minimal and corrections of the biological measurements were usually unnecessary. Similar measurement and recording techniques have recently been reported by Colton and Freeman (10).

Results

Resting and Action Potential

Figure 1 illustrates values of resting membrane potential (RMP) with variations in nitrogen tensions for two neurons in representative experiments. The RMP usually varies over time and pressure, but the fluctuations are random and are not correlated with pressure. Since the RMP does not follow its original pattern on the return to sea level, one must assume

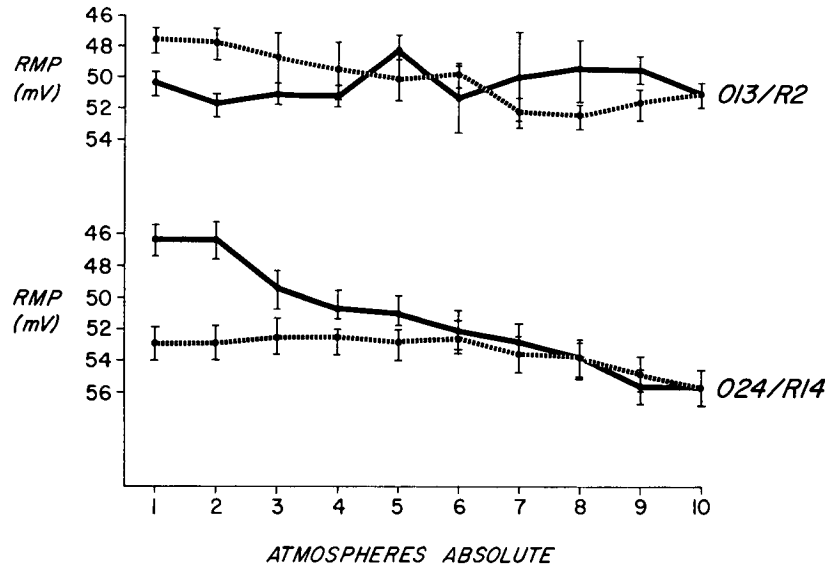

Fig. 1. Resting membrane potential (RMP) values for cells R2 (*above*) and R14 (*below*) in abdominal ganglion of *Aplysia* from 2 experiments, showing changes as nitrogen tension increased from 1 ATA to 10 ATA and back again in 1-ATA steps. Solid lines indicate increasing pressure, hatched lines decreasing pressure. One SD is shown for each RMP value which represents mean of 300 DC voltage samples over 1 min.

that there is a lack of a systematic influence of pressure. In some cases, the RMP will slowly hyperpolarize throughout much of an experiment (see Fig. 2, *upper panel*). Such slow hyperpolarizations probably reflect a general improvement of the cell over time as it recovers from the trauma of impalement. Eighteen complete 1-10-1 pressure experiments on 15 separate neurons were made to study resting potential changes. An additional 12 experiments (eleven neurons) which were aborted at various pressures greater than 7 ATA because of loss of impalement were used to complement and confirm the reported findings. All the results indicate that the resting potentials of *Aplysia* neurons R2 and R14 are not significantly affected by increases in air pressures through 10 atmospheres absolute.

Both of these neurons are usually "silent cells", that is, they do not fire action potentials. Cell R14 sometimes fires in a regular fashion at 1 to 0.2 per second. R2 is rarely active. The large size and usual silence of these neurons were useful for these analytical procedures; in some experiments, however, they were active, and observations were then made of action potential amplitude and duration and firing pattern. Although there was some variation in all of these parameters, no consistent effect of pressure was seen. In a few experiments on other identified cells which are more active, and some of which had spontaneous bursting rhythms, alterations in activity were observed, but these studies are too incomplete to draw conclusions at this time.

Membrane Resistance and Time Constant

There have been twelve 1-10-1 pressure experiments in which membrane resistance and

time constant were measured in R2 or R14. An additional seven experiments on these or other identified cells, though less complete because of loss of penetration, complement the reported general findings. Figure 2 shows a series of I-V curves for cells R2 and R14 replotted from the computer display for a typical experiment. The curves are graphed in sequence at the pressure at which they were obtained. Also represented on the abscissa is the current for each experiment; zero current is plotted separately for each run at the resting potential intersection with the appropriate pressure. The resting potential values are joined by the heavy line. The best least-squares-fit line for each curve was determined by the computer. The slope of this line (voltage/current) represents the input resistance of the membrane. Similarly constructed relationships were obtained in each experiment and values of membrane resistance were defined as the slope of the I-V relationship. No significant effect of pressure could be seen.

The computer was programmed to calculate the time constant of the membrane as the number of msec necessary for a selected current pulse to drive the membrane potential to 1/e of the final value of potential change induced by that current pulse. Values of time

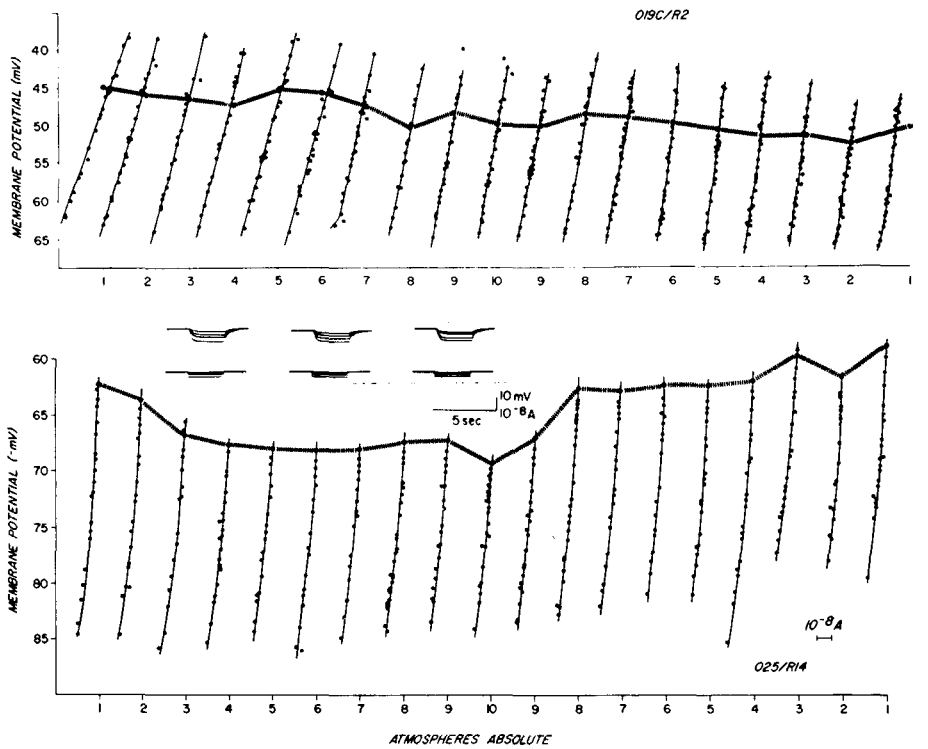

Fig. 2. Plots of current-voltage (I-V) relationships for cells R2 (*above*) and R14 (*below*) from 2 experiments in which nitrogen tension was increased from 1–10 ATA and back (*abscissa*). Heavy hatched line in each graph represents resting potential value, mV (*ordinate*). At each ATA, an I-V plot was generated by passing varying amounts of current through 1 of 2 intracellular microelectrodes and plotting voltage change on *ordinate* and current on *abscissa*. Current calibration for both experiments is at lower right of bottom graph. Zero current was set at the resting potential for each pressure. Insets above lower panel are representative records showing voltage changes induced (*upper records*) for various applied currents (*lower records*) at control (1 ATA), 10, and 5 ATA on descent, respectively.

constants obtained in this manner are shown in Fig. 3. These illustrations summarize the battery of data obtained for representatives of the most complete experiments. Each abscissa shows the absolute pressures at which each value was obtained. The data for each experiment include, from bottom to top: (1) elapsed time, in minutes, at which each value of RMP was obtained; (2) RMP ± one SD; (3) membrane resistance, as defined by the slope of each I-V curve; (4) the time constant; and (5) the temperature of the bath surrounding the ganglion. The solid lines of each curve and the top row of elapsed times refer to values obtained from sea level (control, 1 ATA) through 10 ATA. The dotted curves and lower row of times correspond to values obtained as the pressure was lowered back to sea level.

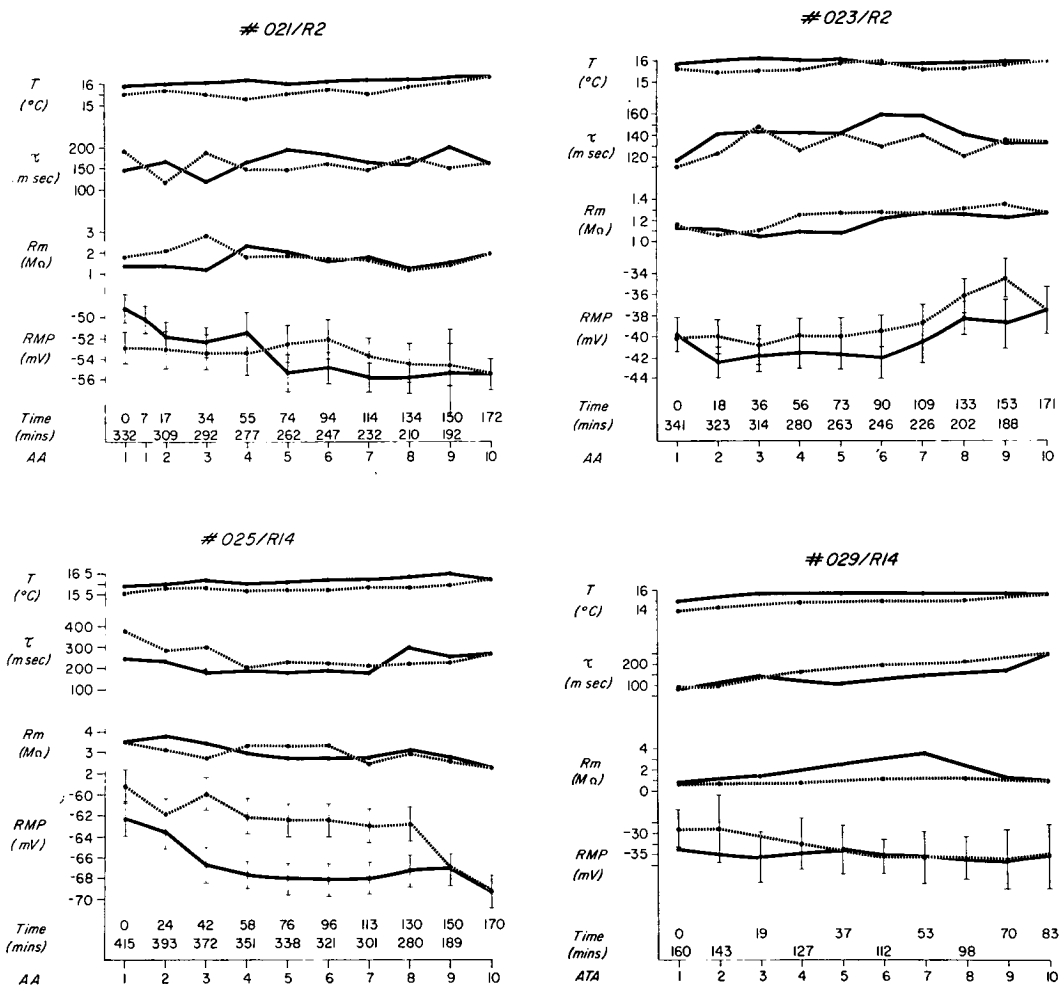

Fig. 3. Results of 4 experiments (cell R2 *above*, R14 *below*) showing data plotted against nitrogen tension in ATA (*abscissa*). Lowest record is elapsed time in minutes; top row and solid lines are increasing pressure, bottom row and hatched lines are decreasing pressure. Resting membrane potential (RMP) in mV is plotted with 1 SD for each value. Input resistance of membrane (Rm) is given in mega-ohms, derived from computer determination of slope of I-V relationships. Time constant, τ, is plotted in msec, and temperature of bath surrounding ganglion is given in degrees C.

It should be noted that the limited changes in temperature are neither consistently correlated with nor adequate to account for the fluctuations seen in resting potential, time constant, or resistance (6, 7, 18, 19). In addition, the values obtained for membrane resistance and time constant are in the range of those normally reported for neurons in *Aplysia* (7, 14, 17, 18, 27) and closely related species (19).

More important, there is no clear-cut correlation between any of these biological parameters and pressure. In most experiments, variations in time constant or resistance were either within experimental error or were simply not large enough to be considered beyond the range of normal biological fluctuation. Some suggestive changes within one experiment, such as the tendency for the resting potential to depolarize at the higher pressures (as in 023/R2), are cancelled out by the totally different response of this cell in another experiment (021/R2).

Discussion

These results indicate that the passive electrophysiological properties and action potentials of neurons in the abdominal ganglion of *Aplysia* are unaffected by increases in nitrogen tensions to 10 ATA. Since no significantly large or consistent alteration occurred in resting potential, action potential, membrane resistance or time constant, it is concluded that these membrane parameters and their underlying conductance states (for sodium and potassium) are not affected by nitrogen tensions through 10 ATA. This would indicate that these conductance states are stable and well controlled in these neurons at this pressure. Whether such stability would persist at greater nitrogen tensions, or whether vertebrate excitable cells demonstrate the same stability remains to be tested. The fact that *Aplysia* is considered an intertidal animal not reportedly found at depths greater than 200 feet (11, 23, R. Fay, personal communication), indicates that this animal's nervous system was only mildly stressed by taking it to depths equivalent to some 300 feet of seawater.

Although more subtle influences of pressure and high nitrogen tensions on synaptic mechanisms in *Aplysia* should be studied, the membrane properties of vertebrate systems in the pressure range where behavioral and psychological influences on man are clearly impaired should also be examined (3, 4).

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BEHAVIORAL EVALUATION OF PHARMACOLOGICAL AGENTS IN HYPERBARIC AIR AND HELIUM-OXYGEN

J. R. Thomas and J. M. Walsh

Knowledge of the behavioral effects of pharmacological agents under hyperbaric conditions is at present practically unavailable. There is need for systematic evaluation of these effects of pharmacological agents and chemical compounds under hyperbaric conditions both in relation to their application for therapeutic use and to aid in determining the basic mechanisms of behavioral change. Although there have been numerous studies of pharmacological and chemical agents under increased pressures (2, 7), none of these has focused upon behavioral aspects. An understanding of the behavioral action of drugs under elevated pressures is becoming more important with the increasing frequency of military, industry, and sport diving (17). Because of the numerous metabolic and physiological changes produced in organisms by high pressures, drugs under hyperbaric conditions are not acting on a normal organism and drug responses should differ from those of normal atmospheric conditions.

Pharmacological research in high pressure conditions has been concerned principally with the identification of prophylactic agents for the prevention or reduction of high pressure problems, such as decompression sickness, oxygen toxicity, inert gas narcosis, and aseptic bone necrosis. Relatively little has been done, however, to evaluate drugs per se for their efficacy or relative safety in hyperbaric conditions, although significant research has begun in this area (3, 4, 5, 7, 8, 15). At present there is almost no research under way concerning the behavioral evaluation of pharmacological agents under high pressures, although the ability of a drug to interfere with or modify the ongoing behavior of organisms is one of the most important actions of a drug. Of particular importance are those drugs known to involve the central nervous system (CNS) directly, and which have specific behavioral actions, i.e., are psychoactive.

The research reported here was concerned with the behavioral evaluation of several representative members of psychoactive drug classes under high pressure conditions. The drug classes evaluated under hyperbaric conditions were: stimulants (amphetamine), phenothiazines (chlorpromazine), benzodiazepines (chlordiazepoxide and diazepam), barbiturates (pentobarbital) and alcohol (ethanol). To assess the effects of drug regimens under both normal and hyperbaric conditions, operant conditioning techniques were used to develop complex patterns of behavior in experimental animals over extended time periods. The techniques and methodology of operant conditioning have been used extensively in the screening and assessment of the behavioral effects of drugs (14). The methodology has also been used effectively in the analysis of hyperbaric behavior (6, 9, 11, 13, 18). Complex patterns of

behavior, once established in research animals, can be used to assess the effects of drug regimens under both normal atmospheric and increased pressure conditions.

The general experimental approach to the behavioral evaluation of drugs under hyperbaric pressure involves a two-phase process: (1) toxicological evaluations of a broad dose range of each drug to detect changes in acute toxicity due to the drug-pressure interaction, which enable a viable dose range to be established to evaluate the behavioral effects of the drug. (2) Behavioral evaluations in which the effects of drugs are determined by imposing the drug variable on well-established behavioral base lines at different pressures and under a variety of gas mixtures to ascertain the behavioral toxicity of drug compounds in the various conditions. (Behavioral toxicity refers to the ability of a drug to interfere with the ongoing behavior of an organism.)

Toxicological Evaluation

During our pre-evaluation toxicity trials to determine dose ranges, it was observed that the benzodiazepine derivative, diazepam, appeared to have some protective properties against decompression sickness. The following investigation was designed to explore this possibility.

Male Sprague-Dawley rats were divided into five groups, a control and four drug groups. The control group animals were subdivided into three conditions: *a*) no injection, *b*) saline injection, *c*) diazepam vehicle injection. Animals in the drug groups received either 0.5, 1.0, 5.0, or 10.0 mg/kg diazepam. All injections were given intraperitoneally. Sixteen animals at a time were compressed at a rate of 1 ATA/min in a dry hyperbaric chamber to 7 ATA breathing compressed air; four groups were equally represented on each dive. Subjects remained at 7 ATA for 1 hour and then were explosively decompressed to the surface.

The results of this study are illustrated in Fig. 1. Since there were no differences between the three control conditions, these data were combined for comparison with the drug conditions. Clearly, the diazepam-treated animals were protected against decompression sickness at all dose levels ($\chi^2 = 39.7$; $P < .001$). Although the 5.0 and 10.0 mg/kg dose appeared to be more effective than the lower doses, these differences were not statistically significant.

The same experimental conditions were repeated with additional subjects, compressed to 10 ATA. Subjects remained at 10 ATA for 1 hour, followed by explosive decompression to the surface. The results of the 10-ATA study (Fig. 2) show that at this pressure there were no differences between control and drug group survival. The protective effect of diazepam seen on the 7-ATA decompression was not evident at the 10-ATA pressure. Subsequent excursions to pressures as high as 10 ATA with normal decompression (12) using diazepam (1.0–20.0 mg/kg) produced no toxic effects.

In other toxicological evaluations using normal decompression tables (12), no toxic effects for the dose range tested were observed for chlorpromazine, chlordiazepoxide, alcohol, or pentobarbital. D-amphetamine sulfate, however, was found to be considerably more toxic at pressure than under normobaric conditions; more instances of fatal decompression sickness were observed in decompression with amphetamine-treated subjects.

Behavioral Evaluation

Most of the behavioral effects of drugs and drugs under pressure examined in this paper

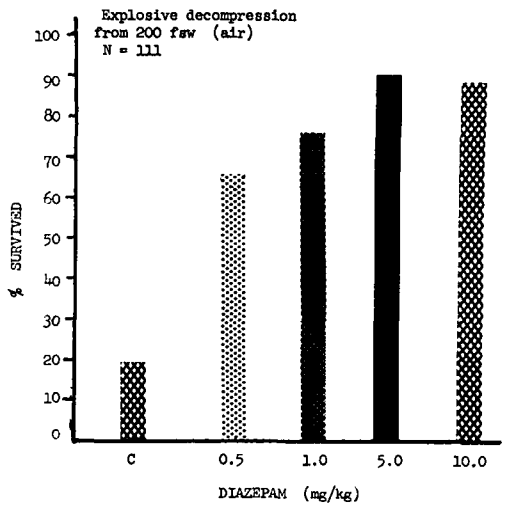

Fig. 1. Percentage change in survival rates after explosive decompression from 7 ATA for control and diazepam-treated animals.

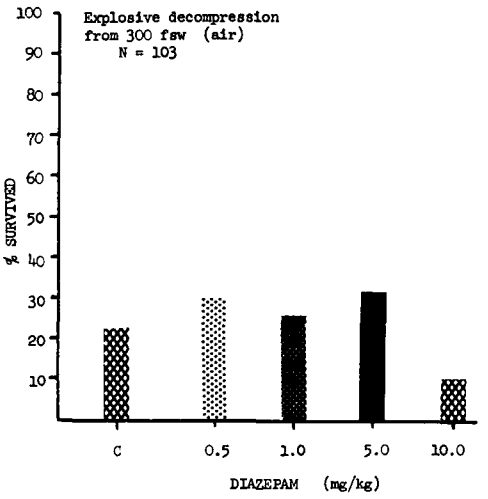

Fig. 2. Percentage change in survival rates after explosive decompression from 10 ATA for control and diazepam-treated animals.

were analyzed by dose-response curves expressed as the cumulative percentage of behavioral change at each particular dose level. The result of such a plot of cumulative percentage change as a function of drug dose is usually a curve similar to that seen in Fig. 3 (middle curve). The data is plotted in this way to allow convenient comparison by visual inspection of the behavioral changes produced by drugs under pressure and drugs alone over a wide dose range. If the behavioral effects of a drug under increased pressure were essentially the same as behavioral effects at normal surface pressure, the plotted function would fall along the same curve found for normal pressure. If the behavioral changes produced by a drug were enhanced or potentiated under increased pressures, the plotted function would be shifted towards the left as shown in Fig. 3. This shift would indicate that a smaller dose of a drug under increased pressure produces the same degree of behavioral change as a larger dose under normal atmospheric pressure. If the behavioral changes produced by a drug were

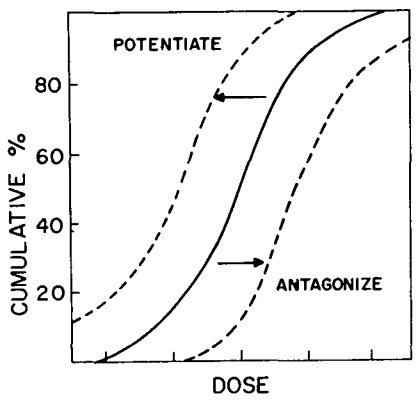

Fig. 3. Illustration of dose-response curves expressed as cumulative percentage of behavior at each dose. Synergistic effects produced by pressure shift curve to left, while antagonistic effects displace curve to right.

antagonized by increased pressure, i.e., the behavioral effects of the drug were lessened under pressure, the curve would be shifted toward the right.

AMPHETAMINE

Behavioral evaluation of CNS stimulants was focused on a representative of the sympathomimetic amines, d-amphetamine. Based on the assumption that inert gas narcosis is related to a general slowing of CNS activity, it appeared possible that the pharmacological enhancement of CNS activity might provide some insight into the mechanisms of narcosis. Amphetamine was also initially evaluated because of its use in antimotion sickness compounds. The dose-response functions obtained for d-amphetamine under normal pressure and under three hyperbaric conditions are shown in Fig. 4. When amphetamine was evaluated at 8.6 ATA breathing air and at 20.7 ATA breathing helium (Po_2 = 0.2 ATA), an increase in behavioral change above that found for the drug alone was obtained. Amphetamine at 8.6 ATA breathing heliox (80% helium-20% oxygen) produced behavioral changes that did not differ from the behavioral changes of the drug at normal atmospheric pressure. The shifted dose-response curves at 8.6 ATA breathing air and at 20.7 ATA breathing helium indicate that these gases at these two pressures potentiate the behavioral effects of amphetamine. The potentiation at 8.6 ATA under air is apparently due to the increased pressures of nitrogen rather than the raised partial pressures of oxygen, because the oxygen pressure was the same at 8.6 ATA breathing heliox, where no potentiation of drug effects was observed. The enhancement of amphetamine-produced behavioral changes by raised nitrogen pressures has been previously described (1, 10, 16). A similar enhancement is apparently produced by raised pressures of helium approximating 20 atmospheres.

CHLORPROMAZINE

As a representative of major tranquilizers, chlorpromazine (generally considered the prototype of phenothiazine compounds) was behaviorally evaluated under hyperbaric conditions. Chlorpromazine was investigated initially at 8.6 ATA breathing compressed air at doses from 0.25 to 2.5 mg/kg. As shown in Fig. 5, the dose-response function obtained

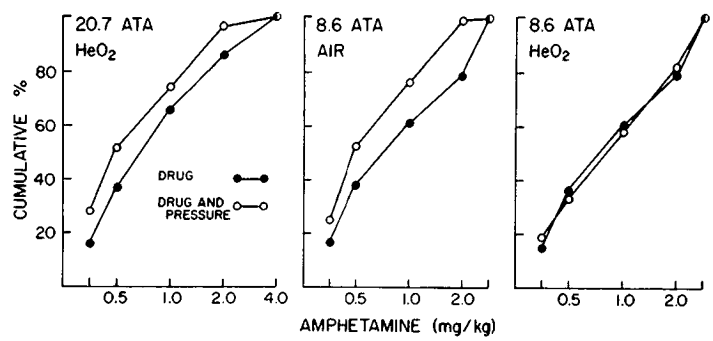

Fig. 4. Cumulative percent change in behavior plotted for amphetamine at surface and at depth (8.6 ATA and 20.7 ATA) using breathing mixtures of compressed air or on an 80% helium-20% oxygen ($He-O_2$) mix.

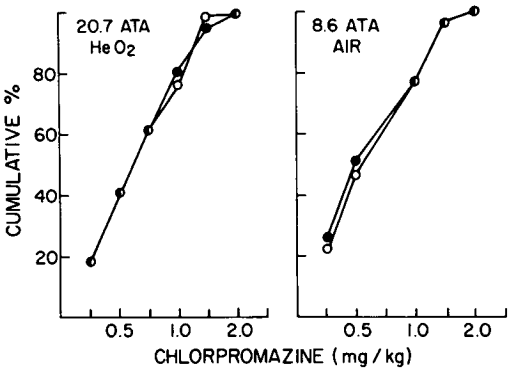

Fig. 5. Cumulative percent change in behavior plotted for chlorpromazine at surface and at depth (8.6 ATA breathing air and 20.7 ATA breathing 80% helium-20% oxygen).

for chlorpromazine at 8.6 ATA did not indicate any behavioral changes that differed from those obtained for the drug under normal atmospheric conditions. Further investigation of a range of doses of chlorpromazine at 20.7 ATA breathing helium (P_{O_2} = 0.2 ATA) indicated that the dose-response function at this pressure also remained essentially unaltered from that obtained at normal pressure.

Chlordiazepoxide and Diazepam

Two representatives of the benzodiazepine class, chlordiazepoxide and diazepam, were extensively evaluated under hyperbaric conditions. As mentioned earlier, diazepam was found to afford significant protection against decompression sickness. Both of these drugs produce changes in ongoing behavior under normal pressure conditions.

Figure 6 compares the effects of chlordiazepoxide over a dose range of 1.0–20.0 mg/kg on behavior with the effects of the drug under conditions of breathing air and helium at elevated pressures. The dose-effect function at 8.6 ATA is shifted toward the left from the dose-effect function at normal atmospheric pressure, which indicates that the raised pressure of air enhanced the effects of chlordiazepoxide on behavior. The enhancement at 8.6 ATA breathing compressed air is probably the result of the raised nitrogen partial pressure, since similar

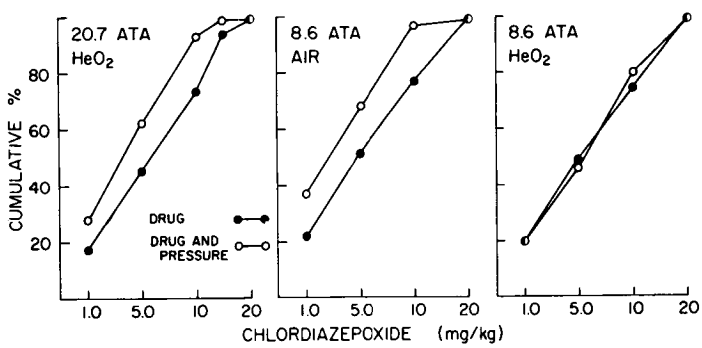

Fig. 6. Cumulative percent change in behavior plotted for chlordiazepoxide at surface and at depth (8.6 ATA and 20.7 ATA), using breathing mixtures of compressed air or an 80% helium-20% oxygen ($He\text{-}O_2$) mix.

changes in the dose-response curve were not obtained when helium replaced the nitrogen component of the gas mixture. Helium breathed at 20.7 ATA did produce changes in the dose-response curve from the normal pressure curve. The modification of the drug effects on behavior by 20.7 ATA of helium was similar to that of nitrogen at 8.6 ATA and indicates that helium at 20 times normal atmospheric pressure is capable of potentiating the behavioral effects of chlordiazepoxide.

Behavioral evaluations of diazepam in the dose range of 0.1 to 5.0 mg/kg indicated that both performance and accuracy on behavioral tasks decreased with increasing doses. When the drug was evaluated at 8 ATA in compressed air, the pressure and drug effects interacted synergistically to produce greater behavioral decrements than seen with the drug on surface trials (Fig. 7*A* and *B*).

Pentobarbital

Sodium pentobarbital was selected as the representative compound of the barbiturate class. A dose range of 1.0–15.0 mg/kg was used in these determinations. The results of the pentobarbital evaluations are shown in Fig. 8*A* and *B*. Behavioral decrements in gross performance across the dose range (Fig. 8*A*) increased at 8 ATA when compared with the drug under normal pressure. The accuracy of behavior that did occur, however, improved somewhat at 8 ATA, especially at the 1.0 and 3.0 mg/kg dose levels (Fig. 8*B*).

Ethanol

Ethyl alcohol, or ethanol, is another agent known to have CNS effects. It is widely self-administered, yet little is known of its action under hyperbaric conditions. Ethanol was examined for its behavioral effects over a dose range of 0.5 to 2.0 g/kg. At present, we have evaluated ethanol over this dose range only at 7.1 ATA in compressed air. The dose-response function for ethanol at increased pressure is presented in Fig. 9 and is compared to the dose-response curve obtained at normal pressure. Particularly at higher doses, the dose-response

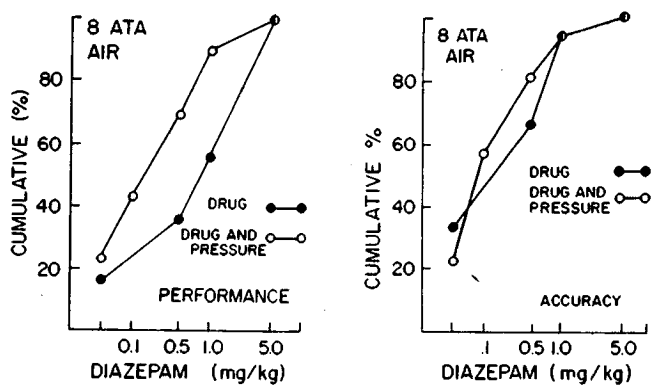

Fig. 7. *A*: cumulative percent change in performance plotted for diazepam at surface and at 8 ATA breathing air; *B*: cumulative percent change in accuracy plotted for diazepam at surface and at 8 ATA breathing air.

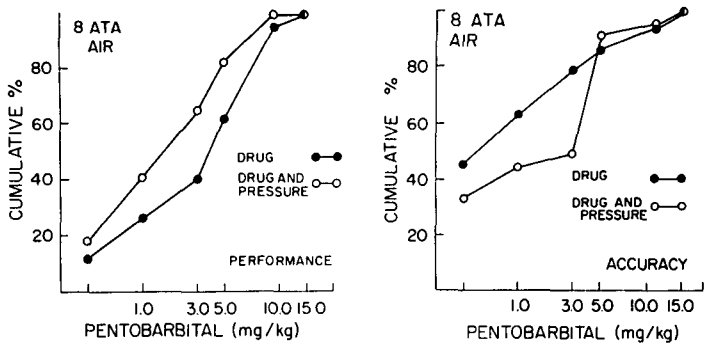

Fig. 8. *A*: cumulative percent change in performance plotted for pentobarbital at surface and at 8 ATA breathing air; *B*: cumulative percent change in accuracy plotted for pentobarbital at surface and at 8 ATA breathing air.

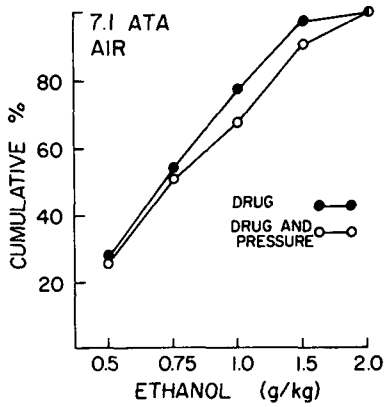

Fig. 9. Cumulative percent change in behavior plotted for ethanol at surface and at 7.1 ATA breathing air.

function for ethanol under pressure appears to be shifted to the right of the ethanol function obtained under normal pressure. This shift indicated that the behavioral effects of ethanol are somewhat lessened under elevated pressure. The lessening of the behavioral effects of ethanol under raised pressure is one of the few examples of reversal of pharmacological effects by a hyperbaric condition observed in our studies of psychoactive agents under increased pressures.

Conclusions

The techniques of the experimental analysis of behavior have proven to be sensitive research tools for the evaluation of pressure-produced modification of pharmacological effects on behavior. The use of these techniques has allowed for the identification and measurement of extremely subtle changes in drug effects under conditions of increased pressures. The application of these behavioral techniques under hyperbaric conditions, which has been

successful in the examination of psychoactive compounds, can certainly be extended to a wide and diverse range of pharmacological and chemical agents.

The evaluation of a number of psychoactive drug classes under hyperbaric conditions has focused attention on two major aspects of research findings. The first and perhaps principal finding of the present psychopharmacological studies is that the nature of the behavioral action of a drug under pressure is not predictable from its normobaric characteristics. A drug's effect on behavior may be enhanced or antagonized by hyperbaric conditions and at present there is no basis on which to make predictions of such changes in drug action. The second major finding is that the drug-produced changes in behavior under increased pressure are not always in accord with the changes which would be expected by our current understanding and theorizing about the nature of inert gas narcosis. Narcosis is generally viewed as a depression or slowing of CNS activity. Amphetamine, a CNS stimulant, was found to potentiate rather than antagonize the behavioral effects of elevated gas pressures. Another unexpected result was that depressants, like alcohol and pentobarbital, were the only drugs to show any reduction in behavioral deficits under pressure. The behavioral changes produced by pressure-drug interactions suggest that inert gas narcosis is not simply a generalized depression of CNS functioning, because CNS stimulants enhance its effects and depressants reduce them.

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The animals used in this study were handled in accordance with the provisions of Public Law 89-44 as amended by Public Law 91-579, the "Animal Welfare Act of 1970" and the principles outlined in the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

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BLOOD-BRAIN BARRIER DYSFUNCTION IN EXPERIMENTAL GAS EMBOLISM

Barbro Johansson

The modern concept of the blood-brain barrier (BBB) is complex and comprises a number of mechanisms regulating blood brain transfer of different substances. Plasma protein cannot penetrate into the brain due to unique characteristics of the endothelial cells in the brain vessels. These cells are joined by tight junctions which prevent passage of substances between the cells, and the vesicular transport from the luminal to the parenchymal side of the endothelium is insignificant (17). A specific enzymatic barrier to catecholamine precursors exists (1). On the other hand, the brain endothelial cells have active transport mechanisms facilitating blood brain exchange and uptake of amino acids and glucose (6, 12, 14).

It is thus evident that blood-brain barrier dysfunction can be revealed in different ways. The increased permeability to protein and dyes has been extensively studied in experimental BBB lesions. In a few pathological conditions damage to specific transport mechanisms resulting in a decreased uptake of amino acids and glucose has been demonstrated (15, 18).

The Blood-Brain Barrier to Protein and to some Dyes in Experimental Gas Embolism

Broman (2) described perivascular tissue staining by trypan blue in vessels blocked by air embolism for 10 minutes or more. The same kind of damage was seen in pial as in intracerebral arteries. Different aspects of the blood-brain barrier changes to dyes and protein have been reported by Lee and Olszewski (13), Jeppsson (9), and Broman et al. (5). In most of these studies a large amount of gas was given which has been found to result in disturbances of the systemic blood pressure and particularly of the cerebral blood flow. It is preferable to give a small amount of gas if the injurious effect of gas per se on the blood vessels is to be studied.

In one series of rabbits, 0.01–0.05 ml of air was injected into the left common carotid artery via a polyethylene catheter after ligation of the external carotid branch. During the experiment the brain surface was inspected under a Zeiss stereomicroscope through a trephine opening in the left parietal region (cf. 5). Trypan blue or Evans blue, both of which bind to serum albumin and can be traced in fluorescence microscopy due to a red fluorescence emitted by the dye protein complex (7, 20), was given i.v. immediately after the gas exposure and the extension and spread of the tracer studied in fluorescence microscopy at different time intervals after killing the animal by perfusion fixation. Microscopic examination was also carried out on surface sections of the pia-cortex inspected intravitally. In a few ex-

periments the tracer was given before the gas and the animals killed within one minute. A gas exposure time of only a few seconds resulted in a staining of arterial and arteriolar vessel walls of the exposed hemisphere and this staining of the vessels was the most striking feature. Extravasation of the dye occurred particularly in some experiments when large amounts of air were injected (0.1–0.5 ml). These experiments have been published in more detail elsewhere (10).

In long-term experiments it was found that the vessel damage lasted for approximately 24 hours. Most animals with a survival time of 2 days or more did not show any lesions. Similar lesions occurred when CO_2 and O_2 were given, but they were less pronounced after CO_2 injection.

The marked staining of the vessel wall with little extravasation of the tracer presents a picture that is not common in other types of experimental blood-brain barrier lesions. The lesions of the endothelial cells occur immediately after contact of the gas and cannot be caused by ischemia. Perfusion with saline does not produce any lesions and it has repeatedly been shown that the blood-brain barrier to protein is not damaged by ischemia of short duration (3, 4, 8, 11). In small vessels where gas is trapped for a longer period of time ischemic lesions of the brain parenchyma may occur naturally but some other factor must be responsible for the permeability changes in the endothelial cells.

The Blood-Brain Barrier to Amino Acids

Damage of arteries and arterioles is most striking but in electron-microscopic examination the protein tracer peroxidase is also seen in capillaries. To study whether the vessel changes have some implication for specific transport functions of the blood-brain barrier, the following experiments were performed. Air (0.05 ml) was injected through a thin needle into the common carotid artery after ligation of the external carotid branch in 12 rabbits. The needle was then removed and 3 ml of 2% Evans blue solution in saline/kg was injected i.v. The animals were killed 1, 6, or 24 hours later. Five minutes before the death of the animals, ^{75}Se L-methionine was injected in a dose of 30 μCi/kg. Because the uptake of amino acid depends on the blood flow, ^{125}I-antipyrine (15 μCi/kg) was given in a continuous infusion as a blood flow tracer (cf. 19) the last 30 seconds. The brain was inspected to ensure that the lesion was unilateral, and symmetric specimens from the hemispheres were removed, weighed, and counted for radioactivity in a well scintillation counter. The results showed that blood flow as well as uptake of amino acid was symmetrical in the two hemispheres. Thus no evidence for changed amino acid net uptake could be demonstrated with this method, which might, however, not be sensitive enough to detect minor changes.

Studies on Isolated Capillaries

In a preliminary series of experiments, air was injected as above and the rabbits were killed 2 hours later. Capillary-enriched tissue fractions were prepared separately from the left and right brain hemisphere, according to the method of Orlowski et al. (16), with small modifications (Blomstrand and Johansson, unpublished observations). The fractions were routinely checked under light microscopy after methylene blue staining. They were satisfactorily pure, and contained capillaries without adhering glia primarily, but also some arterioles. The endo-

genous respiratory activity was measured by means of Clark's oxygen electrode in a phosphate-buffered medium (pH 7.4) without any added oxidizable substrate. The capillary fractions of the exposed and nonexposed hemispheres were compared. In all the animals examined, the vessels from the exposed hemisphere showed more than twice as much oxygen consumption as those from the contralateral hemisphere. Further studies are needed to evaluate the mechanisms which underlie this markedly enhanced respiration, and to determine the time course of this phenomenon.

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PART I. DISRUPTION OF NERVOUS SYSTEM AND PERFORMANCE AT INCREASED PRESSURE

DISCUSSION

R. Naquet, Chairman

Dr. Brauer: I noted that during the decompression from Sagittaire IV, Physalie VI, and in several of the others, there is a very marked enhancement of delta activity in some cases. Is this a universal thing, and is it associated in your dives, as it was in some of our early ones, with behavioral changes?

Dr. Rostain: Yes. During the beginning of the two decompressions there was an increase of delta and theta activity and this increase lasted from the bottom to 400 meters; the decrease was observed after 400 meters and generally disappeared between 200 and 100 meters. For Sagittaire IV the increase of theta and delta activity persisted for several days after the divers left the chamber. I have no explanation for this modification at the beginning of the decompression, but I think that its persistence during Sagittaire IV was due to the big increase during the stay at bottom, because a 2,000–4,000% increase in theta also occurred in Sagittaire II, where the return to normal took place after the end of the decompression.

Dr. Bennett: I wonder, Dr. Rostain, if you have noticed any connection at all between the increase of EEG theta activity and what you call microsleep or narcolepsy. We have just finished some fast dives which we made at RNPL in England to 1300 feet and 1525 feet and there was indeed an increase in theta activity which many of us have seen associated with diver fatigue, which we know you have encountered in your own dives. Have you found that the increase in theta activity is associated with the onset of narcolepsy or microsleep in an individual?

Dr. Rostain: No. They are two different things. In some divers there is only an increase of theta activity in the frontal region or middle regions. In other divers there are increases of theta activity and of microsleep, but these are two different things.

Dr. Bennett: I would also like to express some concern, now that we have seen these very excellent data to 2,001 feet. I think Dr. Rostain commented that the men were reasonably normal, but it is quite evident from the EEG changes and from the other measurements that they were in fact not normal; yet phenomenal times were taken to put these men down to these depths, with stages as long as 49 hours, in some cases. One wonders about the practicality of this kind of diving. Many of us have been involved in trying to decide the limits of deep diving, and certainly these data give me considerable concern. Whether or not trimix will enable us to get very much deeper remains to be decided, I think. Certainly from our own experiments, 1,000 feet is effective.

Dr. Naquet: I think we know now that man is able to work at 300 meters under water. Fifteen days ago two experiments were done by French people in the Mediterranean and in Labrador and men were able to work at 300 meters breathing heliox. The point is, however, that in the deep simulated dive with heliox, at 600 meters, we didn't find any modification of the EEG or paroxysmal discharges or signs of hyperexcitability. In contrast, Hugon's group found hyperexcitability of the medulla at the same depth, and we don't know how to correlate their data with ours. On the other hand, we have found paroxysmal EEG changes in some people with trimix, and we don't know whether there is hyperexcitability at the medullar level or not. Does Professor Hugon have some data on trimix?

Dr. Hugon: We have not had the time to run that experiment yet. Trimix dives are rather short and the experiments are time consuming, so there is no possibility for extensive spinal research. But there are two ways to look at the problem—in man and in monkeys. Short and critical observations will be performed on man, and extensive chronic experimentation is planned on monkeys. We plan to explore the neurophysiological properties of the spinal and vestibular motor system, and also to depict defects in motor performance, including tremor and equilibrium, and record cortical and subcortical activities. We hope to be able to understand the specific effects of helium and nitrogen in trimix for normal or experimental dives. This program requires a lot of experimental work during the next few years, in cooperation with the electroencephalographists.

Dr. Bennett: Last May at the Annual Meeting of the Undersea Medical Society, Dr. Farmer proposed a theoretical concept for the mechanism for HPNS involving the loss of cerebellar inhibitory control of the vestibular nuclei. There is no nystagmus with HPNS so that it is definitely of central origin; it is tempting to think that if nitrogen does in fact act as a depressant in this area, as Professor Naquet has told us, that nitrogen could also act to suppress HPNS. I think, however, that there is some difficulty here. We are probably looking at a number of different effects with different mechanisms: whereas nitrogen may very well act on this particular mechanism to control tremor, ocular tremors, nausea, dizziness, and so on, EEG changes do not seem to be affected by the nitrogen.

Dr. Schaefer: I have a question for Dr. Dimov. You found amazing physiological changes in this 18-day exposure to 3.1 atmosphere air. You also mentioned that you observed a respiratory and nonrespiratory acidosis. You suggested that the effects of exposure to increased pressure might have been related to the acidosis but you did not report the degree or timing of the respiratory and nonrespiratory acidosis. Will you comment on this?

Dr. Dimov: This acidosis lasted for the first three days. The pH was 7.28, 7.30 and thereafter gradually increased to 7.35.*

Dr. Bennett: I must say that as one who has done a lot of EEG recordings in the compressed air environment, I was quite amazed to see this large amount of slow theta activity in the EEG. I began to feel as if I were looking at helium dives instead of very shallow nitrogen dives. This is the first time I have ever seen this kind of theta activity in very shallow nitrogen dives. I have no explanation for it although the oxygen is relatively high. I wonder about nitrogen enhancement of oxygen toxicity, but there was no documentation of any pre-oxygen-seizure EEG changes, so this does not seem to have been a factor and the nitrogen seems far too low to have had any effect. I wonder if there was not some special characteristic of your diver which led to these kinds of results. Perhaps he had epileptic tendencies or was he hypoglycemic?

Dr. Dimov: We were also surprised to see these changes. Only one of these divers had some neurologic defects after these experiments. Tests showed that after three or four months all of our people were in good condition. It is really difficult to explain these changes. I think it is something very complex compounded by the large problem with fatigue because these divers were very active in the water for the first 3 or 4 days.

Dr. Behnke: I am impressed by the species difference between the small mammal and man. Mice are apparently normal at 2500 feet. A second point I raise is this: all of these studies have been neurological, primarily. When men and animals are compressed to 30, 40, 50, 60 atmospheres, there is certainly a high concentration of all substances in the gastrointestinal tract, such as ammonia, hydrogen sulfide, methane, and other substances. Have there been any studies utilizing mass spectrometry or gas chromatography of various trace substances diffusing from the body during the first part of a dive which would be eliminated subsequently during the apparent period of adaptation?

Dr. Dimov: I think this is mainly a respiratory problem. Mass spectrometry has been done, but I don't think it explained any of these effects.

Dr. Naquet: To conclude the first session, we know that man is now able to go to 600 meters. There were EEG changes and tremors, but the men were still able to work. Because of the hyperexcitability of the medulla, it will be unsafe to go deeper with heliox without new experiments with animals. We will learn in the next few years what we can do with trimix. In 1962 when we reached 120 meters we thought that was the deepest we could go; in 1968 we thought that 365 meters was the deepest level we could reach; now we have reached 600 meters. We know now to be careful when we give a limitation.

Dr. Örnhagen: We have seen convulsions in hypothermic liquid-breathing mice with a temperature between 22°–31°. We couldn't see any change in the convulsion threshold: it was roughly 85 atmospheres. However, we noted that the cooler the animal the more prolonged the individual seizure, and at a temperature about 27° we had great difficulty in seeing these seizures because they were as short as breathing actions. I wonder, Dr. Cromer, could you see any change in the length of the individual seizure when you cooled your animals down?

Dr. Cromer: The length of the seizures varied from 10 to 20–25 seconds. We did not see a significant difference in the animals in the euthermic and hyperthermic conditions, and of course we did not see seizures at all in our hypothermic animals. But the difference was due more to individual difference than to temperature differences.

Discussant: I would like to ask Dr. Johansson how he actually achieved micro-air embolization. In particular, how does one technically inject the animals?

Dr. Johansson: In the first series where I studied and correlated the exposure time with the results, I inserted a polyethylene catheter into the common carotid artery and tied the external carotid branch. Of course in the latest

* Dr. Dimov's paper is not included in this volume.

studies when I was interested in metabolism, I didn't want to have any difference in blood supply between the left and right hemispheres, so I just injected the gas directly into the common carotid artery with a thin needle after ligation of the external carotid branch.

Discussant: Did you have any problem with small bubbles being absorbed by the hyposaturation of the blood before it got to the target?

Dr. Johansson: I didn't particularly look for that. All I can say is I saw gas in the vessels under the trephine opening in all of the experiments.

Dr. Chryssanthou: I believe it would be relevant to mention some of our observations which are consistent with those of Dr. Johansson. In experiments which are now in progress in our laboratories, we have observed that conditions which produce decompression sickness in rabbits break the blood-lung barrier and in several animals we noted modification of the blood-brain barrier. In those cases we have seen gas bubbles in systemic circulation but no gas bubbles could be detected in brain vessels. In view of this, do you believe that modification of the blood-brain barrier could be caused by vasoactive substances which are released or activated by gas-blood interactions?

Dr. Johansson: I can only say that some vasoactive substances can produce blood-brain lesions by an increase in blood pressure. Whether vasoactive substances can per se increase the permeability in cerebral vessels is a controversial question. Some of these substances seem to increase the pinocytotic activity. However, the type of blood-brain barrier lesion we see after gas embolism occurs only in vessels exposed to the gas; these lesions cannot be reproduced by any vasoactive substances.

Dr. Hills: Could I ask Dr. Johansson if he injected his gas as a bolus or as bubbles? Observing through a cranial window, we do find quite a difference in mechanical behavior if it was injected as ultramicro bubbles; could this have an effect?

Dr. Johansson: I have only used a bolus; I know about your very interesting work and there may be quite a difference.

Dr. Winter: I would like to ask Dr. Thomas to enlarge upon his interesting initial finding in which he demonstrated that diazepam offers protection against decompression sickness. I don't believe he even hypothesized about an explanation.

Dr. Thomas: Well, that is because I honestly don't have one. This was an observation we made in doing our initial toxicological evaluations and then homed in on this study as I presented it. What type of mechanism or action is going on that allows for this decrease in decompression sickness, in this case lethal effects, I really don't know. We played around with the idea of primary muscle relaxant properties of diazepam, and there are many studies in the literature which demonstrate that agents which have effects on stimulation or relaxation of muscle agents have a protective effect. Whether this is in terms of helping the takeup and rapid elimination of nitrogen, I don't know. This is merely a guess at this time.

Dr. Brauer: I would like to comment on Dr. Cromer's interesting presentation. You remember the animals he looked at were warm-blooded. Obviously, an animal such as a rat will have a certain amount of difficulty in making its nerves function at 20, 22, or 24°C. We have experiments with lizards and with very young mice, both of which are essentially poikilothermic. In both of these cases, over the range from 24 to 36° (but not to 40°C), there is no change in HPNS convulsion threshold. I think it is interesting to note that as we go from the very cold-blooded species to warm-blooded, there is an additional change in the pattern of the HPNS, as in other convulsants.

From the discussion it sounded as though there is a smooth relation between temperature and HPNS convulsion thresholds in the rat. In fact, this is not the case. There is a plateau that stretches from about 30° to at least 35° rectal temperature. Over this range, the convulsion thresholds do not change with the temperature. As one falls below those, or as we get into hyperpyretic temperatures, they become excessively convulsion-prone. Thus, we rapidly reach levels where the animals can no longer produce a convulsion.

Dr. Cromer: I would agree to a point with Dr. Brauer. We were looking at severe stresses and the results here would perhaps be limited to that, but the EEG changes certainly were compatible with what we found in the EEG literature, particularly in the hyperthermic series; we had not seen this previously applied in hyperbaric literature.

Dr. R. Smith: I am interested in Dr. Thomas' pharmacology study with depressant drugs and amphetamines, where the pharmacology appears to be all wrong when you consider the depressant effects of helium and nitrogen in terms of their solubility relations. If you are implying some kind of equivalence between about 8½ atmospheres of air and 20 or so atmospheres of helium, are you speaking of an equivalence in terms of pressure per se or are you thinking of an antagonism simply in terms of the solubility-related depressant effects of these agents? Do you have some kind of idea what your handle is on this problem?

Dr. Thomas: The equivalence that we are speaking of in the data is strictly behavioral equivalence: at what depth or what pressure do you see the same types of effects that you see at another depth or another pressure with

nitrogen. The types of changes we saw at the shallow depths, 7 to 8 atmospheres, with compressed air, which is also seen with the nitrogen alone, these types of changes in behavior do not show up with helium until you reach 20 atmospheres. Our only handle, our only gauge, is relative behavioral change, when the behavior is the same and when it is not the same.

Dr. Madsen: I have a question for Dr. Brauer. In the slide where you compared baby mice and grown-up mice with respect to the relationship between compression rates and convulsion thresholds, the baby mice were only studied at 2 compression rates. If you had only observed the grown-up mice at the same compression rates, you wouldn't have found any dependency of convulsion threshold on compression rate. Therefore, my question is, do you have any additional data on the baby mice to show whether the lack of dependency in this group is a fact or a random finding?

Dr. Brauer: The answer is yes, I have more data. I have complete curves on these. The fascinating thing is that compression-rate dependence of convulsion thresholds appears in the mice at about 10 days and grows over the next few days. It reaches a substantially adult level by the time they are 22 days or so old. It is a progressive change in the mouse, but fairly sharp in the rat. In the rat, there is a dramatic change in behavior between day 9 and day 11, with the doubling of convulsion thresholds. We have an extensive study underway concerning the possible mechanisms or the possible structural substrate for this, but I am not yet prepared to resolve that.

Dr. Naquet: Just a question. Why between 9 and 11 days? Do you have an explanation? These animals become adult, and it is at this time that they become sensitive to sound and convulse easily.

Dr. Brauer: I'm willing to guess. One of the things that happens in the rat at that period is that there is a big jump in the choline-acetylase activity and this is a possible target, but there are obviously others.

Dr. Miller: I would like to ask Dr. Blankenship a question. In frog skin there is also no apparent inert gas effect in the range of 1 to 10 atmospheres' pressure. I noticed in your current-voltage plots there appeared to be some indication of electrical rectification. I wonder if you have looked at rectification and if there were any rectification changes. When you have your plots drawn as vertically as they were, it is a bit difficult to detect rectification, but it appears you had higher resistance at higher voltages. Is that correct?

Dr. Blankenship: It works the other way around. The resistance goes down with higher voltages. Yes, rectification was observed in several experiments. We had to compress the scales to get 20 records across the figure. Rectification does not seem to be affected in *Aplysia* cells up to 10 atmospheres.

Dr. R. Smith: We have studied decompression sickness in our lab. We have been looking at catecholamines and primarily serotonin; we have also had some very good results giving antiserotonin drugs. I wonder if Dr. Brauer has checked any serotonin or catecholamine levels in his animals?

Dr. Brauer: You notice that in my statements I very carefully talked about monoamines, which is a polite way of saying I am not willing to commit myself. We have tried to manipulate serotonin. The problem is that whatever effect we are looking at seems to require rather extensive blockade of the transmitter mechanism, and I don't have really good specific agents that would single that out. The same thing applies to the time course data where the absolute monoamine levels do not tell us what we want to know; we would have to run rather sophisticated incorporation rate studies. So far we have not done so, but we are preparing to.

Dr. Kitano: I have a question for Dr. Johansson. Would you please tell me whether the wall of the veins and glial cells are stained by EDA?

Dr. Johansson: The walls of the veins are not stained. Particularly if you give a large amount of gas you can see fluorescent neurons. In the slide where I show some fluorescent neurons there were some glial cells too. But this is usually not seen when you give a small amount of gas. Arterioles and capillaries are stained, but never veins.

Dr. Libber: I would like to ask Dr. Blankenship if *Aplysia* lives at a depth greater than 10 atmospheres normally; second, have you ever tried nitrous oxide on your *Aplysia* preparation?

Dr. Blankenship: *Aplysia* have never been reported in depths greater than 200 feet. In fact, that is probably an extreme range for the animal. They are normally considered to be intertidal. We really don't feel, however, that the pressures we are working at in the neighborhood of 300 feet are really that much of a stress to the animal; we may not have pushed this particular animal as far as we needed to see changes that it would be unable to cope with. We have not tried nitrous oxide directly on the animal, but there are several kinds of common anesthetics that do work to relax the animal. I frankly don't know if nitrous oxide works or not, but since the neural mechanisms, conductance states, etc., in these nerve cells are quite comparable to those of nerve cells in other animals, I'm sure it does.

Dr. Bennett: I just want to add something here which may be relevant. In the *Second Symposium,* Frank Carpenter reported work on sciatic nerve using argon; he looked at membrane potential. You may remember he had

to go to something like 310 atmospheres of argon to get any effect at all. Because we are dealing here with a narcotic which is twice as potent as nitrogen it would be necessary to pressurize to some 600 atmospheres. That is, in the mammalian system and in an invertebrate one may need much more than that. So I think you may need to try nitrous oxide or some anesthetic like that, perhaps xenon.

Dr. Frattali: I have a quick question for Dr. Blankenship. Do you have any similar statements to make on synaptic activity in *Aplysia* and what type of a synapse you are dealing with?

Dr. Blankenship: We have not done enough yet to be able to give any results. We are looking at two different things. The first is a study of a unitary monosynaptic EPSP produced in cell R15, the parabolic bursting cell, with stimulation of the right connective. This particular connection has been very well described in terms of frequency-dependent facilitation and depression. Also, the effects of changes in calcium and magnesium concentrations in the bath have been well studied. We are currently examining whether increased nitrogen tensions influence the frequency- and ionic-dependent properties of this connection. The second, more complex, system we plan to study involves an investigation of the synaptic connections made by a well-known interneuron in the ganglion, cell L10. This cholinergic interneuron makes a variety of well-understood monosynaptic connections onto several identified neurons in the ganglion. We will examine the influence of increased nitrogen tensions on the characteristics of these connections by recording simultaneously from L10 and several of its follower cells. Because of the relative ease in stimulating the sensitive cell bodies of *Aplysia* neurons with iontophoresed synaptic transmitters such as acetylcholine, we hope to be able to differentiate between pre- and postsynaptic effects of pressure of the follower cells on L10.

Part II. EVOLUTION OF LOW TEMPERATURE DIVING

ENERGY BALANCE OF DIVERS AT WORK IN HELIUM-OXYGEN ATMOSPHERES

P. Varene, H. Vieillefond, J. Timbal, H. Guenard, C. Boutelier and C. Lemaire

Hyperbaric helium atmospheres may alter the energy balance of divers in at least three ways: a modification in metabolism, an increase in skin convective heat exchange, and an increase in tracheobronchial heat exchange. Previous papers have reported the results obtained in divers at rest (11, 13, 15). The present experiments were conducted to study the thermal balance of working divers.

Methods

The subjects were three male deep-sea divers and one physician (Table I). Measurements were made during compression in a helium-oxygen atmosphere with a constant oxygen partial pressure (P_{O_2} = 226.6 ± 7.5 mmHg) at four levels of pressure (Table II). Each pressure level was maintained for two days. The subjects were tested each time in the same order: *subject GD* during the morning of the first day, *subject FF* during the morning of the second day, *subject RG* during the afternoon of the first day, and *subject CP* during the afternoon of the second day. After 30 minutes of bed rest, the subjects exercised two times at a level of 80 watts on a cycle ergometer: the first run for 45 minutes breathing ambient gas at ambient temperature, and the second run for 30 minutes breathing ambient gas at a lower temperature. The two runs were separated by a 40-minute rest period.

The classical method of partition calorimetry was used with minor adjustments for the measurements and computations of metabolism (M) as well as skin heat losses by evaporation (E), radiation (R), and convection (C). Conductive heat losses were neglected.

TABLE I

CHARACTERISTICS OF SUBJECTS

Subjects	Occupation	Age, yr	Weight, kg	Height, m
G D	Diver	26	75.5	1.75
F F	Diver	24	72.5	1.79
R G	Diver	32	72.5	1.65
C P	Physician	30	78.5	1.69

TABLE II

ENVIRONMENTAL CHARACTERISTICS

Seawater Depth, m	P, mb	P_{H_2O}, mb	P_{O_2}, mb	P_{CO_2}, mb	P_{He}, mb	Density, $kg \cdot m^{-3}$
8	1,823	29	292	1.25	1,501	0.70
100	11,082	26	293	0.99	10,762	1.98
200	21,131	27	311	3.60	20,790	3.77
300	31,190	25	299	1.65	30,864	5.57

Metabolism was calculated from the rate of oxygen consumption ($\dot{V}_{O_2}$). Expired gases were collected during three periods of 5 minutes each at the end of each run. Ventilation ($\dot{V}_E$) was measured at ambient temperature and pressure, using the collected gases. Three samples of inspired and expired gas were taken from the chamber through a pressure-reducing valve. The oxygen and carbon dioxide fractions were analyzed twice. The inspired and expired oxygen fractions were measured by means of an electrochemical cell (Westinghouse Model 209 Oxygen Analyzer), and CO_2 was measured by infrared absorption (Hartmann and Braun Analyzer, Type URAS 4); both CO_2 and O_2 were then analyzed by gas chromatography. No significant difference between the two methods was observed.

The subjects were weighed at the beginning and end of each run. To obtain the other components of the body heat balance equation, rectal, skin, ambient and wall temperature as well as inspired and expired gas temperatures were measured. Copper-constantan thermocouples were used, with melting ice as the cold junction.

Both a thermistor and a bulb thermometer graduated to 0.02 °C and reliable at high pressure (Richter and Wiese) controlled the accuracy of zero temperature. The reference junction was placed inside the pressure chamber with the subjects; connections through the walls were by copper wire. Amplification and recording were performed outside the chamber by a potentiometric system. Measurements were made continuously for gas temperature and each minute for skin, rectal, and environmental temperatures.

Our techniques differed from those of Timbal et al. (13) only with regard to estimations of the radiant surface and the partition ratio between core and skin temperature. The ratio AR/AD of radiant surface over total body surface is lower at exercise than at rest. In this study, the exercise value of 0.75 instead of the resting value of 0.84 was used (2). A partition ratio of 0.8:0.2 was chosen for core over skin temperature (4). Heat losses by respiratory convection were computed as previously described (9).

Results

Ambient and body temperature results are given in Table III. Ambient (T_a) and wall (T_w) temperatures increased as a function of pressure. Wall temperature followed T_a, which was increased to maintain thermal comfort at the request of the subjects. Thermal comfort was confirmed by skin temperature values at rest, before the beginning of exercise. During exercise skin and rectal temperatures increased. These increases were higher at 1.8 ATA than at

TABLE III

EFFECTS OF AMBIENT PRESSURE CHANGE ON RECTAL AND SKIN TEMPERATURE OF SUBJECTS EXERCISING AT 80 WATTS

Environmental Conditions			Temperatures of Subjects			
Ambient Pressure, ATA	Ambient Temperature, °C	Wall Temperature, °C	Initial Rectal Temperature, °C	Final Rectal Temperature, °C	Initial Skin Temperature, °C	Final Skin Temperature, °C
1.8	28 ± 0.72	24.75 ± 1.63	37.14 ± 0.09	37.96 ± 0.07	33.41 ± 0.82	34.77 ± 0.45
10.9	29.01 ± 0.58	24.57 ± 1.03	37.20 ± 0.12	37.90 ± 0.13	33.18 ± 0.39	34.22 ± 0.52
20.9	29.98 ± 0.57	26.94 ± 1.69	37.16 ± 0.18	37.96 ± 0.21	32.77 ± 0.36	33.99 ± 0.43
30.8	31.02 ± 1.39	29.16 ± 1.91	37.44 ± 0.39	38.06 ± 0.36	33.54 ± 1.21	34.20 ± 1.18

30.8 ATA. Similarly, the standard deviation of rectal as well as skin temperature increased with pressure.

Table IV shows the mean respiratory values measured during the first exercise test while subjects breathed gas at ambient temperature. Ventilation ($\dot{V}$) and breathing frequency (f) decreased with pressure while tidal volume (V_T) increased, but these variations were not significant except for *subject FF* ($P < 0.005$). As far as oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$) and respiratory quotient (R) were concerned, no systematic variations were observed with pressure.

TABLE IV

RESPIRATORY DATA BY SUBJECT AND PRESSURE

Subjects	Pressure, ATA	$\dot{V}_{BTPS}$, dm^3mn^{-1}	Respiratory Rate, Cmn^{-1}	V_T, liters	$\dot{V}_{O_2}$, dm^3mn^{-1}	$\dot{V}_{CO_2}$, dm^3mn^{-1}	Respiratory Quotient	Metabolic Level, watts
G D	1.80	42.766	21.95	1.948	1.495	1.329	0.890	511
	10.94	36.432	15.85	2.284	1.596	1.188	0.743	526
	20.86	45.634	18.60	2.453	1.765	1.615	0.919	608
	30.79	37.913	17.20	2.204	1.534	1.169	0.762	508
F F	1.80	40.392	26.87	1.503	1.516	1.367	.902	520
	10.94	40.935	22.88	1.789	1.609	1.650	—	566
	20.86	38.213	16.00	2.388	—	—	—	—
	30.79	34.222	15.86	2.158	1.429	1.254	0.880	488
R G	1.80	39.642	23.63	1.678	1.400	1.177	0.836	473
	10.94	34.941	17.50	1.997	1.428	1.168	0.816	480
	20.86	38.794	20.68	1.876	1.638	1.231	0.752	541
	30.79	37.071	15.13	2.450	1.333	1.115	0.837	450
C P	1.80	38.158	20.50	1.861	1.493	1.360	0.910	513
	10.94	39.257	28.50	1.377	1.750	1.399	0.798	585
	20.86	35.132	15.77	2.228	1.348	1.339	0.993	473
	30.79	41.220	18.80	2.193	1.470	1.305	0.888	503

Exercise level = 80 watts; temperature of inspired gas = ambient.

Discussion

Results of this study which show no or very little decrease in ventilation with pressure are in agreement with our previous results for resting subjects (13). They also agree with the data published in other studies (3, 12) for higher levels of muscular exercise and the same range of ambient pressure.

As in numerous previous studies, f decreased and V_T increased slightly with pressure. These classical responses are probably explained by the increase in breathing gas density (Table II). Assuming that dead space does not vary with pressure, it is possible to compute the alveolar ventilation from these data (1): it does not vary with pressure.

The oxygen consumption ($\dot{V}_{O_2}$) measured in these experiments involved a total metabolic activity of approximately 500 watts. No systematic variations were observed with pressure. This fact is in agreement with our previous studies but disagrees with many other studies. Bradley and his co-workers (3), Salzano et al. (12), and Fagraeus (7) found an increase in $\dot{V}_{O_2}$ with pressure for the same level of exercise. They explained the increase of $\dot{V}_{O_2}$ with pressure by the increase of ventilatory power. Computed from these authors' data, the increase of chemical power that the respiratory muscles are supposed to dissipate[1] is 80 watts in Bradley's experiments ($\dot{V}_E$ = 55 dm^3mn^{-1}, P_B = 19 ATA), 112 watts in Salzano's experiments ($\dot{V}_E$ = 40 dm^3mn^{-1}, P_B = 31 ATA) and 70 watts in Fagraeus' experiments ($\dot{V}_E$ = 45 dm^3mn^{-1}, P_B = 4.5 ATA in air). The comparison of these figures with those which can be directly measured at the lung level with methods used in the exploration of the mechanics of breathing (15) shows that it is necessary to assume a very low (1-3%) efficiency of ventilatory muscle to explain the overall increase in oxygen consumption by the increase in the work of breathing. Although estimates of ventilatory muscle efficiency varying from 1 to 25 percent can be found in the literature, it seems unlikely that the increase in oxygen consumption measured by these authors is related to the increased work of breathing alone. Instead, it appears that the very low computed values of ventilatory muscle efficiency for a ventilation around 50 dm^3mn^{-1} are related primarily to contraction of accessory muscles involved in strenuous effort rather than to the phasic contraction of ventilatory muscles.

Our view is also supported by the fact that such an increase has not been found in all the diving experiments. These latest findings are in agreement with the studies published by Flook et al. (8) and Demedts and his co-workers (6), who did not find any increase in oxygen consumption in exercising subjects when resistances were added at the mouth.

The increase of $\dot{V}_{O_2}$ while $\dot{V}_E$ remained constant as pressure increased leads to the computation of a $\dot{V}_E/\dot{V}_{O_2}$ ratio which decreases with pressure. This finding is generally interpreted to mean that pulmonary ventilation improves when gas density increases. In this study the extraction coefficient for O_2 was calculated as $E = \dot{V}_{O_2}/\dot{V}_{E(STPD)} \cdot F_{I_{O_2}}$ (5). According to previously published data, the extraction coefficient increases in three out of four subjects between 1.8 and 30.8 ATA.

Table V shows the mean values of energy expenditure. Because no true steady state was reached during these experiments, the values are expressed in units of energy instead of power, and cover the 45 minutes of exercise.

[1] 20 joules = 1 cm^3 O_2.

TABLE V

EFFECTS OF AMBIENT PRESSURE CHANGE ON ENERGY DISSIPATION DURING EXERCISE FOR 45 MINUTES AT 80 WATTS

Ambient Pressure, ATA	Radiation, kJ/m²	Evaporation, kJ/m²	Storage, kJ/m²	Skin Convection, kJ/m²	Coefficient of Heat Exchange, W/m²/°C
1.8	120.1 ± 9.9	429.1 ± 31.4	130.2 ± 15.6	—	—
10.9	116.5 ± 7.3	272.4 ± 49.4	106.2 ± 13.3	131.1 ± 26.2	10.1 ± 2.3
20.9	82.7 ± 9.0	201.9 ± 29.4	123.8 ± 19.5	184.5 ± 56.9	21.9 ± 8.3
30.8	60.9 ± 4.4	273.6 ± 73.5	86.2 ± 12.2	186.6 ± 19.9	26.7 ± 5.1

Data are means ± SD.

The increase in the wall temperature of the chamber as a function of pressure and the relative stability of skin temperature explain the radiant heat loss (R) as a function of pressure. The decrease in skin temperature differences observed between the beginning and the end of exercise has no influence on R but should be taken into account to explain the relative decrease of heat storage (S) with pressure. A large part of the decrease in S (80%) is due to a reduction in the rate of rectal temperature increase during the 45 minutes of exercise at high pressure, as compared to the increase at low pressure. Evaporative heat loss (E) is obtained by calculations based on the measure of body water lost by evaporation. This latter measure is derived from the difference in body weight before and after the exposure. The value for E at 1.8 ATA is probably an artifact resulting from the inclusion of the weight of a substantial amount of sweat that dripped on the floor but had of necessity to be included in the evaporative weight loss figures. If the value obtained at 1.8 ATA is omitted, E appears to be stable as a function of pressure. That it is impossible to compute a positive value for C at that level confirms the probable overestimation of E at 1.8 ATA. The main point demonstrated by Table V is the increase of the energy lost by convection with increasing pressure.

The convection exchange coefficient (hc) has also been computed (Table V). The values found agree with those computed by the formula proposed (13) for resting subjects, if an increase of 50% due to the effect of pedaling is taken into account (10).

Table VI gives the value of heat lost through respiratory gas convection. It shows the well-known increase of C_{res} as a function of P_B, as well as the decrease in inspired gas temperature (T_i). Its value is proportional to STPD ventilation, so that at a given P_B and T_i, the ratio C_{res} to $\dot{V}O_2$ or C_{res} to M is approximately the same for exercise and rest. An equation to calculate the energy lost by ventilatory convection as a function of T_i is: $\dot{W}er/\dot{V} = 0.42 - 0.012\,T_i$, where $\dot{W}er$ is the total energy lost by ventilatory convection in watts, $\dot{V}$ the STPD ventilation in liters and T_i the inspiratory temperature in °C. From this equation it can be seen that the ventilatory convective heat expenditure equals zero when T_i equals 35 °C.

In conclusion it appears that the part played by convection exchanges in thermal balance is greater at high pressure than at sea level. The opposite may be observed at altitude, where energy lost by evaporation accounts for the main part of energy balance at exercise (14). These observed facts do not explain the exact adjustment mechanism of the different pathways of heat loss.

TABLE VI

EFFECTS OF AMBIENT PRESSURE CHANGE ON RESPIRATORY CONVECTIVE HEAT LOSS AT TWO LEVELS OF INSPIRED GAS TEMPERATURE

Pressure, ATA	Inspired Gas Temperature, °C	Respiratory Heat Loss W/m², by subject			
		GD	*FF*	*RG*	*CP*
	29-30	1.9	2.2	2.0	2.2
1.8	14-17	-	8.7	8.5	8.7
	28-30	19.7	12.0	11.5	11.7
10.9	17-19	44.3	32.0	33.3	38.3
	30-31	25.3	26.6	25.5	24.8
20.9	20-23	64.9	55.0	60.9	63.2
	31-33	27.3	41.9	35.0	28.9
30.8	23-25	84.0	69.4	78.2	68.5

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PHYSIOLOGICAL FUNCTIONS DURING A 10-DAY HELIOX SATURATION DIVE TO 7 ATA

H. Nakayama, M. Matsuda, A. Itoh, N. Kirigaya, F. K. Kurata, R. H. Strauss, J. R. Claybaugh and S. K. Hong

Many saturation diving experiments carried out in the past clearly indicate alterations in certain physiological functions, such as heart rate (3, 4, 7, 8, 11, 12), body heat balance (7, 8, 9, 14) and body fluid balance (1, 4, 7). These changes are admittedly subtle, but if sustained, they could affect man's performance at depth.

The present investigation was undertaken to document systematically: (1) cardiovascular functions; (2) energy metabolism and body heat exchange; and (3) body fluid balance during a 10-day stay in a dry hyperbaric heliox environment. The present study represents the first cooperative dive between the Japan Marine Science and Technology Center (JAMSTEC) and the University of Hawaii (UH). The dive was carefully planned by both groups and was carried out in Yokosuka, Japan, under the auspices of JAMSTEC during July and August, 1973.

Methods

Seven male Japanese subjects (22–27 years old, average height 164 cm, average weight 58 kg) were selected from a number of applicants on the basis of rigorous physical and physiological examinations. All subjects were trained SCUBA divers, and one had been employed in a 2-day dry saturation dive to 4 ATA in 1972.

The hyperbaric chamber (7 m long, 2 m wide, 2 m high) was equipped with temperature and humidity control units as well as other life support systems.

The dive profile and environmental parameters are shown in Fig. 1. Following a 3-day predive control period (*Period I*) the chamber was compressed to 7 ATA, first with air to a depth of 4 m and then with pure helium to a depth of 60 m (7 ATA). The total pressure of 7 ATA at depth was attributed to He (5.5 ATA), N_2 (1.2 ATA), and O_2 (0.3 ATA). The chamber pressure was maintained at 7 ATA for 7 days, but chamber temperature was maintained at about 28 °C during the first 3 days (*Period II*), then lowered to about 25 °C for 2 days (*Period III*), with a return to 28 °C during the last 2 days (*Period IV*). Following a 3-day decompression (*Period V*), a 2-day postdive control period (*Period VI*) was imposed.

Throughout the dive periods all subjects adhered to the daily activity schedule shown in Table I. A complete record of food and fluid consumption was kept to compute the daily

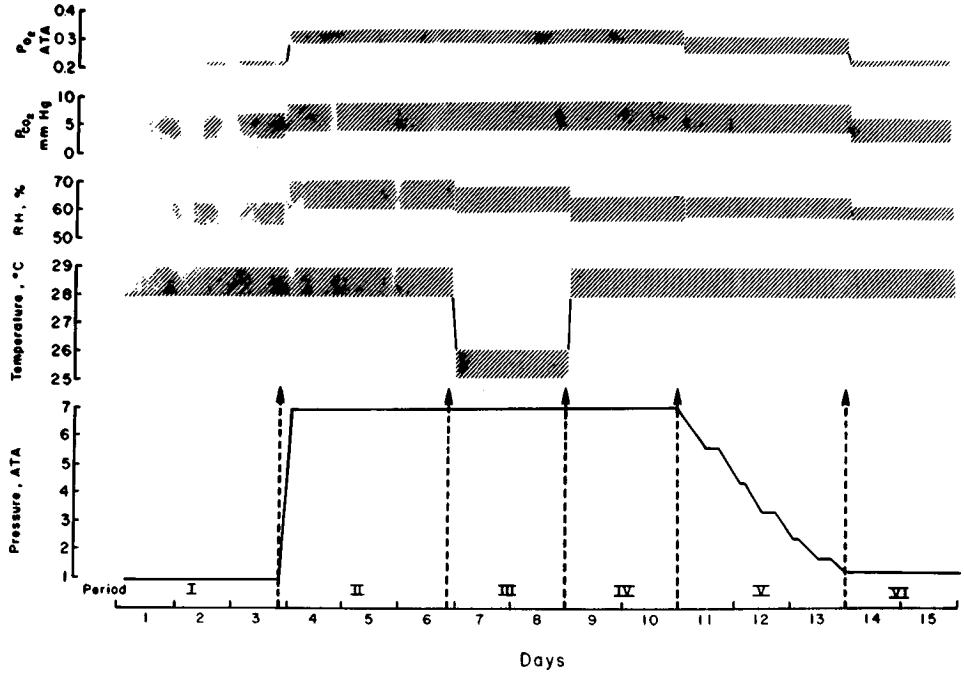

Fig. 1. Dive profile and environmental parameters. (From (5), with permission of the publisher.)

TABLE I

DAILY ACTIVITY SCHEDULE INSIDE THE CHAMBER

Hour	Activity
0600	Rise; empty urinary bladder; measurements of body weight, skinfold thickness, pulse rate, blood pressure, rectal and skin temperatures, and oxygen consumption; draw venous blood sample (10 ml)
0730	Breakfast
1030	Empty urinary bladder; measurements of pulse rate, blood pressure, rectal and skin temperatures and oxygen consumption
1200	Lunch
1500	Empty urinary bladder; repeat same measurements carried out at 1030
1630	Supper
1800	Submaximal exercise test (once in each period except *Period V*)
1930	Repeat same measurements carried out at 1030
2030	Snack
2200	Empty urinary bladder; retire

From Matsuda et al. (5), with permission of the publisher.

caloric, water, Na and K intake. Urine was collected daily to determine the excretion of solute, aldosterone and antidiuretic hormone (ADH).

Certain physiological parameters such as heart rate, blood pressure, body temperature, O_2 consumption and urine flow were measured 4 times a day, and the average for each period was used for statistical analysis. A far more detailed description of the methods appears in the articles by Matsuda et al. (5, 6).

Results and Discussion

Cardiovascular Functions

Average heart rate and blood pressure for the various dive periods are shown in Fig. 2. Upon compression to 7 ATA without changing the environmental temperature (*Period II*), the pulse rate decreased by approximately 20% and remained at this level even when the chamber temperature was lowered to 25 °C (*Period III*). This suggests that the chamber temperature does not play a significant role in inducing hyperbaric bradycardia. This is at variance with the report of Moore et al. (7) who observed an attenuation of bradycardia upon raising the chamber temperature from 27.8 to 29 °C at a 500-ft depth. It is also of interest to note that the heart rate during *Period IV* is significantly higher than that during *Period II* ($P < 0.05$). It is conceivable that had the subjects stayed at 7 ATA for longer than

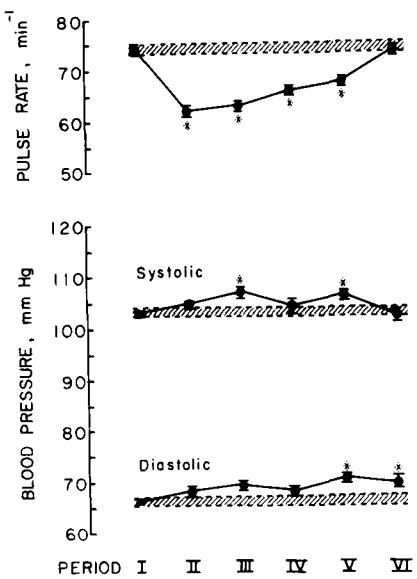

Fig. 2. Heart rate and blood pressures during various dive periods. In this and following figures, each point represents mean ± SE of measurements taken 4 times daily on 7 subjects during each period; shaded area indicates predive control values (mean ± SE) taken during *Period I*; * denotes significant difference ($P < 0.05$ on non-paired *t*-test) from corresponding control (*Period I*). (From (5), with permission of the publisher.)

7 days, the bradycardia might have disappeared. Although blood pressure showed significant changes during the dive, these changes were very small and therefore hard to interpret without knowing the stroke volume and peripheral blood flow.

Energy and Body Heat Balance

Average caloric intake was approximately 3,000 kcal per day throughout the entire dive. Neither body weight nor skinfold thickness showed any significant change throughout the dive.

Originally, the chamber temperature of 28 °C at 7 ATA was selected because it is close to a temperature which is comfortable, according to a prediction by Webb (14). However, as shown in Fig. 3, there were small but significant reductions in both rectal and skin tempera-

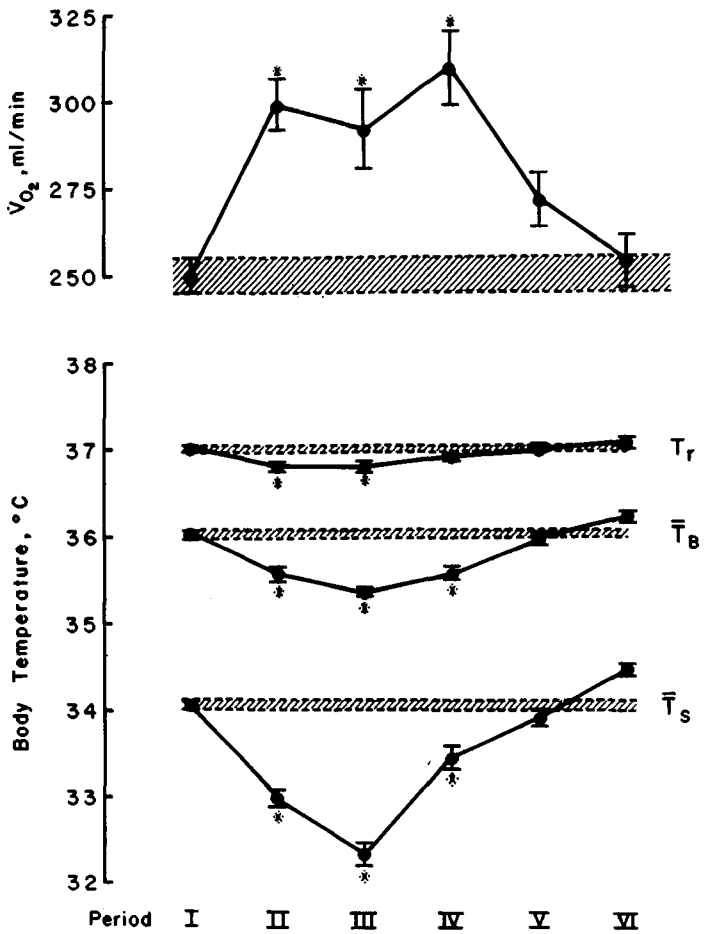

Fig. 3. Oxygen consumption and body temperatures during various dive periods. (From (6), with permission of the publisher.)

ture during *Period I*. During *Period III* when the chamber temperature was lowered to 25 °C, there was a further reduction in mean skin temperature while the rectal temperature remained unchanged. As compared to *Period II*, the rectal and mean skin temperature were significantly higher during *Period IV* ($P < 0.05$).

This negative heat balance at 7 ATA was associated with a significant increase in O_2 consumption at 7 ATA which was approximately 20% of the predive level. Interestingly, the O_2 consumption during *Period III* was not different from that during *Periods II* and *IV*.

Body Fluid Balance

Average urine flow of 3 subjects during various dive periods is shown in Fig. 4, with the corresponding urine osmolality and endogenous creatinine excretion. With compression, urine flow increased within a few hours and was maintained at a higher level while the daily fluid and salt intake remained unchanged throughout the hyperbaric periods. The magnitude of increase in urine flow over the predive level was approximately 300 ml/day during *Periods II* and *IV*, and 600 ml/day during *Period III*. This "hyperbaric diuresis" was accompanied by reductions in both urine osmolality and rate of endogenous creatinine excretion. Since the serum creatinine level remained fairly constant during the dive, the above results indicate that hyperbaric diuresis was present in the face of a reduced glomerular filtration rate. In other words, the inhibition of tubular reabsorption of water at depth appears to have been responsible for the observed diuresis.

The excretion of solutes per 100 mg creatinine excreted, shown in Fig. 5, indicates that the fractional excretion of filtered solutes, especially urea and Na, is also significantly increased. This means that there is also an inhibition of the tubular reabsorption of solutes at depth which in turn should inhibit the tubular reabsorption of water.

These findings indicate that the observed increase in urine flow at depth may have components of both water and osmotic diuresis. The fact that the magnitude of diuresis was greatest during *Period III* and that all subjects in the present dive were in a state of negative heat balance at depth (Fig. 3) strongly suggest that the hyperbaric diuresis may be associated with cold stress. A subtle cold stress could suppress both the ADH and reninaldosterone systems, thus inhibiting the tubular reabsorption of Na and water (2, 13). There is also a possibility that the so-called "third factors" could be activated in a hyperbaric environment. Attempts to demonstrate the inhibition of ADH and aldosterone during the dive, however, were not successful. The daily excretion of both hormones did not change significantly throughout the dive.

Although the degree of hyperbaric diuresis was rather modest, it is estimated that approximately 7,000 ml of extra water were lost during the 10 days of hyperbarism. As stated earlier, fluid intake remained fairly constant throughout the dive and daily caloric intake and dietary composition did not change significantly throughout the dive. Moreover, there was no clinical information to suggest alteration of fecal characteristics throughout the dive. These considerations lead to the conclusion that the subjects should have been in a state of negative water balance amounting to approximately 7,000 ml by the end of the 10-day stay in the hyperbaric environment.

It was therefore very puzzling to note that: (1) the subjects failed to drink more; (2) there was no reduction in the body weight; and (3) neither the serum osmolality nor the protein

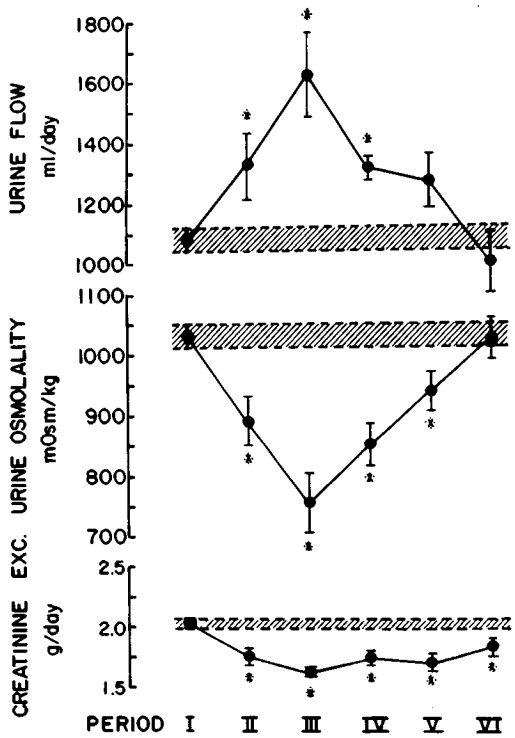

Fig. 4. Urine flow and osmolality and endogenous creatinine excretion during various dive periods. (From (6), with permission of the publisher.)

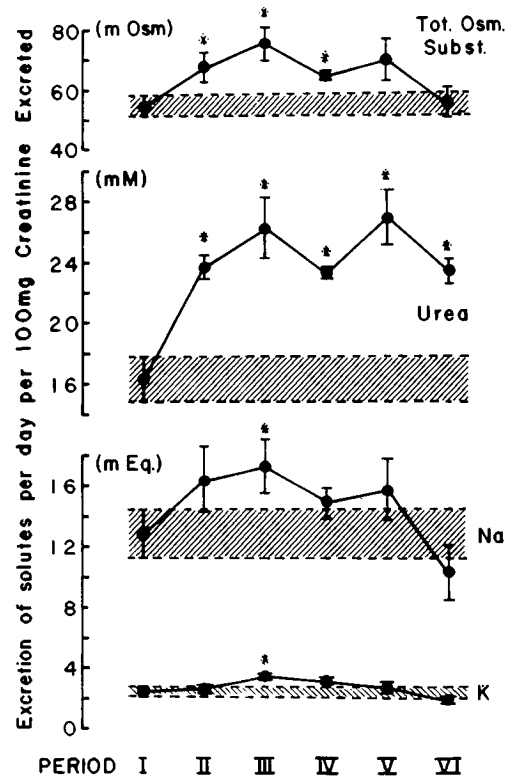

Fig. 5. Urinary excretion of total osmotic substances, urea, Na and K/100 mg creatinine excreted during various dive periods. (From (6), with permission of the publisher.)

concentration increased significantly. In other words, contrary to the predicted negative water balance, the subjects appeared to be maintaining normal water balance. This seemingly contradictory observation can be explained only if the avenues of body water exchange are altered in the hyperbaric environment. It is possible that insensible water loss decreases in the hyperbaric environment because of the significant reduction in skin temperature (Fig. 3) as well as in the binary diffusion constant of water vapor stipulated by the Chapman-Enskog theory (10).

Obviously, more systematic experiments are needed to substantiate these views, but it is significant to note that Raymond et al. (8) observed a 40% reduction in insensible water loss in a heliox saturation dive to 453 ft., which has also been confirmed in a recent saturation dive to 1,600 ft. (9).

It therefore appears that hyperbaric diuresis is primarily due to the retention of water resulting from a suppression of insensible water loss. Theoretically, such a retention of water should inhibit the ADH system but the inhibition may be too small to be detected.

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CALORIMETRIC ANALYSIS OF COLD EXPOSURE IN DIVING

Paul Webb

When a diver operates in cold water and becomes chilled, we usually judge how cold he is in one of several ways: estimating his ability to continue to perform; observing how hard he is shivering, or accepting his judgment of how cold he is. If we want to be more objective, we can take his rectal temperature. Unfortunately, however, considerable heat loss takes place before there is a major decrease in rectal temperature, and a serious decrement in performance may already have occurred. In our laboratory we wanted to investigate this important first stage of cooling which happens before serious hypothermia develops. We therefore exposed subjects to strong cooling, similar to that encountered by divers, while we measured heat loss by direct calorimetry, and body temperatures and metabolic response to shivering.

As a guide to what rate and amount of cooling to apply in the calorimeter, we first carried out a study of cold exposure on three divers clad in lightweight dry suits who swam underwater in a tank at water temperatures of 5, 10, and 15 °C until they reached a voluntary tolerance limit for cold. We then rewarmed them in our calorimeter and counted the calories needed to restore initial heat content, as previously reported (2).

On the average, the subjects had tolerated a loss of 210 kcal of body heat (as judged from rewarming), with a drop of 0.3 to 1.1 °C in rectal temperature, and a drop of 0.4 to 0.75 °C in esophageal temperature, including in both cases the familiar afterdrop of temperature in the early stage of rewarming. There was no clear correlation between change in these body temperatures and the quantity of heat lost as measured by the calorimeter, nor was correlation any better when we compared heat loss with change in mean skin temperature, subcutaneous temperature, or the mean body temperature calculated from weighted core and surface temperatures. All the body temperatures were lower at the end of the dives in colder water, but the exposure times had been shorter, and it appeared that heat loss at tolerance was the same at all three water temperatures. Put another way, the coldest water temperature caused the most rapid loss of body heat for the shortest time, and body temperature fell further when the cooling was fast, but the quantity of heat lost at tolerance was about the same at all cooling rates.

In the work reported here, the experiments were laboratory simulations of cold water exposure followed by rewarming. Both cooling and heating were done in the suit calorimeter. A cooling period lasted until the subject reached his tolerance for being cold. Since the whole procedure was carried out in the suit, we could directly measure calories lost during cooling and calories gained during rewarming. This report will relate heat loss and heat gain to changes in body temperature and metabolic rate.

Methods

The calorimeter is a garment assembly consisting of a water-cooled suit and heavy outer insulation which controls the subject's heat exchange. Water temperature and flow are regulated so that a man can be kept in the thermoneutral zone and his heat loss accurately measured from the heat appearing in the water stream (3). Alternatively, the water can be made cold to cool the man, or warm to heat him. The same flow and temperature measurements provide an accurate indication of heat lost or gained by the man. At the same time metabolic rate is continuously known from oxygen consumption, which we measure with our metabolic rate monitor (4), so that net heat loss or gain is known from the algebraic sum of metabolic free energy conversion (M) and heat transfer (ΣQ). When M and ΣQ are equal and there is no net heat loss or gain, the man is in heat balance. During cooling, ΣQ is strongly negative and greater than M, while during warming ΣQ becomes positive so that M and ΣQ add together.

During the cooling phase of these experiments, the rate of heat loss was typically about 6 kcal/min, and M initially was 1.5–2 kcal/min, so net heat loss was initially rapid. But as the subject became cold, he shivered more and more strongly, and M became 3, 4, or 5 kcal/min. Since the suit could not extract heat any faster, the net cooling rate fell from more than 3 kcal/min to 1 kcal/min, a rather slow rate, which resulted in cooling periods 1.5 to 2 times longer than they had been during the cold dives in the tank. This limitation of the suit could not be overcome without a complete redesign, so we had to be content with the results of these relatively slow cooling experiments.

Body temperatures continuously measured were: rectal (T_{re}), with a thermistor inserted 10 cm past the anal sphincter; ear canal temperature (T_{ac}), with a thermistor suspended from an insulating foam plug; and skin temperature at 16 sites with thermistors, which were combined electrically to provide a mean skin temperature ($\bar{T}_{sk}$).

The mean body temperature ($\bar{T}_b$) was calculated in two ways. Using calorimeter data, a change in mean body temperature ($\Delta\bar{T}_b$) was computed from

$$\Delta\bar{T}_b = \frac{\Delta H}{0.83\ m_b} \qquad (1)$$

where 0.83 is the specific heat of the body; m_b is body mass in kg; and ΔH is enthalpy change in kcal from net heat loss or net heat gain for a specific period of time. Using body temperature data, we also computed by the standard method

$$\Delta\bar{T}_b = 0.65\ \Delta T_{re} + 0.35\ \Delta\bar{T}_{sk} \qquad (2)$$

We preferred to use T_{re} for deep body temperature rather than T_{ac}, since it was evident that T_{ac} was influenced by the cooling or heating of the scalp by the water-cooled suit.

Three subjects were studied, the same three men who had done the earlier dive experiments; they had the physical characteristics shown in Table I.

The procedure was to have the subject begin an experiment in mid- to late-morning, with the first 1.5–2 hours spent sitting quietly in the calorimeter until a steady state of heat balance was established, i.e., $M = \Sigma Q$ at a normal resting level of metabolism. Then cooling

TABLE I

DESCRIPTION OF SUBJECTS

	A	*B*	*C*
Age, yr	23	41	50
Height, cm	173	183	173
Weight, kg	67	71	77
$VO_{2\,max}$, ml/kg-min	50	49	44
Skinfold thickness, mm			
Triceps	4	6	7
Upper chest	9	10	24
Abdomen	11	9	28
Calf	9	7	6

was begun by lowering the temperature of the water entering the suit (T_{wi}) from its comfort level of 27–28 °C down to 15 °C for about 20 minutes, then further lowering T_{wi} to 5 °C over another 20 minutes, and holding that level until the end of the cooling period. Water flow rate was 2.2–2.4 liter/min. When the subject called for a halt to cooling, which occurred on the average in 96 minutes (range 70–120 minutes), T_{wi} was raised quickly to between 40 and 43 °C to start the rewarming period. As body temperatures began to rise, T_{wi} was gradually lowered to avoid overheating, and after 45–50 minutes of rewarming, T_{wi} was back to its original level of 27–28 °C. Rewarming was complete when a steady state of heat balance reappeared (2).

Results

In eight experiments the average net heat loss at tolerance, or ΔH, was −217 kcal in the average time of 96 minutes. Shivering in bursts usually began 10–15 minutes after the start of cooling, and became more and more pronounced until it became continuous and quite distracting. After an hour or more of hard shivering, the subjects felt fatigue, and this contributed heavily to their eventual decision to terminate. *Subject A* usually terminated soonest and at a smaller net heat loss than the others, while *subject C* usually endured the greatest heat losses, as had been true in the cold water dives.

There was a clear relationship between net heat loss and metabolic (shivering) response, as shown in Fig. 1. Each data point represents a 10-min average level of M versus the accumulated ΔH for that time in the cooling period. From the regression line on the figure, it can be seen that M doubled, on the average, for a ΔH of −130 kcal, and tripled at ΔH of −260. If the maximum metabolic response is five times resting, as Iampietro et al. (1) have shown, this should occur at a ΔH of −520 kcal.

The nonlinear relationship between enthalpy change and rectal temperature during cooling is shown in Fig. 2. Notice that in the early stages of cooling T_{re} rose, remained unchanged, or decreased only slightly. Had the experimental procedure been different, such that at a ΔH of −100 kcal cooling had stopped and that level of heat loss been maintained, T_{re} would probably have decreased consistently in time. But since cooling was continuous, the slow-

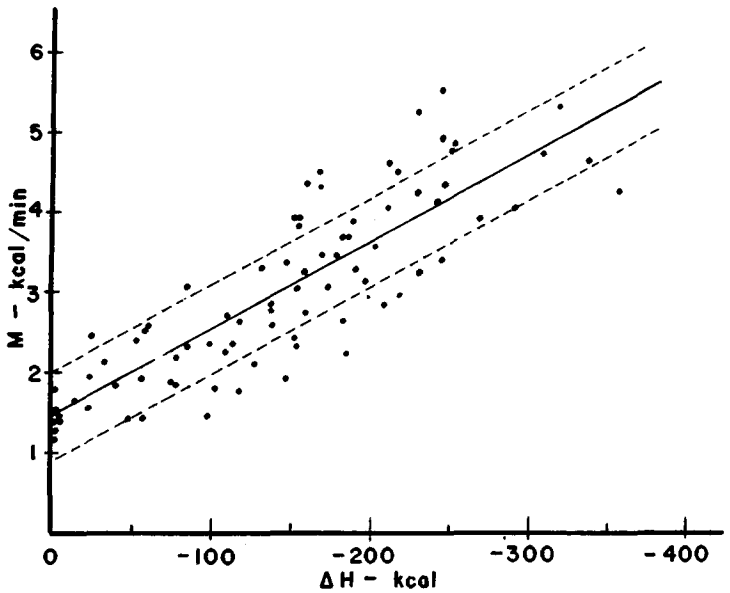

Fig. 1. Relationship between enthalpy change (ΔH) (net cooling accumulated for various periods) and metabolic free energy conversion rate (M). Each dot represents one 10-min value for M, calculated from oxygen consumption for the period and level of ΔH reached during same period. From 74 data points, correlation between M and ΔH was 0.85. Linear regression is shown by solid line, plus or minus its SE (dashed lines). Regression equation is $y = 1.349 - 0.011x$.

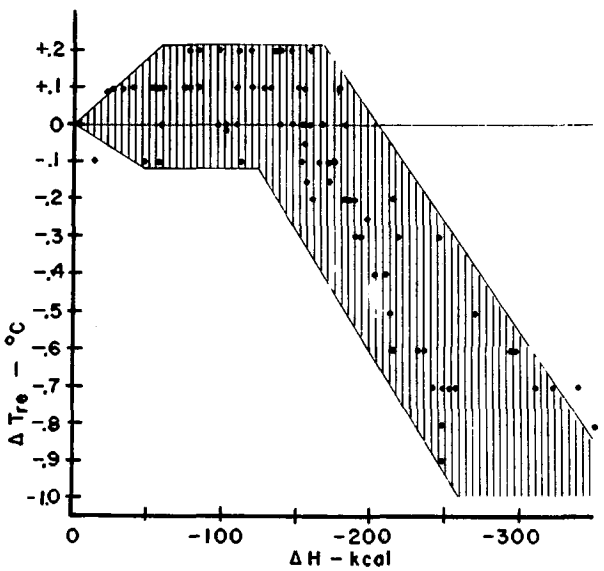

Fig. 2. Relationship between change in rectal temperature (ΔT_{re}) and enthalpy change during continuous cooling.

reacting temperature in the rectum always lagged behind temperature changes in superficial tissues.

Figure 3 shows how the mean body temperature changed during cooling. The dashed line shows true $\bar{T}_b$ computed from ΔH (*Eq. 1*) as a function of ΔH. The solid line shows $\Delta\bar{T}_b$ calculated from ΔT_{re} and $\Delta\bar{T}_{sk}$ (*Eq. 2*), which shows a linear relationship between $\Delta\bar{T}_b$ and ΔH after cooling has produced a deficit of more than 100 kcal. At least part of the discrepancy between the true $\bar{T}_b$ from calorimetry and that calculated from body temperatures is caused by the same phase lag in ΔT_{re} referred to in the preceding paragraph. Whether the weighting factors of *Eq. 2* are correct, whether they change with body heat content, or indeed whether we can hope to know $\bar{T}_b$ from only surface and core temperatures, has not yet been established.

The relationship between ΔH and change in body temperature during rewarming of an already cold subject is shown in Fig. 4. The data reflect the well-known phenomenon of afterdrop, apparently caused by the slowness of T_{re} to respond to changes of temperature on the surface. Thus while the subject is gaining heat from the suit and from his own heat production, T_{re} continues to fall at first as if cooling were continuing.

Figure 5 shows how the mean body temperature varies with ΔH during rewarming. Again, the true values of $\Delta\bar{T}_b$ from ΔH by calorimetry are shown as a dashed line, and those from body temperatures are shown as a solid line.

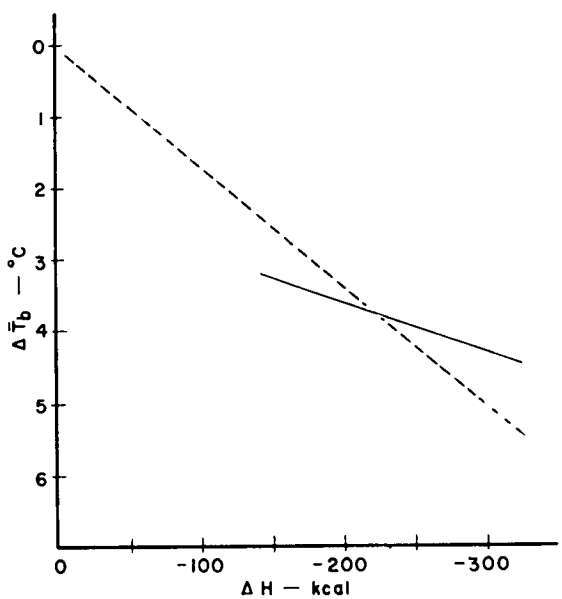

Fig. 3. Change in mean body temperature ($\Delta\bar{T}_b$) with loss of body heat during cooling. Dashed line is true change in $\bar{T}_b$ calculated from ΔH, body weight, and specific heat; solid line is estimate of $\Delta\bar{T}_b$ from body temperatures. Regression equation for solid line is $y = 2.244 - 0.007x$; correlation of x and y values is 0.57.

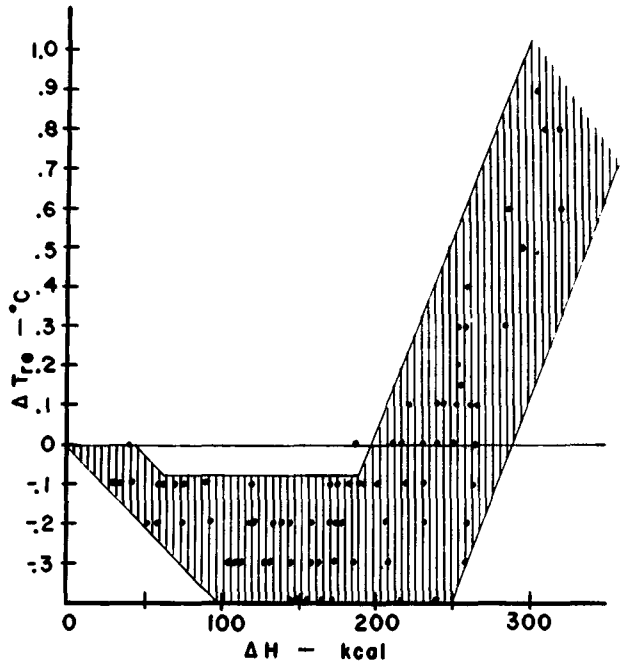

Fig. 4. Change in rectal temperature (ΔT_{re}) during rewarming of an already cold subject.

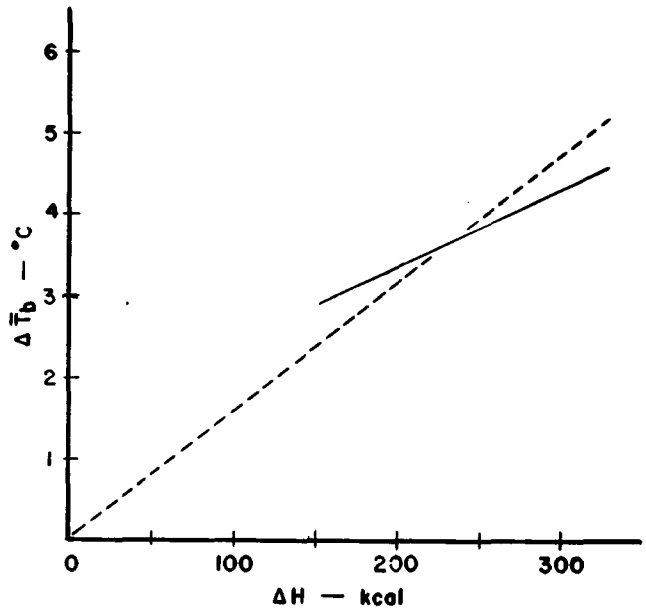

Fig. 5. True change in mean body temperature ($\Delta \bar{T}_b$) is shown by dashed line as heat is restored to an already cold man, while that estimated from body temperatures is shown by solid line. Regression equation for the solid line is $y = 1.489 + 0.009x$; correlation of x and y values is 0.82.

Discussion

It is not surprising that these experiments show serious discrepancies between quantities of heat lost during cooling and changes in both rectal temperature and mean body temperature as it is usually calculated. There was no chance for a steady state to develop during cooling to voluntary tolerance, so there was no time for the slow-moving rectal temperature to catch up during the cooling transient. Yet the cooling rates we used were somewhat slower than those that occur often in diving, which suggests that T_{re} during or after a dive is not a useful indicator of how much heat was lost. We would like to be able to relate enthalpy change to body temperature change, but this relationship is evidently strongly influenced by the cooling rate. Evidently the faster the cooling the greater the separation between ΔH and T_{re}.

It was interesting that the mean value for enthalpy change at the voluntary tolerance limit, -217 kcal, was rather close to that defined in the earlier cold water dives, -210 kcal, which had somewhat faster cooling. There are even faster cooling rates in men who are accidentally exposed to cold water, and perhaps in certain diving situations, and there are certainly slower cooling rates than those we used. These different cooling rates should be explored to see if some method could be found for estimating ΔH from T_{re} by adjusting for speed of cooling (time to voluntary tolerance).

The regular relationship which exists between metabolic response and enthalpy change shown in Fig. 1 suggests the possibility that there is a physiological control aimed at preserving body heat content rather than a thermostatic or setpoint control for maintaining body temperature. The physiologists' argument over the dominance of central versus peripheral temperature signals in thermal control may be resolved if metabolic responses are compared to levels of heat loss rather than to temperature change.

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PHYSIOLOGICAL RESPONSES OF MEN WORKING IN 25.5°C WATER, BREATHING AIR OR HELIUM TRI-MIX

P. F. Hoar, L. W. Raymond, H. C. Langworthy, R. E. Johnsonbaugh and J. Sode

Abstract*

Fourteen scuba divers in swim trunks did ergometer work while breathing air at 3 m in 25.5 °C water. They were stressed by work and cold. Exercise produced increases in heart rate, minute ventilation (V_E), oxygen consumption (V_{O2}), and catecholamine excretion. Cold lowered rectal temperature (T_{re}) despite exercise, and contributed to the increase in V_{O2} and catecholamine excretion. Immersion, cutaneous vasoconstriction, work, and scuba breathing contributed to a brisk diuresis, probably by centralizing blood volume and thus stimulating central vascular volume receptors. Similar exercise in 25.5 °C water, breathing helium tri-mix (gas density less than air), produced higher V_E but lower V_{O2} when compared to air breathing. Tri-mix scuba breathing resulted in a smaller diuresis, perhaps because its lower density leads to lesser atrial distension during work. The fall in T_{re} during work in 25.5 °C water was identical whether air or helium tri-mix was respired, since helium does not accentuate respiratory convective heat transfer.

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COMPARISONS OF METABOLIC, THERMAL, AND CARDIOVASCULAR RESPONSES OF ACCLIMATED AND UNACCLIMATED NAVY DIVERS

B. Clingman and E. Evonuk

The original intent of our study was to investigate energy costs in Navy divers submerged in 0 °C water at two and three atmospheres absolute. We used the hyperbaric facilities at the diving locker at the Naval Torpedo Station, Keyport, Washington.

To simulate test conditions we fabricated a wet pot by placing a metal water tank inside the chamber, and at the beginning of each day's run this immersion tank was filled with water and 200 lbs of crushed ice. To prevent thermal-layering, a perforated air line extended to its full length was attached to the bottom of the tub, and compressed air was continually bubbled through this line into the ice water. Each subject was wired with EKG electrodes, six thermistors for skin temperature, and one rectal thermistor for core heat data.

Oxygen consumption and respiratory minute volume were measured by collecting expired air in Douglas bags via a four-way valve attached to the hoses and mouthpiece taken from a U.S. Dive Aquamaster regulator.

After each subject was dressed in a neoprene exposure suit (¼ in. thick) and the subjects' base measurements returned to normal and were recorded, the subjects were immersed in the water bath and pressurized to the required depth.

The first two runs went as expected; the O_2 uptake was relatively high, as was the respiratory minute volume, and the classic bradycardia diver reflex was noted.

While testing the third subject, however, a significant increase in heart rate occurred on bottoming out at the required depth. Later examination of the subject's background indicated he had recently transferred to Keyport from New London and had not had a previous opportunity to dive in the cold waters of Puget Sound.

Could this apparent bradycardial override have been caused by lack of cold acclimation? To test this premise we flew three additional Navy divers in from San Francisco to participate in a revamped study designed to compare cold and warm water divers.

We considered four of the subjects acclimated to cold water diving, while the remaining four were picked because they were not acclimated. The criterion for determining cold acclimation was based on diving activities of the subjects. If a subject had been diving in waters of 10 °C or colder for an average of three times a week for six months or longer, he was considered acclimated. Subjects who were accustomed to diving in waters warmer than 22 °C, or who averaged fewer than two dives a month in waters colder than 22 °C for at least six months were considered unacclimated. Each of the subjects was a graduate of the Navy Deep Sea Diving School, and the Base Medical Officer prounounced all subjects in excellent physical

and mental health. All subjects had extensive training and experience in the use of SCUBA equipment as well as conventional heavy gear.

Results

Oxygen Consumption

Oxygen consumption at 2 ATA for both the acclimated and unacclimated subjects is presented in Fig. 1*A*. When compared to the surface reading, the reading at the end of the dive showed a 190 ml/min increase in oxygen consumption for the acclimated group, while the unacclimated group recorded a 672 ml/min increase, a difference of 482 ml/min. The acclimated subjects increased their O_2 consumption by 187 ml/min. The unacclimated subjects, from start to finish, showed an increase of 848 ml/min, a difference of 661 ml/min (Fig. 1*B*). The unacclimated divers had a 26.2 percent greater oxygen consumption at 3 ATA than they had at 2 ATA, while there was no appreciable increase in O_2 uptake for the acclimated subjects for the same increase in pressure (Fig. 1*B*).

Respiratory Minute Volume

The mean and standard error of the mean for the respiratory minute volume at 2 ATA for both the acclimated and unacclimated subjects are shown in Fig. 2*A*. Comparing surface data to that at the end of the dive, the mean respiratory minute volume for the acclimated group increased 126.0%, while the unacclimated subjects had an increase of 232.5%.

Measuring the subjects at 3 ATA (Fig. 2*B*) showed that the acclimated group's respiratory minute volume increased by 222% while the unacclimated subjects had a 3.3-fold increase in their RMV.

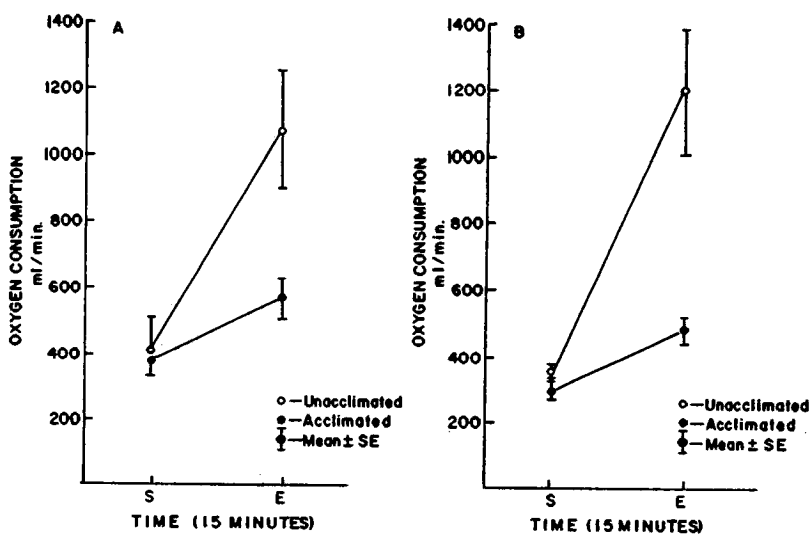

Fig. 1. Oxygen consumption at 2 ATA (*A*) and 3 ATA (*B*); values are means, ± SE. On *abscissa*, S represents control values prior to submersion and pressurization; E represents value at end of 15-min dive. On *ordinate*, O_2 consumption is plotted in ml/min.

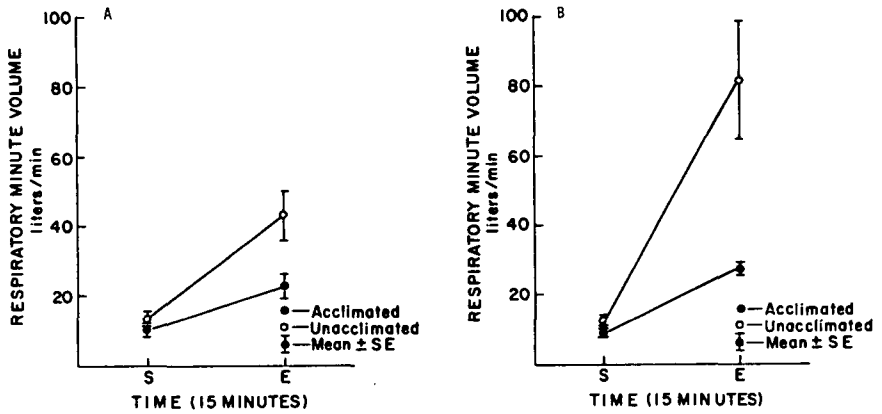

Fig. 2. Respiratory minute volume at 2 ATA (*A*) and 3 ATA (*B*); values are means ± SE. On *abscissa,* control values; on *ordinate,* respiratory minute volume in liters/min.

Heat Debt

The mean heat debt for both groups of subjects is presented in Fig. 3. At 2 ATA, there was no appreciable difference in heat debt between the two groups. At 3 ATA, however, the unacclimated subjects had a 9.6 kcal/m² greater mean heat debt than the acclimated group.

During the course of the dive, the acclimated subjects appeared to have a greater tolerance to thermal stress, whereas the unacclimated subjects shivered violently 3-5 minutes after submersion.

Respiration Rate

Figure 4 represents the mean respiratory rate for both groups at 2 and 3 ATA, respectively. No apparent difference in the respiration rate was found in either group at either depth.

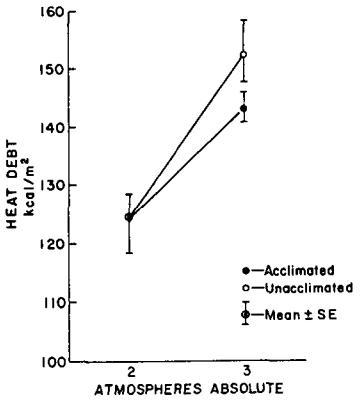

Fig. 3. Mean heat debt for both acclimated and unacclimated groups. On *ordinate,* heat debt is plotted in kcal/m².

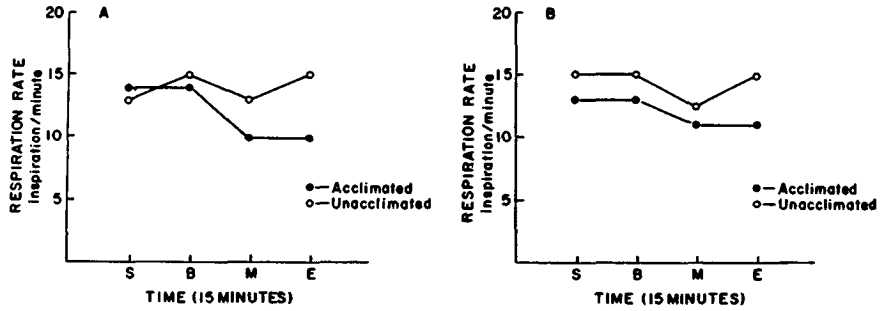

Fig. 4. Mean respiratory rate for both groups at 2 ATA (*A*) and 3 ATA (*B*).

Heart Rate

The mean and standard error of the mean for the heart rate of both the acclimated and unacclimated groups at 2 ATA are presented in Fig. 5*A*.

For the acclimated group at 2 ATA, the heart rate decreased as expected from a control value of 94 beats per minute (bpm) to 79 and 75 bpm at the bottom and midpoint of the dive, respectively. From the midpoint to the end of the dive, the heart rate increased to 84 bpm. However, for the unacclimated subjects, the heart rate unexpectedly increased to 108 bpm at the bottom, then slowed to 93 bpm at the midpoint of the dive; from the midpoint to the end of the dive, the heart rate increased to 104 bpm.

A similar override was noted at 3 ATA, as indicated in Fig. 5*B*. The acclimated subjects exhibited the typical diving bradycardia reflex, while the unacclimated group again recorded an increase in heart rate from a control value of 94 beats to 112 bpm, after reaching the bottom of the simulated dive. From the bottom to the midpoint of the dive the heart rate decreased to 82 bpm, and at the end of the simulated dive the heart rate increased to 97 bpm.

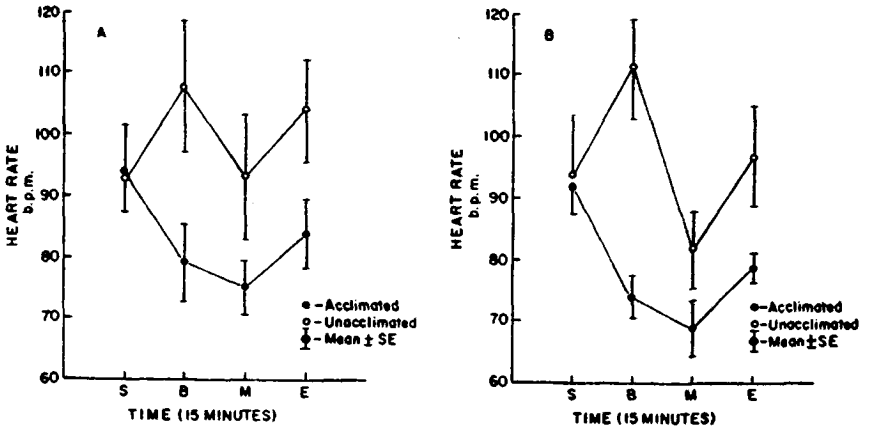

Fig. 5. Heart rate for both groups at 2 ATA (*A*) and 3 ATA (*B*); values are means ± SE. On *abscissa*, S represents control values prior to submersion and pressurization. On *ordinate*, heart rate in bpm. At point B, subject is completely submerged and under pressure; M is midpoint of dive; E is end of dive.

Discussion

It is evident that there were distinct variations in metabolic, thermal and cardiovascular parameters between cold-water-acclimated and unacclimated divers. It is also possible to delineate the effects of pressure per se when its effects are superimposed on thermal stress.

The heat debt at 2 ATA for both groups was essentially the same, while at 3 ATA the heat debt for the unacclimated subjects was 9.6 kcal/m^2 greater than the acclimated group. This small difference is attributed to the much greater increase in heat production by the unacclimated subjects. This was reflected by the two- to threefold increase in O_2 consumption seen in the unacclimated group compared to the acclimated groups at 2 and 3 ATA, respectively. The unacclimated subjects increased their heat production, for the most part, by moderate to violent shivering, which began from 3-5 minutes after submersion and pressurization. The slight-to-moderate increase in oxygen consumption for the acclimated subjects can be attributed to undetectable shivering, or primarily to nonshivering thermogenesis.

The most marked variation between the unacclimated and acclimated subjects was the difference in heart rate response after submersion and pressurization. At both pressures, the mean resting predive heart rate for the two groups was essentially the same. However, the first minute after submersion and pressurization, the heart rate of the acclimated group decreased 20.1% while the unacclimated group mean heart rate increased 14.8%. This decrease of heart rate in the acclimated group demonstrated a typical diving bradycardia reflex, and the pattern was the same at both pressures. For the unacclimated group, it is apparent that the initial response to thermal stress had an overriding effect on the diving bradycardia reflex. At the end of the dive, the heart rate for both groups and at both pressures increased approximately 10 to 15% from the midpoint value. This increase was apparently due to the response to thermal respiratory minute volume.

The delineation of the effects of pressure when superimposed on cold stress is apparent from our findings. This was demonstrated by an increase in all the physiological variables measured, with the exception of respiratory rate, with an increase of pressure from 2 to 3 ATA. The increase in heat debt, heart rate, oxygen consumption and respiratory minute volume at 3 ATA was apparently caused by the squeeze effect of the diving suit which reduced the insulation and thermal protection.

From this study it is apparent that the combined effects of cold and pressure severely limit the efficiency and bottom time of divers, especially with increasing pressures. This would be particularly true for the unacclimated diver. The increase in oxygen consumption and the considerable increase in respiratory minute volume would effectively limit bottom time. The early thermogenic response to cold and pressure, increased intensity of metabolic function and initial tachycardia displayed by unacclimated subjects exposed to cold and pressure could be used as a diagnostic tool to evaluate the degree of acclimation.

STEADY-STATE MODEL OF LOCKOUT SUBMERSIBLE AND CREW THERMAL REQUIREMENTS

L. A. Kuehn, T. J. Smith and D. G. Bell

At present, the deployment of lockout submersibles in deep diving operations is severely limited by several problems, one of which is crew member heat loss. This problem is particularly acute for lockout divers and those individuals under compressed gas conditions. This paper describes a biophysical model, Cold Diver, for calculating the steady-state thermal comfort requirements of crew members in various operations.

A lockout submersible consists of two spherical pressure chambers, a forward and an aft sphere, which may be connected by a small tunnel. Such vehicles are used to transport men and instruments at one atmosphere internal gas pressure and/or to maintain and support divers at an underwater work site. Such vehicles may be used in nonlockout or submersible operations to depths beyond 600 meters, and in lockout operations to depths of 300 meters.

The control and life support facilities of the submersible are usually located in the forward sphere where the crew complement consists of a pilot, co-pilot and observer, all in air at one atmosphere pressure. Two modes of operation are possible in the aft sphere. It can be used in the submersible mode to carry additional observers or equipment at one atmosphere of air, or it can be used in the lockout mode to carry two lockout divers and a tender in a compressed gas environment. During lockout operations, a lower hatch permits exit of the divers into the water at the same hydrostatic pressure as is present in the aft sphere, which leaves the tender to monitor the divers' progress and ensure their safety.

When aided by a diving-support ship, lockout operations have the following features. The divers and tender are compressed in the ship-borne deck decompression chamber (DDC) to the hydrostatic pressure of the intended work site. They then enter the aft sphere under pressure via a mating tunnel. The submersible is launched and the divers are transported to the work site, where they leave the aft sphere. After a predetermined time or completion of the work, the divers return to the lockout sphere and are transported to the DDC for decompression or rest. All crew members may thus be considered to be at approximately constant pressures throughout any operation.

There are three thermal environments associated with a lockout submersible, as shown in Fig. 1. These are:

(1) Forward sphere: the individuals in this chamber lose heat by conduction to the inner surface of the sphere, respiration of cold air, radiation to the enclosing sphere, and convection to circulating air. Crew members usually work at sedentary rates and wear work overalls in the summer and insulated suits in the winter.

Fig. 1. Thermal environments associated with lockout submersibles, showing major heat transfer avenues for each environment.

(2) Aft sphere: when in compressed gas atmospheres such as oxygen-helium, the occupants of the aft sphere experience conduction and radiation heat losses similar to those within the forward sphere. Respiratory and convection heat losses are increased because of the increased density and higher thermal conductivity of the compressed gas medium. The divers and tender all wear diving clothing during transport to and from the work site.

(3) Lockout phase: during immersion in cold water, lockout divers experience great respiratory and convective heat losses. Radiative heat loss is minimal and heat loss via conduction is included as part of the convective heat loss.

For the purpose of Cold Diver calculations, the specifications and characteristics of the Canadian Forces Submersible Diver Lockout Vehicle SDL-1 were used. The overall length of this submersible is 6.1 meters and the pressure chambers are made from high-tensile-strength steel; the forward sphere is 2.1 meters in diameter and the aft sphere 1.7 meters in diameter. Ten circular viewports, each 12.7 cm in outside diameter, permit visibility from the forward sphere. The existing onboard power supply consists of three lead acid batteries of 120 volts, 28 volts and 12 volts for which the cell capacity is rated at 300 ampere-hours, based on a 6-hour discharge.

The Cold Diver Model

The Cold Diver model is a steady-state digital computer model of the thermal losses of a man in any of the thermal environments associated with a lockout submersible. It determines the energy required to keep an individual at a thermally comfortable body core temperature of 37 °C. If this thermal demand is not met, the individual loses heat gradually, which results in lower body core temperatures. The rate of decline of core temperature depends on the severity of the thermal demand. The model does not take into consideration any transient heat loss that must occur during initial deployment of the submersible in cold water.

The scope of the model's applicability includes water temperatures of −2 to 37 °C and

depths from 0 to 300 meters. Thermal losses for each environment were separated into types: natural convection; forced convection; radiation; and respiration.

Theory of Diver's Heat Loss

By using a simple energy balance equation, the heat loss from a diver's body may be examined in detail. In the lockout mode, a diver's body is entirely surrounded by water, and the breathing gas mixture which is usually at ambient water temperature contains no water vapor. In the submersible mode or in the aft sphere before or after a lockout operation, the diver is surrounded by a gaseous mixture except for that part of his body in direct contact with the inner surface of the sphere. Usually the gaseous mixture is at ambient water temperature and has a relative humidity level of 100% (caused by cooling of the sphere and enclosed gas to temperatures below the dew point of the temperate air in the submersible at the surface).

The energy balance equation that is pertinent to the gas mode is

$$M - W - S = R + C + V \tag{1}$$

where M = metabolic heat production, W = work output, S = heat storage or depletion in the diver, R = radiative heat loss, C = convective heat loss, and V = respiratory heat loss. For the water mode the radiation term can be considered negligible and the pertinent equation is

$$M - W - S = C + V \tag{2}$$

The function of the Cold Diver model is to calculate the right hand side of *Eqs. 1* and *2*. Metabolic heat generation and work output are known and can be measured for given conditions. If a diver's thermal equilibrium is defined as a heat storage change of zero for a body core temperature of 37 °C, the amount of heat required to sustain this condition can be calculated, allowing the supplementary heat required to be calculated.

Radiative Heat Loss

Assuming that the exterior surface of each individual crew member is at an average temperature, $\bar{T}_c$, and is radiating energy to the surroundings which are assumed to possess a mean radiation temperature, T_{mrt}, equivalent to the ambient water temperature, the net radiation heat loss, R, for each individual is given by the equation

$$R = \frac{\sigma \varepsilon_i A_r (\bar{T}_c^4 - T_{mrt}^4)}{(A_r/A_e)[(1/\varepsilon_2) - 1]} \tag{3}$$

where σ = Stefan-Boltzman constant, A_r = radiative area of the body, assumed to be 80% of the total body area to account for interbody radiation exchange, A_e = surface area of enclosure, ε_i = emissivity of surface clothing of crew member, taken as 0.95 for maximal estimation, and ε_2 = emissivity of inner surface of sphere.

The Cold Diver model employs values of $\bar{T}_c$ calculated from the total heat flux through the individual's clothing and the convective heat losses from the surface of the clothing. Body area is calculated from the Dubois formula (13) and corrections for body posture were made to A_r using the data of Guibert et al. (15).

Convective Heat Loss

Natural or forced convection or a combination of both occurs in the submersible modes of operation. Each of these is calculated in the Cold Diver program with the application of McAdams' Rule (21), i.e., that the greater of the two calculations is accepted as the total convective heat loss.

Both natural and forced convective heat losses to the fluid surrounding an individual's body are calculated from the formula

$$C = U A (\bar{T}_s - T_f) \tag{4}$$

where C = convective heat loss, A = total body area, $\bar{T}_s$ = mean skin temperature, T_f = ambient fluid temperature, and U = universal heat transfer coefficient.

The individual is represented in these calculations by a cylinder 1.85 m long and 0.305 m in diameter with a uniform temperature of $\bar{T}_s$ and an area of 1.8 m^2, as suggested by Tauber et al. (32). In these calculations, $\bar{T}_s$ is assumed to be constant at 33 °C.

The coefficient, U, obeys the following relationship (26)

$$U = \frac{1}{1/h_c + x/k + 1/C_i} \tag{5}$$

where h_c = suit-water boundary layer heat transfer coefficient, x = thickness of diving suit material, k = thermal conductivity of diving suit material, and $1/C_i$ = thermal resistance of skin-suit interface, assumed to be 20% of the suit thermal resistance x/k, as suggested by Nevins et al. (26).

In all Cold Diver calculations of convective heat loss, the thickness of foam-formed diver clothing was assumed to have a pressure-dependent function for thermal insulation, as documented by Butler and Payne (11). In the calculation of natural and forced convection heat loss, the equation defining h_c in the gas mode was that used by Tauber et al. (32), while that for h_c in the water mode was that used by Witherspoon et al. (36). Because the mean temperature of the surface of the clothing was a factor in these formulas, the value of this temperature was obtained by iteration, matching the heat flux through the suit to that across the boundary layer.

Respiratory Heat Loss

The calculations in Cold Diver that pertain to respiratory heat loss account for the energy required to warm inhaled gas and the addition of water vapor from the respiratory system to this gas. The inhaled gas is assumed to be at a temperature equal to that of the water. When the diver is in the water mode, the breathing gas is further assumed to be completely dry; in the gas mode in either sphere, it is assumed to be water-saturated. The exhalation temperature, T_e, of the diver is assumed to be that documented by Goodman et al. (14)

$$T_e = 22 + 0.649\, T_i \tag{6}$$

where T_i = the gas inhalation temperature.

In the forward and aft spheres during nonlockout operations, the gas mixture is always assumed to be air. During lockout operations and in the water mode, the gas mixture in the aft sphere is always assumed to be oxygen-helium with an oxygen partial pressure of 0.3 ATA,

regardless of the pressure.

Calculations were performed for a range of respiratory minute volumes from 5 to 50 liters/min, according to the equation

$$V = R_{mv}\rho\, C_p\,(T_e - T_i) + R_{mv}\rho\, h_{fg}\,(W_e - W_i) \qquad (7)$$

where R_{mv} = respiratory minute volume, ρ = density of breathing gas, C_p = specific heat of breathing gas, W_e = humidity ratio of gas at temperature T_e, W_i = humidity ratio of gas at temperature T_i, and h_{fg} = latent heat of vaporization of water.

Results

Figure 2 shows the calculated heat requirement per individual for various steady-state conditions of pressure and ambient temperature. Four pairs of heat loss curves are shown, for ambient temperatures 0°, 10°, 20° and 30°C. One line of each pair represents heat requirement in water while breathing oxygen-helium, and the other line the heat requirement in an oxygen-helium mixture in the sphere, both at ambient pressures and temperatures. It is assumed that each individual has a sedentary rate of respiration equivalent to a volume rate of gas exchange of 20 liters/min.

For each ambient temperature, the heat requirement during water immersion is approximately twice that in the gas mode at the same pressure for depths in excess of 10 atmospheres of pressure. This difference becomes smaller at shallower depths, the two modes being approximately equal in thermal stress at the surface. If lines are drawn on Fig. 2 parallel to the x-axis at 100 watts, the metabolic heat equivalent to basal metabolism, and at 300 watts, a

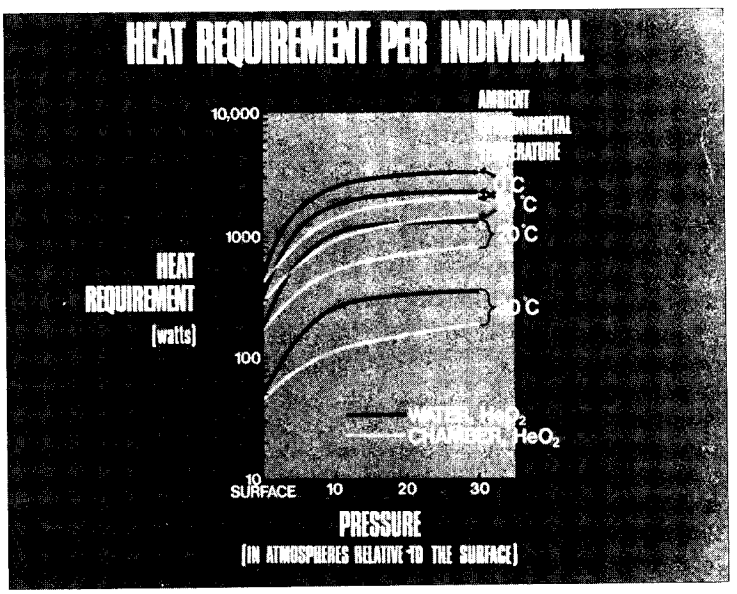

Fig. 2. Heat loss per individual for various steady-state conditions of pressure and ambient temperature; individual assumed to be wearing 'dry' diving suit with 2 layers of nylon pile underwear and a diving helmet, metabolic rate considered to be sedentary, with respiratory minute volume of 20l.

moderate work rate, it can be seen that neither activity level can maintain thermal equilibrium for all pressure-temperature environments of interest, particularly those in the ambient temperature range of 0–10 °C. For thermal comfort of the crew in these thermal environments, it is necessary to supply heat actively to the individual members in the water in compressed gas modes.

The curves displayed in Fig. 2 are in agreement with values of diver heat loss reported in the literature. Many of the values pertinent to shallow depths (4, 5, 23, 31) concern divers wearing wet suits; however, the Cold Diver results which pertain solely to dry suits are significantly lower. At greater depths (in excess of 100 m), the Cold Diver results are in excellent agreement with literature values (8, 20, 28). These predict that at a depth of 300 m in water of 0 °C, approximately 3000 watts would be required for individual thermal comfort.

A substantial testing program was carried out in laboratory and field conditions to verify the Cold Diver model. In the laboratory testing, thermal exchanges of divers in water of −1.2, 4.4 and 10 °C temperatures and simulated depths of 0, 10, 30.5, 61, and 91 m of seawater were measured in human hyperbaric chambers. As reported by Bell (6), various dry diving suits as well as different working conditions were studied. Field measurements of the rate of heat loss of operational divers were also made at Halifax, Nova Scotia, to depths of 43 m, and at Resolute, Cornwallis Island, in the Canadian Arctic, to depths of 76 m.

In these experiments, two different techniques for measuring thermal exchange were applied. One was that of classical indirect partitional calorimetry developed by Burton (10) for men in an air environment and used effectively with divers by such workers as Milan (22), Reins and Shampine (29) and Boutelier et al. (9). This method involves measurement of body core and mean skin temperatures to assess the rate of change of body heat storage. This technique has been criticized by Craig (12) and Webb (34) because there is no evidence that the equations and weighting coefficients which pertain to air can be applied to water experiments.

The proper technique for use in direct measurement of heat loss from immersed human subjects is that of direct calorimetry, which has been successfully applied by few workers, most notably Craig (12) and Webb et al. (35). This technique, while accurate, is difficult to perform in laboratory experiments and field or operational environments.

The second technique used to verify Cold Diver results was that of heat flow discs (16, 27), which are small thermopiles that measure heat loss directly. Physiologists have recently applied such techniques in cold water studies with some success (6, 17, 19, 25). A suitable sampling scheme for measuring heat flow from the body is the use of the same subdivision of body area as is used for skin temperature measurement. Application of the two techniques has led to verification of the Cold Diver model to depths as great as 91 m and water temperatures as cold as −1.2 °C. Such validation has been extensively reported elsewhere (6) but Table I shows a summary of the results.

Predicting Thermal Stress for Lockout Submersible Operations

A typical nonlockout operation for a lockout submersible would consist of a 30-min predive systems check, a launch and tow action of 1-hour duration, a descent to the work site lasting 30 minutes, a period of observation and/or work lasting 5 hours, an ascent time of 30 minutes, and a tow and recovery operation of 1 hour. Deployment of the same submersible

TABLE I

MEASUREMENTS OF DIVER HEAT LOSS IN WATTS

Temperature, °C	0 ATA			1 ATA			3 ATA		10 ATA	
	RV	S	WV	RV	S	WV	RV	WV	RV	WV
10	129	37	37	163	136	159	184	165	—	—
4.4	140	88	71	226	141	208	274	210	—	—
−1.2	225	—	155	—		241	329	275	475	486

Data are averages obtained from 2-7 subjects. RV = resting vertical position; S = swimming horizontally; WV = working vertical position.

on a typical lockout mission would be virtually identical, except that the 5-hour observation/work period would be replaced by a 2-hour lockout period.

Figures 3 and 4 show the heat required for the submersible crew for various pressure/temperature conditions for both types of operation. On each figure are several lines indicating the capability of the crew members and the submersible itself (using the Canadian Forces SDL-1 as an example) to supply this thermal demand. In the case of the nonlockout operation, the resting metabolic rates of the crew are insufficient to sustain thermal comfort in water at 0–10 °C temperatures. However, the thermal demand on the crew is not excessive, and the process of hypothermia will take much longer than the time of the mission. The submersible energy supply can meet this thermal demand if there is a method of heating either the crew or the submersible. In the case of lockout operations, neither metabolic rates nor the submersible power source itself is sufficient to maintain thermal comfort in cold water (0–10 °C) except at very shallow depths.

Figure 5 shows the thermal energy required to maintain the crew's thermal comfort in a survival situation, without rescue in less than 72 hours. On this figure there are three horizontal lines indicating the capability of the crew's resting metabolism, the submersible power source (unaffected by cold) and the combination of these two energy sources to meet this thermal demand. It is obvious that in the 72-hour exposure in water from 0–10 °C, all crew members would suffer hypothermia. At shallower depths it is likely that all crew members, particularly those in the forward sphere, would be alive but suffering from advanced hypothermia after 72 hours. For greater depths (more than 50 m) it is not likely that any crew members in the pressurized aft sphere would survive.

Discussion

On the basis of Cold Diver calculations, operating unheated lockout submersibles with lead acid power sources in cold water is limited at great depths and for long missions by the thermal drain on crew members. To ensure thermal comfort for the crew to the depth and time limitations for which these submersibles were intended, it is necessary to provide both improved energy sources and an efficient method of conveying thermal energy from the source to the crew. As much as two or three times as much heat is required to heat the entire submersible as to heat the men individually with electrically heated or special hot-water-con-

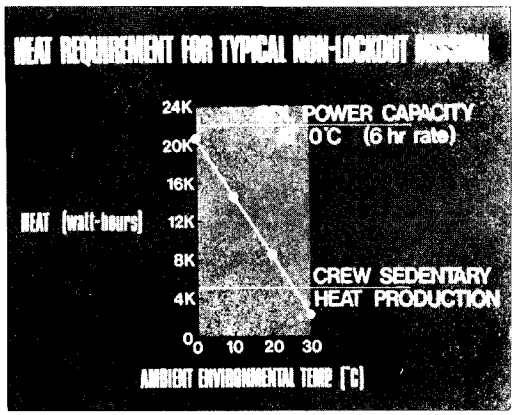

Fig. 3. Heat required for submersible crew on 8½-h nonlockout mission for various steady-state conditions of ambient temperature, showing lines indicating capacity of 6-man crew and submersible itself to supply thermal demand. Crew members considered to be engaged in sedentary activity, wearing work overalls.

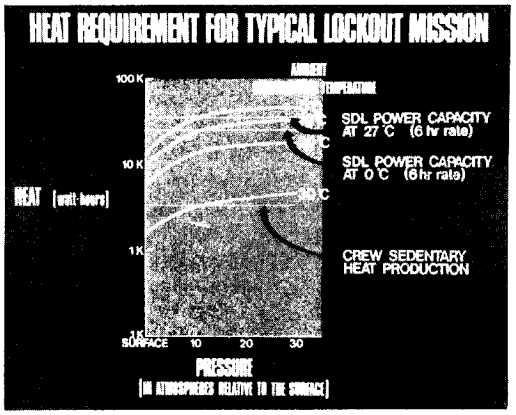

Fig. 4. Heat required for submersible crew on 5-h lockout operation for various steady-state conditions of pressure and ambient temperature, showing lines indicating capacity of 6-man crew and submersible itself to supply thermal demand. Crew members are considered to be engaged in sedentary activity; occupants of fore sphere are assumed to be wearing work coveralls; occupants of aft sphere are assumed to be dressed in diver clothing.

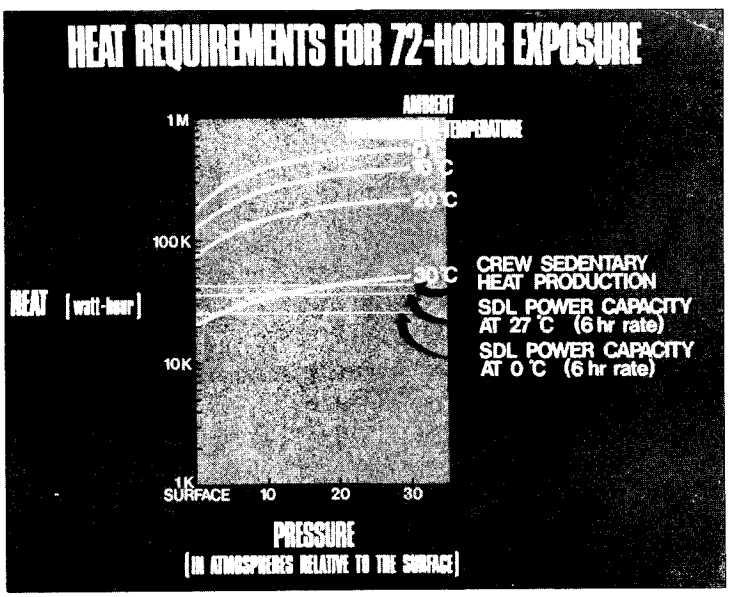

Fig. 5. Heat required for 3-day survival of submersible crew for various steady-state conditions of pressure and ambient temperature, showing lines indicating capacity of 6-man crew and submersible itself to supply thermal demand. Crew members are considered to be engaged in sedentary activity; occupants of fore sphere assumed to be wearing work coveralls; occupants of aft sphere are assumed to be dressed in diver clothing.

ditioned suits. Passive insulation such as a 1-inch thickness of solid neoprene or syntactic foam on each submersible sphere can reduce submersible heat loss by one half (28, 30, 32, 33) but until lockout submersibles are built with double hulls with an insulation space between, they will require supplementary heating on the order of tens of kilowatts (1, 28, 30, 32, 33).

Potential thermal power sources include huge power density batteries, isotope power generators, thermionic heaters, thermoelectric generators, catalytic fuel cells and devices utilizing exothermic chemical reactions. These sources have been reviewed extensively by Beckman (5). Of particular interest are the inert cathode/magnesium anode seawater batteries described by Black et al. (7) and the lithium sulphur primary battery described by Holland (18).

One potential heat source that may be readily applicable to lockout submersibles would be an adaption of the chemical heat engine (24) developed by the Centre des Etudes Nucleaires (CENG) at Grenoble, France. Known as a 'climataseur,' this device consists of an insulated metallic 'thermos bottle,' containing molten lithium salts, which is used to heat water flowing through channels in an incompressible dry suit. Tests now indicate that this system may be used to heat divers for extended periods of time in cold water. We suggest that application of this technology may suffice for the thermal needs of a submersible crew.

A second potential energy source could be an extension of the underwater automotive engine such as that being developed by the Naval Coastal Systems Laboratory in Panama City, Florida, or in Japan (2, 3). This method involves the use of an internal combustion or diesel engine, which is so arranged that its exhaust is cleaned of pollutants and fed back into the engine intake with the addition of fuel and oxygen. Running in a closed-cycle mode, the only gas to circulate through the engine is inert nitrogen. Use of such 'clean' fuels as propane or hydrogen permits a simple process of cleaning or scrubbing of the exhaust. The whole unit could be enclosed in a pressure vessel and used to supply energy requirements for propulsion, electrical power and thermal heating.

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PART II. EVOLUTION OF LOW TEMPERATURE DIVING

DISCUSSION

S. K. Hong, Chairman

Dr. Hong: We have heard six very interesting papers this afternoon, and all the papers are now open for discussion, comments, and questions.

Dr. Johnson: Dr. Webb, I would like to ask you about the equations for calculating skin temperatures from mean body temperature. You indicated on your graphs that they were somewhat different from your experimental results. Have you ample experimental data so that you can correct these equations and provide us with more agreeable ones with the experimental results?

Dr. Webb: I'm not quite sure I understood the whole question, but the problem seems to be in the calculation of mean body temperature. We calculate mean skin temperature like anybody else. We have 16 thermistors and they are area weighted. We get a mean skin temperature from that. Mean body temperature is perhaps more important. We have been using the standard Burton numbers for weighting because there isn't anything better. You can change them from 0.6 to 0.8 or 0.9 or whatever you want, it doesn't help. We have tried every conceivable combination of weighting factors, and you can get a set that will work in one situation and won't work in another. So there is no universal here for how to calculate mean body temperature from body temperature data. I'm sorry to say that at this point I can't promise you anything useful until we get to the steady-state experiments. Then perhaps we can give you some better numbers.

Dr. Kent: I noticed on your graphs that the sedentary metabolic production of heat seemed to vary from one graph to another. Was that for the total number of people?

Dr. Kuehn: It did vary from one graph to another, and in the last two cases the differences were due to the different times involved. There was a 72-hour exposure for the survival case, 8½ hours for the submersible case, and 5½ hours for the diver lockout case. These were all based on 6 men losing heat at the rate of 100 watts; we kept that a constant figure throughout all of these calculations. We assumed that 100 watts represented fairly sedentary activity for each individual.

Dr. Rudell: I would like to ask Dr. Hoar what the name of the reflex is that controls the urine flow by sensing the pressure of exhalation in the lung?

Dr. Hoar: It is referred to as a Gauer-Henry reflex.

Dr. Rudell: Did I understand correctly that this reflex is changed by a trimixture?

Dr. Hoar: We postulated that breathing this less dense gas, that is the trimix, reduced transpulmonary pressure to a lesser extent, leading to lesser stimulation of the atrial stretch receptors. Applying the work of Gauer and Henry to our setting, less atrial stretch would cause less suppression of the antidiuretic hormone (ADH), hence less diuresis with trimix.

Dr. Hong: I have one question along this line. When did you notice this attenuation of diuresis?

Dr. Hoar: We never saw the effects until after the trimix respirations.

Dr. Hong: How soon after the subjects breathed the trimix did the effect on diuresis appear?

Dr. Hoar: I'd say approximately 10 or 15 minutes.

Dr. Hong: Well, in that case it is very difficult to attribute it to the ADH suppression. It will take about 30 minutes because you have to break down circulating ADH, and that just cannot be done in 10 minutes.

Dr. Hoar: The actual urine collection didn't occur until the surface interval. In other words, the individual was breathing the gas for at least 10 minutes, including 4 minutes of rest and 6 minutes of exercise and was then brought to the surface where we collected another urine.

Dr. Hong: How long did it take?

Dr. Hoar: Sometimes we had difficulty getting individuals to urinate. It would probably, I would estimate, go

anywhere from 20 to 20 some odd minutes. But the time that the subject actually respired trimix was only 10 minutes. The immersion cycle consisted of only 10 minutes.

Dr. Hong: You didn't notice any difference in heart rate?

Dr. Hoar: There was no significant statistical difference between the heart rates of the individuals breathing air or those on the trimix combination.

Dr. Johnson: Dr. Clingman, could you briefly describe the method by which you calculated your heat loss, or heat debt, in the divers?

Dr. Clingman: We used a weighted method.

Dr. Johnson: Skin temperatures?

Dr. Clingman: Yes, skin temperature and rectal too.

Dr. Johnson: Dr. Webb, I noticed that in some of your profiles you initially had an increase in body core temperature and then a rather rapid decrease as the experiment went on. It seems to me that we often see an elevated temperature in subjects who are put in a stressful situation. Did you consider this in accounting for this temperature increase, and do you think it might have had some application here?

Dr. Webb: I am not sure I would agree that these people are very heavily stressed. They are sitting comfortably until the cold water comes, and they are used to being cold. Most of these experiments were done on three men who have been through these cold exposures over and over again. There is no indication of increased heart rate or oxygen uptake, for example, until the cold exposure has accumulated considerable heat debt. It is a very common thing to see rectal temperatures go up when you first start cooling somebody.

Dr. Raymond: I address this to Dr. Varene. I'm wondering if you observed an increase in urinary flow rate in your divers during exercise, and if there was an increase in urinary flow rate, did this vary with the ambient pressure or the temperature of the inspired gas?

Dr. Varene: We have not measured the urinary flow rate.

Dr. Raymond: No subjective indication from the divers? Now I have a question for Dr. Nakayama. I find the 7 liter net deficit in fluid balance puzzling, since I can't account for it on a caloric basis. The question concerns urinary osmolality. The men were putting out a dilute urine in the face of a constant level of antidiuretic hormone, as I understand the results. That is, the same vasopressin level but still a dilute urine, which would argue for some tubular effect, some tubular resistance to vasopressant. I wonder if you would comment on that.

Dr. Hong: I will answer this question for Dr. Nakayama. If one starts with a relatively low urine flow as in our case, the urine osmolality is 1,000 milliosmol or so. In that case, if we induce even a slight osmotic diuresis one would expect a reduction in the urine osmolality even with the same level of ADH. So a reduction in urine osmolality could mean either inhibition of ADH or inhibition of the tubular reabsorption of sodium without a change in the ADH level. Of course there is a question of sensitivity of the collecting ducts to ADH, but this is very difficult to test at this stage.

Dr. Bove: This is addressed to Dr. Clingman. I couldn't help looking at the cardiogram you showed of one diver who had a rapid heart rate, which appeared to be a junctional or nodal tachycardia. The question is, did you experience any arrhythmias in your divers, and did you take care to ensure that they had a sinus rhythm when you were measuring their heart rates?

Dr. Clingman: There were some arrhythmias that were noted and we took those kinds of precautions.

Dr. Morlock: Did you calculate a convective heat transfer coefficient while your men were exercising, and if so, how did it compare with the values you reported earlier while resting?

Dr. Varene: The difference in convective coefficient between rest and exercise has been somewhat difficult to measure in this experiment because it was quite impossible to obtain a steady state during muscular exercise. Nevertheless, we tried to make this computation and if we compare the development of this coefficient with pressure, we find the same development at exercise as at rest. The equation for subjects at rest was a power function with a constant coefficient and a power coefficient. At exercise, this constant coefficient has to be increased by 1.5 to achieve a value which corresponds approximately to those during exercise.

Dr. Morlock: You're saying that the constant changed, but the power function was the same.

Dr. Varene: The equation is $h = aP^n$. I don't remember the exact value of the coefficients, but the equation at exercise would be $h = 1.5\ aP^n$. There is an increase of 50 percent in the constant part of the equation.

Dr. Behnke: This presentation is involved, and the question is whether or not there is some simple way to assess the effects of cold. In hypothermic experiments that I observed with Drs. Rodahl and Horvath in Philadelphia, the effect of cold exposure on individuals over a period of days was manifested by a negative nitrogen balance; there was some metabolic derangement that led to a negative nitrogen balance. I would like to know in cold tests what the food intake was, and if extra food was consumed. Fluid balance has been monitored over a period of a week before,

during, and following experiments. It seems to me that the fluid balance and nutritional requirements can be accurately assessed as parameters which I haven't heard discussed.

Dr. Webb: Dr. Behnke, in these rather acute cold exposures, we have not at this point made any effort to control the things you are talking about. The exposures last about one to two hours, so no food is taken and no urine is voided. We could not have the sort of data you need for metabolic balance studies for the cold exposure proper. I agree one would like to know that much about the subjects. You should have them on metabolic control days before and maybe days after the exposure, but the present experiments were very short.

Dr. Behnke: Your tests were not of long duration, but for the 24-hour period was the fluid intake, urine output, and caloric consumption the same as on control days prior to and following the study? What about the nitrogen balance?

Dr. Webb: I'm going to plead innocence. We have not really made the measurements that would allow me to answer your question.

Dr. Mikelionis: I have a very simple question. I wonder if one of you gentlemen could comment on the ordinary charley horse or severe muscular spasm associated with the cold water. I know at least a couple of cases with crippling results. The cramps start usually in one lower extremity, quickly go to the second, and finally involve pelvic muscles and lower trunk. I wonder if so-called undiagnosed cases of divers drowning may eventually fall into this category. Could you comment on the causes, prevention and treatment?

Dr. Webb: I don't believe that heat loss per se has very much to do with the kind of muscle cramp you are talking about, but they certainly would be a problem to someone swimming and unable to bring himself back to shore.

Dr. Hody: This question is addressed to Dr. Webb. I heard the term "thermal steady state" used several times by several speakers, and I'm womdering if you define that term in terms of a constant net rate of heat loss, that is a net rate of decrease in body content, or would you like to define it in terms of a constant temperature distribution, looking at various cross sections through the body?

Dr. Webb: I would be willing to define it either in terms of temperature or heat flow, a net balance where heat outflow and heat production are the same, since I can measure it both ways. In the experiments I have discussed, we had no steady state of either kind during the cooling and rewarming. We had it predive or precooling and only after the end of rewarming.

Dr. Hody: Would there be any theoretical reason to assume that those two definitions would coincide necessarily?

Dr. Webb: They coincide well unless you are looking at a person over a 24-hour day, in which case you get variations in both internal and surface temperature which are not in phase with changes in metabolic rate or heat loss, either one. There are some out-of-phase relationships.

Dr. Townsend: Dr. Clingman, what do you propose as the mechanisms by which the acclimated divers can avoid this cold stress response, mechanisms that the unacclimated divers don't seem to have available to them?

Dr. Clingman: It is unknown to us. Perhaps someone else here has been confronted with the same problem.

Dr. Hong: I might make a comment on this point. You reported everything except one thing, rectal temperature. Obviously you measured the rectal temperature, but I could not find the value. What did you find?

Dr. Clingman: We averaged that, and it was not appreciably different. We weren't submerged that long.

Dr. Hong: What you are saying is that in the face of such a tremendous difference in the oxygen consumption, you did not find any difference in rectal temperature. This means that their heat balance must be different. An acclimated subject must have an increased body temperature. There has to be a difference in core temperature.

Dr. Clingman: There was, and perhaps it was caused by the really violent shivering of the unacclimated.

Dr. Webb: Dr. Matsuda, let me just suggest that while the core temperature did not change in the 15 to 20 minutes of exposure, the surface temperature probably changed enormously, and your acclimated people had a more rapid vasoconstriction and a lesser heat loss, if heat loss and metabolism were related as they were in our data.

Part III. RESPIRATORY-PULMONARY COMPETENCE AT HIGH AMBIENT PRESSURES IN ATMOSPHERE AND WATER, AT REST AND WORK

EFFECT OF HYPERBARIC NITROGEN ON CENTRAL RESPIRATORY RESPONSE TO CO_2

D. Linnarsson and C. M. Hesser

Ventilatory responses to exercise and to CO_2 stimulation have been shown to be depressed in hyperbaric air (5, 6, 9). Increased respiratory resistance per se produces similar effects (3, 10) and it is therefore likely that the increased gas density is responsible, at least in part, for ventilatory depression in hyperbaric environments. It is also known that ventilatory responses can be affected by narcotic depression of the central neural structures involved in respiratory regulation, e.g., after the administration of morphine (8, 11). Whether a narcotic depression of the respiratory centers occurs also in a hyperbaric air environment in the presence of N_2 pressures of sufficient magnitude to impair other nervous functions is not known.

The difficulty in separating gas density effects on respiration from possible narcotic actions in hyperbaric environments arises from the fact that denser gases also tend to be more narcotic. However, the study of respiratory events that do not involve the movement of gas to and from the lungs offers an opportunity to study respiration in hyperbaric environments without influence from the increased gas density. Thus Hesser (7), comparing breath-holding ability with and without the presence of 3.8 ATA N_2, obtained evidence against any nitrogen-dependent depression of the central respiratory drive. In the present study we have used a more direct approach to assess the output of the respiratory centers, namely the inspiratory occlusion-pressure method of Whitelaw, Derenne and Milic-Emili (12) and applied it to study the respiratory response to CO_2 in hyperbaric air.

Methods

Four healthy male students were studied, each performing two CO_2 rebreathing tests with O_2 at 1.3 ATA and with air at 6 ATA (1.3 ATA O_2 + 4.7 ATA N_2), respectively, in a dry recompression chamber. The rebreathing circuit (Fig. 1) was designed to offer minimal respiratory resistance, and the volume of the rebreathing bag was such that end-tidal P_{CO_2} increased approximately 1 mmHg/min during the CO_2 rebreathing periods, which lasted 20–25 min. Mouthpiece pressure, respired P_{CO_2}, and inspiratory flow were measured continuously, and end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) and ventilation ($\dot{V}_E$) were computed breath-by-breath using a special purpose computer. The inspiratory line was occluded during an expiration at intervals of 0.5–1 min by means of an inflatable rubber valve, causing the ensuing inspiratory effort to result in a rapid pressure drop in the airways. A control logic opened

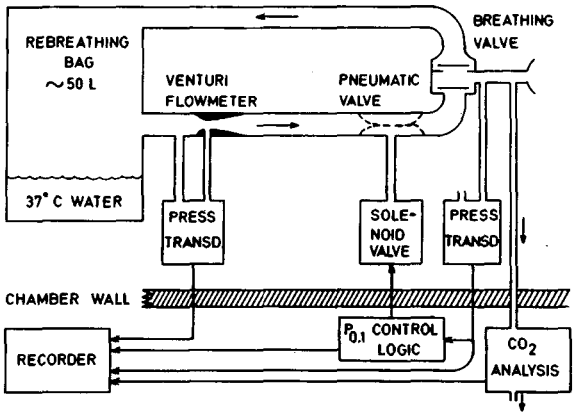

Fig. 1. Schematic diagram showing breathing circuit with occlusion valve placed inside pressure chamber, and various recording instruments outside chamber. Not shown: continuous supply of gas to breathing circuit which compensated for gas sampling flow to CO_2 analyzer.

the valve 0.25 s after the onset of the inspiratory effort (Fig. 2) to minimize the influence of the occlusion on the respiratory pattern. The valve acted silently and could be closed without the subjects' knowledge. The pressure drop generated at 0.1 s after the onset of inspiration ($P_{0.1}$) was obtained from the mouth pressure tracings as shown in Fig. 2. Ventilation ($\dot{V}E$) was determined as the time average over 10-s periods before and after each $P_{0.1}$ determination. Ventilatory and $P_{0.1}$ responses from the two different experimental conditions were compared on an intraindividual basis using the Student *t*-test. The method of least squares was used to construct first- and second-order regression lines relating $P_{0.1}$ and $\dot{V}E$ to PET_{CO_2}. An analysis of variance and an F test were used to determine whether regression lines showed a significant departure from linearity.

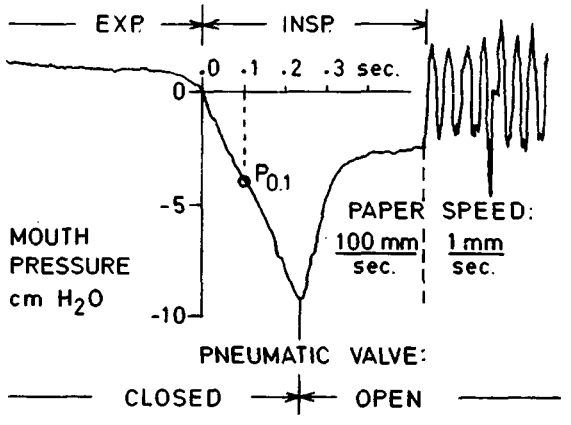

Fig. 2. Original tracing showing time course of mouth pressure during an inspiration when a pneumatic valve occluded inspiratory tubing during initial 0.25-s period. Occlusion pressure generated at 0.1 s after onset of inspiration ($P_{0.1}$) is indicated. Note change of paper speed at end of tracing.

Results

In all experiments P_{ETCO_2} increased almost linearly at a rate of approximately 1 mmHg/min. The hypercapnia was generally better tolerated at 1.3 ATA O_2 than at 6 ATA air. Two subjects thus aborted the tests at P_{ETCO_2} values of 52–53 mmHg due to dizziness during the 6-ATA experiments, while in the other experiments the tests were interrupted by the investigators at P_{ETCO_2} values of about 60 mmHg to avoid excessive hypercapnia.

The $\dot{V}_E$ and $P_{0.1}$ values increased with increasing P_{ETCO_2}. No consistent pattern of linearity or nonlinearity in the responses was found among the subjects. Thus, in the same subject, a linear $\dot{V}_E$ response could be found in one condition and a nonlinear in the other. Linearity of the $P_{0.1}$ responses varied similarly. The results from a subject with nonlinear responses in both variables and in both conditions are shown in Fig. 3. For each subject and condition the values for $\dot{V}_E$, $P_{0.1}$ and $\dot{V}_E/P_{0.1}$ at an arbitrarily chosen P_{ETCO_2} value of 50 mmHg were computed from the corresponding best-fit regression equations (Table I). The slopes of the regression lines relating $\dot{V}_E$ and $P_{0.1}$ to P_{ETCO_2} at P_{CO_2} = 50 mmHg are also given in Table I.

The $P_{0.1}$ values were on the average 46% higher at 6 ATA air than at 1.3 ATA O_2, whereas the ventilatory responses were consistently reduced at 6 ATA. Thus $\dot{V}_E$ was 11% lower, and ventilation per unit $P_{0.1}$ ($\dot{V}_E/P_{0.1}$ at P_{ETCO_2} = 50 mmHg) 38% lower at 6 ATA air as compared to control at 1.3 ATA O_2. The slope of the regression line relating $\dot{V}_E$ to P_{ETCO_2} was also significantly reduced at 6 ATA (Table I), while no consistent change was observed for slope of the regression of $P_{0.1}$ on P_{ETCO_2}.

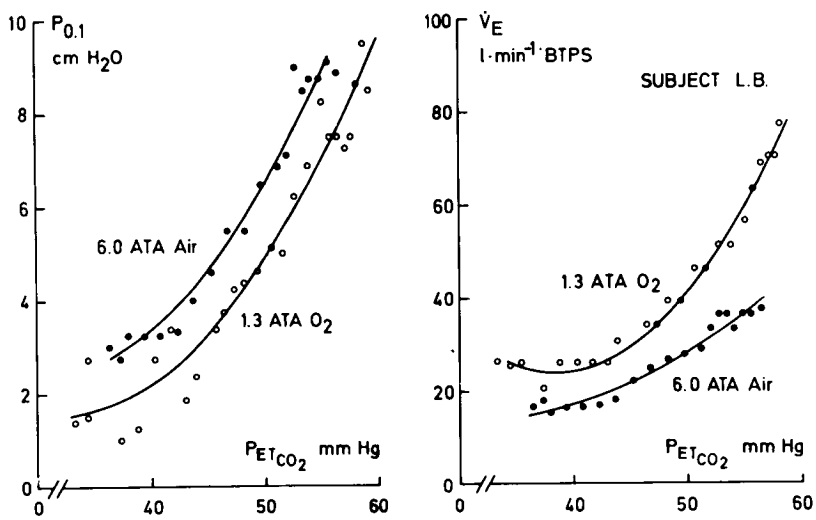

Fig. 3. Typical changes in occlusion pressure generated at 0.1 s after onset of inspiration ($P_{0.1}$, *left*) and in ventilation ($\dot{V}_E$, *right*) as functions of end-tidal P_{CO_2} (P_{ETCO_2}) during CO_2 rebreathing at 1.3 ATA O_2 (open circles) and 6 ATA air (closed circles).

TABLE I

MEAN VALUES AT AN END-TIDAL P_{CO_2} OF 50 MMHG DURING $\overline{CO_2}$ REBREATHING

	1.3 ATA, O_2 (A)	6 ATA, air (B)	Significance of difference between A and B
$P_{0.1}$, cmH_2O	5.3 (3.7-7.1)	7.7 (6.5-10.0)	$P < 0.05$
$\dot{V}_E$, $l \cdot min^{-1}$ BTPS	35.1 (24.0-42.5)	31.2 (28.5-36.3)	NS
$\dot{V}_E/P_{0.1}$, $l \cdot min^{-1}/cmH_2O$	6.6 (4.5-8.5)	4.2 (2.9-5.1)	$P < 0.05$
$d\dot{V}_E/dP_{ET_{CO_2}}$, $l \cdot min^{-1}/mmHg$	2.7 (1.8-3.4)	1.2 (1.1-2.6)	$P < 0.05$
$dP_{0.1}/dP_{ET_{CO_2}}$, $cmH_2O/mmHg$	0.5 (0.3-0.9)	0.5 (0.3-0.7)	NS

The first derivative of regression equations relating $\dot{V}_E$ and $P_{0.1}$ to $P_{ET_{CO_2}}$ at P_{CO_2} = 50 mmHg is given in bottom two rows. n = 4; ranges shown in parentheses. NS = no significant difference.

Discussion

VENTILATORY RESPONSES

The observed ventilatory changes at 6 ATA air are in agreement with previous observations in hyperbaric air (5), i.e., there was a reduction in the slope of the regression of $\dot{V}_E$ on $P_{ET_{CO_2}}$. A similar decrease in the slope of the $\dot{V}_E$ response to hypercapnia has been found when the respiratory resistance is artificially increased (3, 10). A parallel shift to the right of the ventilatory CO_2 response curve is commonly said to be typical of narcotic depression of respiration with morphine-like drugs (8), but a decrease in slope has also been reported (11). In contrast, during nitrous oxide inhalation the $\dot{V}_E$ responses to CO_2 (4) and to exercise (2) have been shown to be enhanced. The observed depression of $\dot{V}_E$ at 6 ATA may thus be ascribed to both a narcotic depression of the respiratory centers and to the influence on ventilation of increased breathing resistance, or to a combination of these two factors. It also seems possible, however, that the output of the respiratory centers at 6 ATA was enhanced as in the case of N_2O inhalation (2, 4), although not to a sufficient degree to maintain $\dot{V}_E$ when the flow resistance in the airways was increased due to the raised gas density.

RESPONSES OF INSPIRATORY OCCLUSION PRESSURE

Occlusion pressure, measured 0.1 s after the onset of an inspiratory effort at the level of relaxed functional residual capacity (FRC) has been shown by Whitelaw, Derenne and Milic-Emili (12) to be a reproducible index of the output of the respiratory centers, and to be independent of mechanical events in the lungs and in the thorax. We have therefore considered it justified to use $P_{0.1}$ measurements to assess the role of changes in central respiratory drive

for the reduction of the ventilatory response to CO_2 that occurs in hyperbaric air and high P_{N_2} environments. The results indicate that the output of the respiratory centers is enhanced rather than depressed in the presence of a P_{N_2} of 4.7 ATA. Such a high N_2 pressure is known to exert a narcotic influence on man, characterized by slowed mental activity and motor incoordination (1). We suggest that one or several of the following mechanisms may be responsible for this enhanced respiratory drive, which was observed during the whole range of P_{CO_2} values at 6 ATA:

(1) A mechanism similar to that responsible for the shift to the left of the CO_2 response curve during nitrous oxide narcosis (4). Similar N_2O-induced changes of the respiratory response to muscular exercise have been ascribed to sensitization of pulmonary stretch receptors (2).

(2) Anxiety with a tendency to hyperventilate. Although the subjects were familiar with the experimental procedures, this possibility cannot be ruled out.

(3) A decreased FRC at 6 ATA, which would enhance the pressure generated by the inspiratory muscles and the elastic forces of the thorax during an occluded breath. This possibility seems unlikely, however, since it has been found that during voluntary hyperventilation and exercise hyperpnea FRC tends to change towards a slightly higher volume when the gas density is increased (Fagraeus, Hesser and Linnarsson, unpublished observations).

Conclusion

The results of the present study support the notion that the reduced ventilatory responses to CO_2 and exercise in high P_{N_2} environments are due to the raised gas density and not to any narcotic depression from high N_2 pressures on the respiratory centers. Instead, the output of the respiratory centers increased. The mechanisms behind this increase are not evident and deserve further study.

ACKNOWLEDGMENTS

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SOME CARDIOPULMONARY RESPONSES TO EXERCISE IN MAN AT AN INCREASED AMBIENT PRESSURE OF OXYGEN-HELIUM

M. M. Winsborough, J. Vorosmarti, Jr. and R. S. McKenzie

The metabolic demands of large groups of exercising muscle are met at the surface by a series of cardiovascular and respiratory adaptations. Under conditions of increased ambient pressure, however, these adaptations are complicated by mechanisms activated by the elevated environmental pressure. Some of these mechanisms, such as the well-known reductions in heart rate and total pulmonary ventilation, would appear to antagonize the normal compensatory adaptations to exercise directly. In a situation where the physics of the environment are such that the ventilation required by the subject to keep alveolar P_{CO_2} within normal limits may closely approach or exceed the MEV, carbon dioxide must be retained and alveolar hypoventilation will ensue. Similarly, a fall in the heart rate means that the stroke volume must increase if the cardiac output is to remain adequate, particularly at heavy exercise. The stroke volume cannot increase to more than twice its resting value (19) and other mechanisms must therefore be brought into play or the minimum exercise capacity of the subject will be drastically reduced by the inability of both the respiratory and cardiovascular systems to supply the oxygen needed to perform heavy work. The main oxygen reserve of the body is provided by hemoglobin. However, in vitro studies by Kiesow (15) have shown that elevated pressures of oxy-helium may have a deleterious effect upon the O_2 dissociation curve, rendering the unloading of oxygen from hemoglobin to the metabolizing tissues more difficult at great depth. A further complication is provided by the possibility that helium at high pressure may lower the tolerance to oxygen, particularly under saturation conditions and when combined with increased ambient pressures of oxygen.

In this study higher levels of work than are usual in the hyperbaric environment were used to assess the degree of compromise between the antagonistic and compensatory effects triggered by exercise at increased ambient pressure.

Methods

The subjects were six healthy male divers who were members of the saturation diving team of the Royal Navy. They were split into 2 teams of 3 in consecutive dives, using identical experimental protocols. They were fit but not in athletic training, and were accustomed to the hyperbaric environment. They were not trained respiratory subjects. Each subject was studied initially at the surface in an oxy-helium environment in the hyperbaric chamber. The same measurements were repeated twice at 250 meters of chamber pressure, after 24 hours and after 1 week of bottom time. The experiment was also repeated at 170, 105, and 40

meters of chamber pressure during decompression. The inspired oxygen concentration was maintained at 0.4 bar throughout the dive, chamber atmosphere being monitored continuously by means of a Servomex O_2 analyzer. The background carbon dioxide level was maintained at less than 0.5 bar surface equivalent. Chamber temperature was maintained at 30°C ± 1.0°C, depending upon diver comfort. The divers were not placed under any dietary restrictions.

The experiments at the surface and at 250 meters were performed approximately 1 hour after a light breakfast. The experiments at 170 m, 105 m and 40 m varied with the time of arrival at the decompression stop. The experimental protocols were identical in all cases. Since it was known that the time available at the decompression stops would be very short, the 15 minutes traditionally allowed to achieve a steady state at rest were shortened to 5 minutes and the resting experiments were regarded as training runs for the exercise studies. This approach was vindicated during the first runs at 250 meters when some of the subjects appeared to develop amnesia for anything remotely resembling respiratory physiology. This appeared to be caused by failure of recent memory rather than motivation; some of the divers were much more affected than others. Exercise was continuous once started. Two consecutive work loads of 50 watts and 200 watts were used. The subjects breathed chamber air through a low-resistance, low-dead-space valve, which delivered expired air to a dry gas meter by means of a short run of large-bore rigid corrugated tubing. Expired air was sampled continuously at the mouth by means of a heated stainless steel capillary from which the mass spectrometer (MS) sampled gas externally. When both the expired air composition and the heart rate were constant the MS probe was switched to a mixing chamber attached to the outlet side of the dry gas meter, and mixed expired air was sampled for 1 minute. The subject then inspired a single breath of a 10% N_2O, 40% O_2, bulk He mixture from residual volume (RV), and rebreathed in an empty rebreathing bag for not more than 10 seconds. End-tidal gas was sampled continuously at the mouth. Shortly afterwards the oxygenated mixed venous P_{CO_2} was estimated by rebreathing a CO_2 mixture chosen to give a period of no gas exchange for CO_2 at equilibrium. The electrical outputs from the spirometer and MS were displayed on a suitable recorder (Mingograph 81). The output from the ECG leads was displayed on a Devices recorder.

The rebreathing gases were fed into the chamber, with suitable valve arrangements, from an external source. All the gases were analyzed at the surface before use by means of the MS and the gas chromatograph. The MS was calibrated with gases previously compared with gravimetric standard gases analyzed by the gas chromatograph. Lloyd-Haldane analysis was not used because of the difficulties inherent in analyzing helium bulk mixtures by a volumetric method. The 95% confidence limits of the analyzers were better than ±0.01% for both the gas chromatograph and the MS.

Cardiac output and pulmonary tissue volume were estimated using the rate of change of alveolar CO_2 and N_2O during rebreathing (22). The CO_2 dissociation curve operative during each experiment was calculated from the mean alveolar mixed venous P_{CO_2} difference using McHardy's (16) in vitro CO_2 dissociation curves for whole blood. Regression analysis was carried out by a digital computer (Olivetti) using the method of least squares.

Calculation of Cardiac Output

Cardiac output was calculated from end-tidal samples measured at the mouth, utilizing the

rate of rise of endogenous CO_2 and the rate of fall of alveolar N_2O. A modified instantaneous differential Fick equation was used to calculate both $\dot{Q}_{CO_2}$ and $\dot{Q}_{N_2O}$ (22,13).

$$\dot{Q}_{CO_2} = \frac{V_A \cdot \lambda \cdot \left[\frac{760}{P_B - H_2O}\right]}{\alpha_b \cdot t_{BH}} \cdot \ln \frac{(\Delta P_{CO_2} t_1)}{(\Delta P_{CO_2} t_2)} \quad (1)$$

$$\dot{Q}_{N_2O} = \frac{V_A \cdot \left[\frac{760}{P_B - PH_2O}\right] \cdot \left[\frac{100}{\text{Intercept value in \%}}\right]}{\alpha_b \cdot t_{BH}} \times \ln \left[\frac{P_x t_1}{P_x t_t}\right] \quad (2)$$

V_A is expressed in ml STPD; α_b (N_2O): the Bunsen solubility coefficient for N_2O at 37 °C in ml STPD per ml of tissue/standard atmosphere; α_b (CO_2): is the slope of the appropriate dissociation curve for whole blood expressed as ml of CO_2/ml of blood/standard ATA of pressure. $\Delta P_{CO_2} t_t$ represents the $P\bar{v}_{CO_2} - P_{ACO_2}$ difference at any chosen time; $P_x t_t$ represents the foreign soluble alveolar gas concentration at any one time, and time is in seconds.

Pulmonary Gas Capacitance

The ability of the lung to release (CO_2) or absorb (N_2O) gas is represented by a depression of the zero intercept of the extrapolated semilogarithmic relationship between P_A/P_{A_0} and time of breath-holding during a simple rebreathing maneuver (4). Pulmonary tissue volume (V_{tis}) can then be calculated using the formula

$$V_{tis} = \frac{V_A}{\alpha_t}\left[\frac{100}{\text{Intercept at zero time in \%}} - 1\right]\left[\frac{760}{P_B - 47}\right] \quad (3)$$

where V_A is in ml STPD and α_t is the Bunsen solubility coefficient of the gas at 37 °C in ml STPD/ml of tissue/standard atmosphere of pressure (4).

Results

The dives were divided into 3 groups for analysis. Measurements were taken at the surface, after compression to 250 m (24-hour and 7th-day measurements), and during decompression (170, 105, and 40 m).

Regression equations relating cardiac output, expired ventilation, and pulmonary gas capacitance to oxygen uptake in the various experimental conditions are given in Tables I, II, and III, and shown graphically in Figs. 1, 2, and 3.

There was no significant difference between any of the regressions of $\dot{Q}_{CO_2}$ plotted against $\dot{V}_{O_2}$ at the surface and under conditions of increased ambient pressure (Table I). There was a significant difference ($P < 0.05$) between $\dot{Q}_{N_2O}$ plotted against $\dot{V}_{O_2}$ at the surface and at 250 meters. However, there was no significant difference between the $\dot{Q}_{N_2O}$ regressions calculated from measurements taken at day 1 and day 7.

The regressions of ventilation plotted against oxygen uptake at 250 m were significantly different from the surface measurements (Table II).

The combined measurements of pulmonary gas capacitance at 250 meters and at 40 meters showed an increase over the surface measurements on the same subjects (Table III). How-

TABLE I

CARDIAC OUTPUT IN THE HYPERBARIC ENVIRONMENT

Conditions	Regression Equation	SD, l/min	r
Surface	$\dot{Q}_{CO_2} = 4.47 + 6.42\ \dot{V}_{O_2}$	0.3	0.98
	$\dot{Q}_{N_2O} = 5.49 + 5.33\ \dot{V}_{O_2}$	2.38	0.91
Compression			
250 m, 24 hour	$\dot{Q}_{CO_2} = 4.63 + 5.65\ \dot{V}_{O_2}$	1.38	0.96
	$\dot{Q}_{N_2O} = 4.75 + 6.65\ \dot{V}_{O_2}$*	2.42	0.92
day 7	$\dot{Q}_{CO_2} = 4.10 + 5.83\ \dot{V}_{O_2}$	1.68	0.95
	$\dot{Q}_{N_2O} = 3.2 + 7.7\ \dot{V}_{O_2}$*	1.49	0.97
Decompression			
170 m	$\dot{Q}_{CO_2} = 5.24 + 6.29\ \dot{V}_{O_2}$	2.11	0.93
	$\dot{Q}_{N_2O} = 4.63 + 6.59\ \dot{V}_{O_2}$	1.21	0.98
105 m	$\dot{Q}_{CO_2} = 4.43 + 5.97\ \dot{V}_{O_2}$	2.14	0.89
	$\dot{Q}_{N_2O} = 4.59 + 5.97\ \dot{V}_{O_2}$	1.33	0.96
40 m	$\dot{Q}_{CO_2} = 5.93 + 5.75\ \dot{V}_{O_2}$	3.0	0.88
	$\dot{Q}_{N_2O} = 4.66 + 6.43\ \dot{V}_{O_2}$	1.39	0.95

Cardiac output ($\dot{Q}$) and oxygen uptake ($\dot{V}_{O_2}$) expressed in liters/min; $r = 6$. * = Significant difference between $\dot{Q}_{N_2O}$ plotted against $\dot{V}_{O_2}$ at surface and at 250 m ($P < 0.05$).

TABLE II

EXPIRED VENTILATION IN THE HYPERBARIC CHAMBER

Conditions	Regression Equation	SD, l/min	r
Surface	$\dot{V}_E = 7.03 + 3.27\ \dot{V}_{O_2} + 9.23\ (\dot{V}_{O_2})^2$	5.8	0.93
Compression			
250 m	$\dot{V}_E = 5.77 + 13.77\ \dot{V}_{O_2}$*	3.33	0.97
250 m	$\dot{V}_E = 6.31 + 12.83\ \dot{V}_{O_2}$*	3.64	0.97
Decompression			
170 m	$\dot{V}_E = -0.83 + 18.17\ \dot{V}_{O_2}$	2.92	0.98
105 m	$\dot{V}_E = 0.325 + 20.77\ \dot{V}_{O_2}$	5.47	0.94
40 m	$\dot{V}_E = -1.68 + 22.48\ \dot{V}_{O_2}$	3.92	0.96

* = Significant difference from surface measurements ($P < 0.025$).

ever, probably because of the large scatter of the results, no significant difference was found at any depth.

HEART RATE IN THE HYPERBARIC CHAMBER

Due to technical difficulties, consistent recordings of heart rate on all subjects at all work loads were not obtained. The mean heart rate fell by 30 beats/min at heavy work at 250 meters, while the mean stroke volume increased by 45 ml/beat.

TABLE III

PULMONARY GAS CAPACITANCE IN THE HYPERBARIC CHAMBER

Conditions	Regression Equation	SD	r
Surface	$\lambda = 1.034 + 0.0054\ \dot{V}_{O_2}$	0.029	0.33
Compression			
250 m	$\lambda = 1.012 + 0.0115\ \dot{V}_{O_2}$	0.022	0.47
250 m	$\lambda = 1.014 + 0.041\ \dot{V}_{O_2}$	0.068	0.50
Decompression			
170 m	$\lambda = 1.0185 + 0.0124\ \dot{V}_{O_2}$	0.024	0.41
105 m	$\lambda = 1.0138 + 0.0047\ \dot{V}_{O_2}$	0.008	0.45
40 m	$\lambda = 1.013 + 0.0318\ \dot{V}_{O_2}$	0.039	0.50

No significant difference found at any depth.

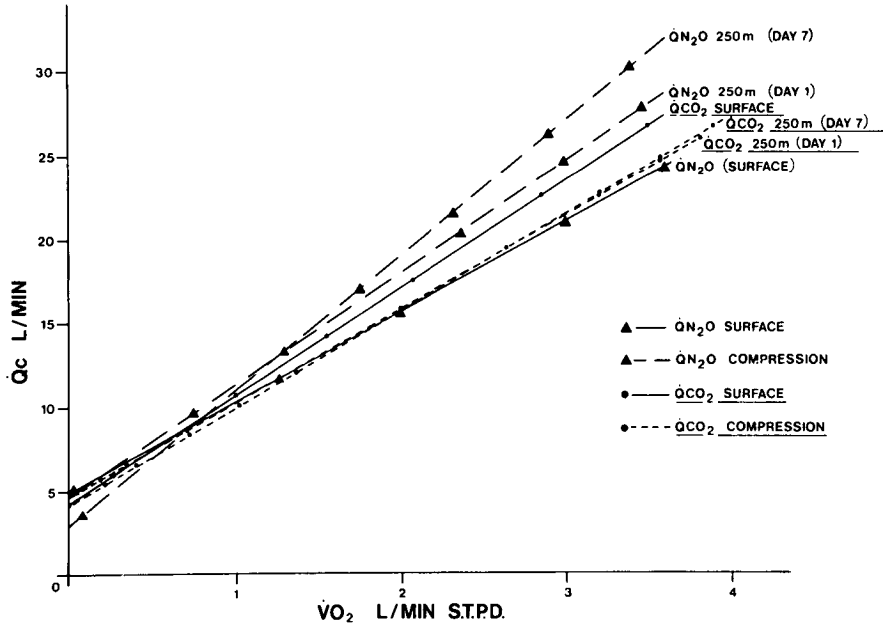


Fig. 1. Pulmonary capillary blood flow, calculated simultaneously from rate of fall of alveolar N_2O (▲) and rate of rise of alveolar CO_2 (—·—·). Solid lines represent surface regressions, broken lines regressions at 250 meters of chamber pressure on day 1 and day 7.

DISTRIBUTION OF VENTILATION AND PERFUSION AT INCREASED AMBIENT PRESSURE

One subject showed an increase in the maldistribution of $\dot{V}_A/\dot{Q}$ 24 hours after compression. All subjects on whom measurements were obtained gave some evidence of increased $\dot{V}_A/\dot{Q}$ inequality, particularly at heavy exercise, after 7 days' bottom time at 250 meters of simulated seawater pressure. A graphical analysis (17) of fast-in, slow-out breaths from 1 subject at rest and at exercise at 250 m are presented in Fig. 4.

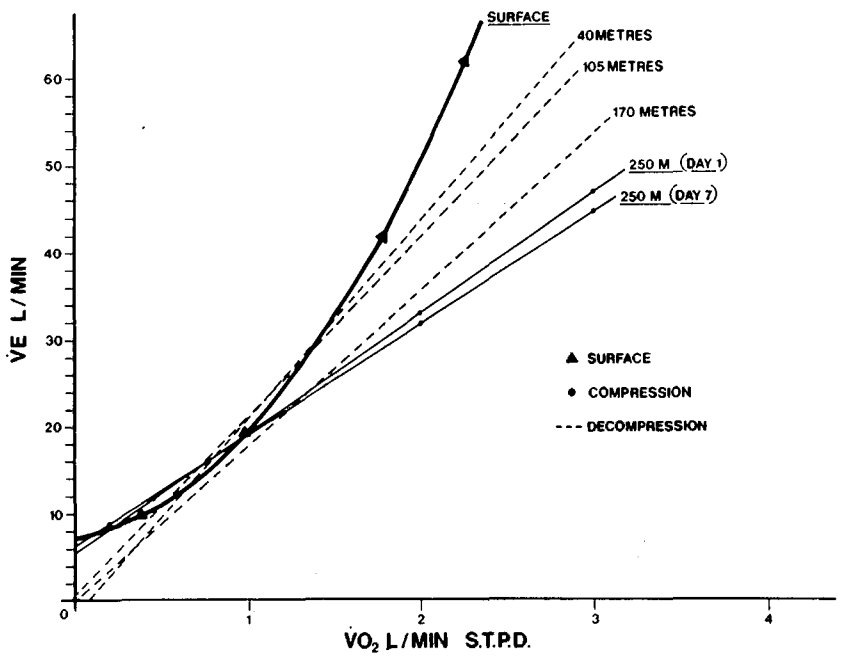


Fig. 2. Expired ventilation plotted against O_2 uptake. Solid line (▲) represents surface regression, solid lines (— · — ·) regressions obtained at 250 meters of chamber pressure on day 1 and day 7; broken lines represent regressions obtained during decompression.

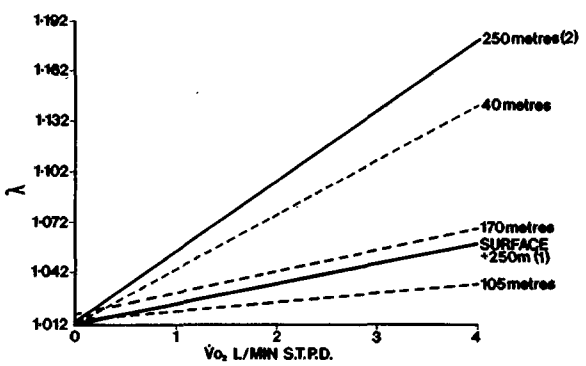
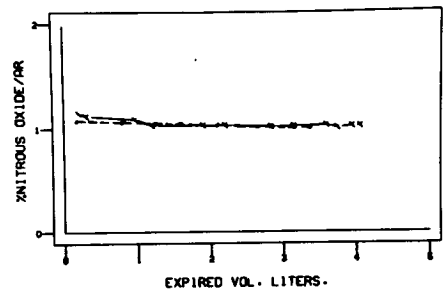
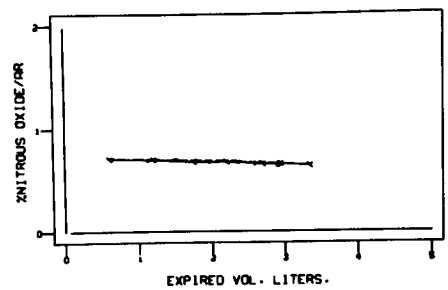


Fig. 3. Pulmonary gas capacitance plotted against O_2 uptake. Solid lines represent regressions obtained at surface and 250 meters of chamber pressure on day 1 and day 7; broken lines represent regressions obtained during decompression.

Discussion

The data obtained during these experiments confirmed the rapidly mounting evidence in the literature describing the complex nature of the physical environment. These seem to affect man in a variety of ways: (1) There is the physical effect of the environment on the mechanics of respiration which reduces the $\dot{V}_E/\dot{V}_{O_2}$ ratio from 30 at the surface to approxi-





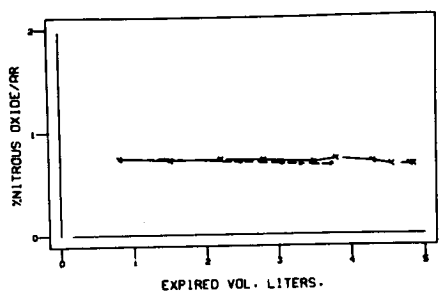


Fig. 4. Distribution of ventilation and perfusion at 250 meters of chamber pressure in subject ME on day 7. Solid line shows distribution of ventilation and perfusion in lung under normal dynamic conditions. Broken line shows distribution of perfusion in same lung with distribution of ventilation evened by rebreathing. Any difference between the two, after initial mixing, represents maldistribution of ventilation and perfusion.

mately 20 at 250 meters chamber pressure of oxy-helium. (2) There is a complicated effect on the heart rate which reduces its output by about 25 beats/minute at heavy work. These two effects, taken in conjunction, imply a considerable loss in the overall mechanical efficiency and physiological reserve of the gas transport system. (3) Changes in the hemoglobin disso-

ciation curve have also been reported and must assume even greater significance when taken in conjunction with mechanical changes.

All the subjects showed the well-known decrease in total pulmonary ventilation and in-increase in $\dot{V}O_2$ associated with an equivalent gas density of approximately 4 atmospheres of air. However, the initial increase in $\dot{V}O_2$ of about 10% which agrees well with published data in the literature (17, 20) was followed by a further 6% increase after 7 days' bottom time. The initial increase can be explained by the increased cost of breathing a dense gas at heavy work, but the secondary rise, if substantiated by further work, is more difficult to rationalize. Kiesow (15) reported a change in both the position and shape of the in vitro oxygen dissociation curves under conditions of increased partial pressures of oxygen and inert gases, with different inert gases affecting the in vitro curves to varying extent. However, the changes he reported would make oxygen delivery to the tissues more difficult and a shift in either the position or shape of the curve would not in itself cause an increase in $\dot{V}O_2$. Disruptive changes in cell function, perhaps caused by invasion of the intracellular macromolecules by helium, could have such a result if the relaxation time of working muscle was prolonged (9). A more likely explanation is that there was a change in lung compliance as a result of the increased pulmonary tissue volume which increased the cost of breathing. This hypothesis is supported by the accompanying changes in $\dot{V}A/\dot{Q}$ which were suggestive of very early pulmonary edema (2). It is possible that there was a more mundane cause, since all the divers developed ear infections during the 7 days at 250 meters of chamber pressure. A central temperature rise of as little as 1° Farenheit would be enough to account for the secondary rise in oxygen uptake, since pulmonary capillary temperature was presumed to be 37°C. The divers were treated with oxytetracycline, which should not have affected gas exchange, but the pharmacological effects of this drug at increased ambient pressure are unknown.

The subjects in this experimental series were able to increase their stroke volume sufficiently to offset the decrement in heart rate, thus keeping their cardiac output within normal limits. Since the partial pressure of oxygen was relatively small compared with the pressure of the diluent gas, an oxygen-dependent decrease in heart rate would be expected, but the principal effect should have been on the non-oxygen-dependent decrease mediated over the sympathetic pathways, possibly combined with an increase in parasympathetic activity (10). This does not imply that the mechanical efficiency of the heart muscle itself was unimpaired, since a decrement in performance, if any existed, could have been masked by greater contractility in response to the increased venous return of exercise.

The divers all showed a widening arteriovenous CO_2 difference at heavy work, with a mean end-tidal P_{CO_2} of 49 mmHg at a mean oxygen uptake of 2.5 liters/min STPD. This agrees well with previous independent investigation within the laboratory (20). The cause of CO_2 retention during heavy work at increased ambient pressure has provided endless discussion for those with a philosophic bent. Movement of the equal pressure point as a result of increased gas density, with resulting $\dot{V}A/\dot{Q}c$ maldistribution and alveolar hypoventilation, has been discredited by a number of workers (17, 23). A simple physical effect of the dense gas which reduces the maximum exercise ventilation to a point where alveolar hypoventilation must ensue is more likely, although CO_2 retention seems to appear well before MEV is reached. There are also other possibilities, one of the most interesting of which is concerned with the change in Henry's law coefficient at increased pressure, described by the equation (18)

$$\frac{d\ln (s/p)}{dP} = \frac{\bar{V}_2}{RT} \quad (4)$$

where $\bar{V}_2$ is the partial molar volume of the gas in solution, (s/p) is the solubility per atmosphere partial pressure, P is the absolute pressure, R is the gas constant and T is the temperature. At 25 atmospheres, reduction of approximately 5% in solubility might be expected. Recent work by Kiesow (15) has shown that the O_2 dissociation curve moves to the left at increased ambient pressure, facilitating uptake and probably offsetting any slowing of diffusion into, or out of, the red cells. However, it is well known that for arterial/alveolar CO_2 equilibrium to take place at heavy work, the pathways for the release of CO_2 must be infinite. Considerable controversy still exists as to whether CO_2 equilibrium does in fact take place during the time course of one stroke volume of blood through the pulmonary capillaries (7). Bosman et al. (3) have shown that the output of CO_2 into the alveoli is only linearly related to blood flow for a very short period during systole at heavy work. Fifty percent of the CO_2 content of one stroke volume of blood can be absorbed by the pulmonary parenchyma, to be released along a time course that is much longer than that of the blood in the pulmonary capillaries. Since CO_2 moves across the alveolar membrane in molecular form and not in ionic form (5, 6), a reduction in solubility could emphasize this effect, particularly if carbonic anhydrase should prove to be affected by pressure. At 100 atmospheres the reduction in solubility could be as much as 20%. The same reasoning would apply to the uptake of oxygen across the alveolar membrane, and a reduction of much less than 20% in the solubility of oxygen could represent a very real diffusion block.

A further diffusion block for oxygen but not necessarily for CO_2 would arise if the lungs became edematous or pre-edematous. V_{tis} can be calculated from the zero intercept of the extrapolated semilogarithmic relationship between F_A/F_{A_0} and the time of breath-holding during a simple rebreathing maneuver (4). Blauser et al. (11) have shown that the Cander and Forster method of estimating pulmonary tissue volume is a sensitive and reliable method for detecting early pulmonary edema. A modification of the Cander and Forster method used in this experiment showed that an increase in pulmonary tissue volume was present after 24 hr in 1 sensitive subject, and in 3 out of the 4 subjects measured after 7 days' exposure to 250 meters of chamber pressure. One subject showed no change throughout the experiment. The subject who showed an immediate increase in V_{tis} also showed an immediate increase in the maldistribution of $\dot{V}_A/\dot{Q}c$ at work. All subjects gave some evidence of decremental changes in the distribution of $\dot{V}_A/\dot{Q}c$ at work after 7 days' exposure to maximum chamber pressure, although one of these subjects did not have a measurable increase in V_{tis}. It would be expected in these circumstances that perfusion would be affected more than ventilation (2), as indeed was the case.

An increase in V_{tis} was not expected during these experiments because the small size of the helium molecule should not have had an osmotic pressure effect on the pulmonary parenchyma (18). However, the subjects were breathing 0.4 bar oxygen, and it is possible that either the high partial pressure of oxygen in combination with the raised helium pressure overcame the compressing effect of the increased ambient pressure on the tissues, enabling critical volume to be reached, or that in some way helium facilitated the toxic effects of oxygen. Ardashnikova et al. (1) reported evidence of a rise in pulmonary vascular resistance and a reduction in pulmonary capillary blood flow in 2 of the subjects after 7 days at 5

atmospheres of oxy-nitrogen. They concluded that an increased partial pressure of oxygen may help divers to perform heavy work adequately during short exposures but that it may be contraindicated during saturation exposures. Harrison et al. (12) exposed three generations of mice to a 20% oxygen-80% helium environment at normal ambient pressure. They found that changes occurred in the alveolar capillary layer of the lung of the animals consistent with early pulmonary edema; these changes had only been seen previously in response to exposure to oxidated environments. Shanklin and Lester (21) also found that helium was a less effective protection against oxygen toxicity than nitrogen. Oxygen toxicity is notorious for its variability (8) and our results, together with those of Ardashnikova, are quite consistent with previous work, if in fact it was very early oxygen toxicity that caused the pulmonary changes in both cases. Further support of this hypothesis is provided by the steady decrease in hemoglobin (Hb) level through the experiment. The changes reported here were differentially reversed during decompression, apart from the Hb effects; such reversal was almost complete by 105 meters of chamber pressure. There was a discrepancy in the measurements obtained at the 40-meter stop on the second dive, when 2 of the subjects showed marked decremental changes in the distribution of $\dot{V}_A/\dot{Q}c$ and an increase in V_{tis}. However, it was later found that the 2 subjects involved had been given an antihistamine-privine spray to reduce nasal congestion. The drug could have had a marked constrictor effect on the pulmonary vasculature if absorbed into the lungs, and an effect of this kind would have been fully consistent with the changes found in the distribution of $\dot{V}_A/\dot{Q}c$. The alteration in V_{tis} may have been due to swelling in the nasopharynx which is included in the V_{tis} measurement, and which would not have been immediately reduced by a vasoconstrictor. If the results from these two subjects had been excluded from the data, the least squares regression at the 40-meter stop for λ plotted against $\dot{V}o_2$ would have been indistinguishable from the surface regression.

It would seem, therefore, that healthy divers are able to perform hard work at 800 ft of simulated seawater pressure, particularly during short exposures. Long exposures appear to lead to secondary changes in pulmonary function, one of the most important of which may be related to increased sensitivity to oxygen in a helium environment. Almost equally important if confirmed by further work is the secondary rise in $\dot{V}o_2$ that was found after 7 days of maximum chamber pressure. Changes have been found in 2-3 diphosphoglycerate after 3 days of exposure to a hyperbaric environment, and it is possible that changes may be taking place in cell structure (18, 15). Apart from insidious myopathal changes, it is interesting to speculate on a decompression schedule able to cope with inert gas molecules that were loosely bound to macromolecules in a manner reversible with pressure, such as hemoglobin. This situation would mean that more inert gas molecules would be available during decompression than simple considerations of solubility would indicate. Oxygen would appear to be the key to the problem, perhaps because of its effect on hemoglobin, but the line between oxygen toxicity and safe, fast decompression may turn out to be very slim.

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CARDIORESPIRATORY FUNCTION DURING ARM EXERCISE IN WATER AT 500 AND 600 FEET

Lennart Fagraeus and Peter B. Bennett

Our present knowledge of man's physiological responses to exercise in a hyperbaric environment is to a large extent based on information obtained during leg exercise, as described in recent reviews by Fagraeus (12) and Lanphier (22). However, at today's extreme operating depths, modern diving techniques frequently require the diver to operate from a submersible vessel, which reduces the amount of leg exercise. Instead the diver is required to perform his tasks almost entirely by using his arms. Since the physiological responses to arm exercise under normal conditions are different from responses to leg exercise (3,6,30), and because to our knowledge there are no relevant hyperbaric data in the literature, we undertook the present investigation to study cardiorespiratory responses to arm exercise in water during short exposures to 500 and 600 feet. Control studies were carried out under similar wet conditions at 1 atmosphere absolute (ATA), and additionally, under dry conditions at 1 ATA to determine the effects of immersion per se.

Method

These experiments took place during dives devoted to the development of subsaturation decompression procedures from depths in excess of 400 feet (4), which is the reason for the less than optimal control of environmental parameters.

Seventeen healthy male volunteers who were experienced divers served as subjects (commercial divers). (See Table I for anthropometric and functional data.) The studies were performed during complete immersion in cold water (mean temperature 11.4°C, range 8.2-16.5°C) in a hyperbaric chamber. Each subject was dressed in commercial diving equipment consisting of a rubberized canvas dry suit with neck dam seal and a helmet with demand regulator and mouthpiece.

The subjects performed steady-state arm exercise in a kneeling position on a cycle-type ergometer, the load of which could be set with a springloaded brake (Fig. 1). The ergometer was not calibrated but was checked in pretrial runs to give various constant loads. The subjects pedaled at a predetermined rate, usually 45 rpm, by following a calibrated tachometer. The experimental sequence consisted of an initial 5-6 minute period during which compression was carried out to the appropriate depth. The diver donned his helmet, entered the water and adjusted his buoyancy, started a 3-minute warm-up period on a light load, and then rested for a 2-minute period. Fifteen minutes after the start of compression, the subject

TABLE I

ANTHROPOMETRIC AND FUNCTIONAL DATA

Subject	Age, yr	Height, cm	Weight, kg	VC, l/min BTPS	MVV l/min BTPS
JM	23	168	78	4.89	161.6
JK	21	173	65	3.80	137.1
WM	24	178	86	6.36	185.6
MF	25	180	70	5.06	143.2
GP	26	173	69	4.81	174.7
EP	27	173	75	4.45	106.6
GK	22	178	75	5.24	160.9
TO	26	180	86	4.90	214.3
MD	23	191	84	5.88	200.2
MH	30	183	82	5.60	230.7
BS	24	183	84	5.59	162.1
DK	27	163	68	3.67	187.6
PB	28	178	83	6.21	150.4
PK	27	183	92	5.59	156.1
JB	25	191	98	6.52	154.5
KM	25	170	81	6.37	126.2
MD	21	183	85	6.60	173.9
Mean	24.9	178.1	80.1	5.38	166.2

VC = vital capacity; MVV = maximal voluntary ventilation (15 s)

began the first work load period of 6 minutes, followed by a 1-minute rest period; after the rest period a second work period of 6 minutes on a heavier load was performed. Total exposure time in each hyperbaric experiment was 30 min (including compression time). At 1 ATA the experimental sequence followed the schedule outlined above, but since time was not crucial three different work loads were performed. In addition to the 1 ATA wet experiments, further control studies were carried out at 1 ATA at room temperature under dry conditions, using the same equipment and procedure as above (except for the dry suit).

The ECG was monitored continuously using telemetry from a bipolar chest lead, and heart rate was recorded on a beat-by-beat basis. Wide-bore tubing attached to the expiratory valve of the helmet allowed collection of expired gas in 200-liter Douglas bags above the water level (Fig. 1). A short, thin-walled collapsible rubber segment was inserted in the underwater part of the sampling circuit to prevent any through-flow of inspirate (23). This flow control valve was attached to the lower front part of the helmet at a "balance" point subjectively chosen by the divers as the most comfortable, and corresponding to a level just above the suprasternal notch. Respiratory rate was obtained from a thermistor probe (Yellow Springs Instrument) in the mouthpiece.

The various gas mixtures breathed were air at 1 ATA (control), 7% O_2 in helium at 500 fsw (16.13 ATA), and 7% O_2 and 10% N_2 in helium at 600 fsw (19.16 ATA). The gas densities at 500 and 600 fsw were 3.81 and 6.33 g/l (BTPS), respectively, as compared to 1.17 g/l (BTPS) during air breathing at 1 ATA. Samples of inspired gas were drawn outside the chamber from the diver's supply line into glass syringes which were sealed with three-way plastic stopcocks and a film of dilute lactic acid between the plunger and barrel. Mixed expired gas

samples were drawn using the same technique during emptying of the Douglas bags through the chamber wall into a 350-liter Tissot gasometer (Fig. 1). The gas samples were immediately analyzed for O_2 and CO_2 by means of a gas chromatograph (Quintron Model H-3).

Reported cardiorespiratory data are the mean values observed during the last minute of each exercise period. Regressions were calculated by the method of least squares and statistical significance was determined by paired sample analysis (Student's *t*-test).

Results

After pretrial work-up dives it was decided to choose work loads sufficient to maintain exercise heart rate (HR) at depth within a range of 100–150 beats/min. Consequently at 500 fsw HR ranged from 94–149 beats/min and at 600 fsw from 97–159 beats/min, while under surface conditions the range was larger: 83–181 beats/min. The relation between HR and oxygen uptake ($\dot{V}O_2$) and their associated regression equations during the four different experimental conditions are shown in Fig. 2 and Table II. Whereas the $\dot{V}O_2$ under surface conditions ranged between 0.44–2.66 l/min STPD, corresponding values at 500 and 600 fsw were 0.60–2.14 and 0.72–2.23 l/min STPD, respectively. Exposure to 500 and 600 fsw did not reveal any changes in the HR response as compared to 1 ATA wet. Under dry surface conditions, however, HR was raised at comparable $\dot{V}O_2$ levels, but because of great variations in slopes and intercepts of individual regression lines in the dry surface condition, no significant differences between the two 1-ATA conditions were obtained. However, it was observed that each subject had a higher exercise heart rate in the dry than in the wet 1-ATA condition and, when comparing heart rates at $\dot{V}O_2$ levels of 1 and 1.5 l/min STPD, highly significant intra-individual differences averaging 17 and 15 beats/min were obtained ($P < 0.001$).

The relationship between ventilation ($\dot{V}E$) and $\dot{V}O_2$ is shown in Fig. 3 and Table II. The slopes of the regression lines at 500 and 600 fsw were significantly lower than at 1 ATA wet ($P < 0.05$ and $P < 0.001$, respectively). Between the two 1-ATA conditions, however, no

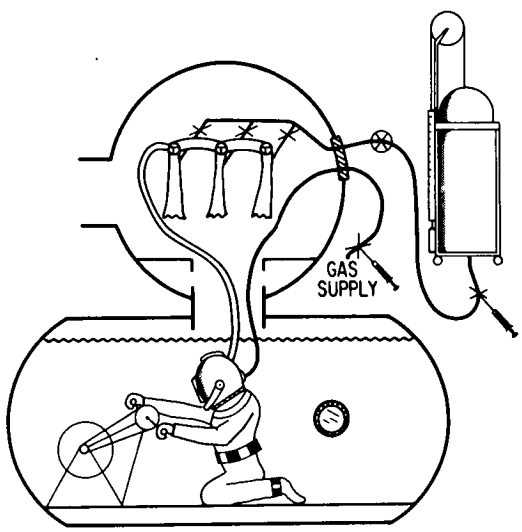

Fig. 1. Experimental equipment with diver in working position under water.

TABLE II

REGRESSION EQUATIONS RELATING OXYGEN UPTAKE, HEART RATE, VENTILATION, RESPIRATORY RATE AND TIDAL VOLUME DURING ARM EXERCISE IN WATER AT 1 ATA, 500 AND 600 FSW, AND AT 1 ATA, DRY

Condition	Regression Equation of Association	r
1 ATA, wet	$HR = 35.1\ \dot{V}O_2 + 80.2$	0.84
500 fsw	$HR = 32.4\ \dot{V}O_2 + 77.4$	0.91
600 fsw	$HR = 30.0\ \dot{V}O_2 + 85.4$	0.82
1 ATA, dry	$HR = 31.7\ \dot{V}O_2 + 100.6$	0.61
1 ATA, wet	$\dot{V}E = 34.8\ \dot{V}O_2 - 7.8$	0.93
500 fsw	$\dot{V}E = 18.6\ \dot{V}O_2 + 6.4$	0.94
600 fsw	$\dot{V}E = 19.3\ \dot{V}O_2 + 6.9$	0.88
1 ATA, dry	$VE = 37.4\ \dot{V}O_2 - 7.0$	0.84
1 ATA, wet	$RR = 10.40\ \dot{V}O_2 + 2.2$	0.83
500 fsw	$RR = 2.6\ \dot{V}O_2 + 12.9$	0.40
600 fsw	$RR = 6.8\ \dot{V}O_2 + 6.8$	0.59
1 ATA, dry	$RR = 7.6\ \dot{V}O_2 + 6.1$	0.69
1 ATA, wet	$VT = 0.5\ \dot{V}O_2 + 1.7$	0.43
500 fsw	$VT = 0.9\ \dot{V}O_2 + 0.7$	0.89
600 fsw	$VT = 0.4\ \dot{V}O_2 + 1.6$	0.34
1 ATA, dry	$VT = 1.2\ \dot{V}O_2 + 1.0$	0.72

Oxygen uptake = $\dot{V}O_2$, l/min STPD; heart rate = HR, beats/min; ventilation = VE, l/min BTPS; respiratory rate = RR, breaths/min; tidal volume = $\dot{V}T$, l/BPTS.

significant differences in the ventilatory responses were observed. The significant ventilatory reductions in the two hyperbaric conditions were brought about with a combination of small (and separately nonsignificant) changes in respiratory rate (RR) (Fig. 4, Table II) and tidal volume (VT) (Fig. 5, Table II).

Discussion

Total immersion in water creates a condition in which the weight of the body tissues and hydrostatic gradients in the vascular system tend to be almost exactly counterbalanced by the wet medium. The implication of weightlessness would appear to indicate that less work would be required to perform a given task. However, to overcome buoyancy problems divers usually wear weights, and this combined with resistance to movement in the watery medium and less than ideal breathing equipment contribute to making underwater work more strenuous than equivalent tasks above water.

Oxygen Uptake

Donald and Davidson (11) studied heavy arm work in oxygen-breathing divers in shallow waters and obtained an average oxygen uptake of 1.15 l/min STPD. In the present study, however, the heavy work loads at 500 and 600 fsw required average oxygen uptakes of 1.55

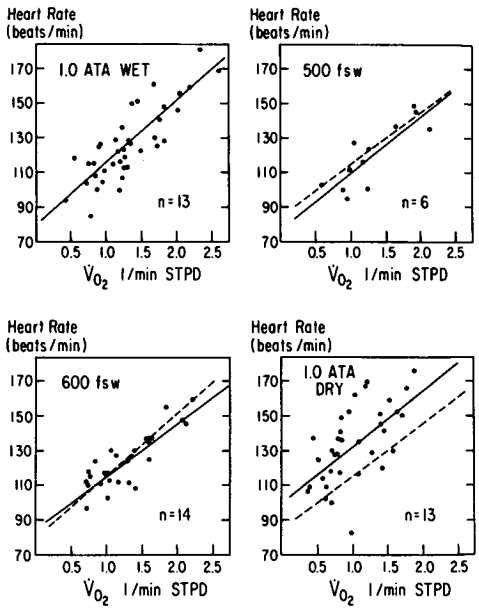

Fig. 2. Relation between heart rate (HR) and oxygen uptake ($\dot{V}O_2$) at 1 ATA wet (control), 500 and 600 fsw, and 1 ATA dry. In all figures, solid lines = regression equations; broken lines = corresponding controls, wet, at 1 ATA. For regression equations see Table II.

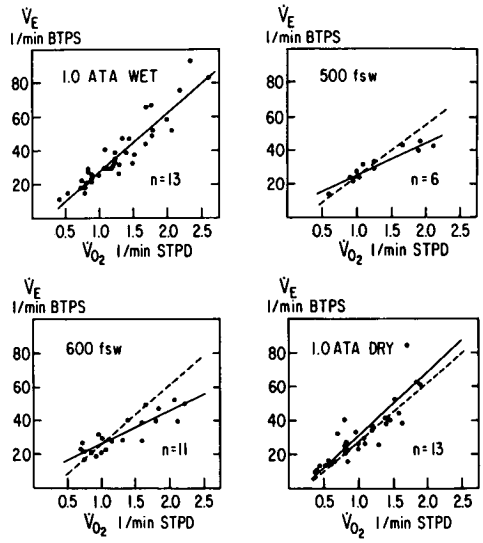

Fig. 3. Relation between ventilation ($\dot{V}E$) and $\dot{V}O_2$ at 1 ATA wet (control), 500 and 600 fsw, and 1 ATA dry.

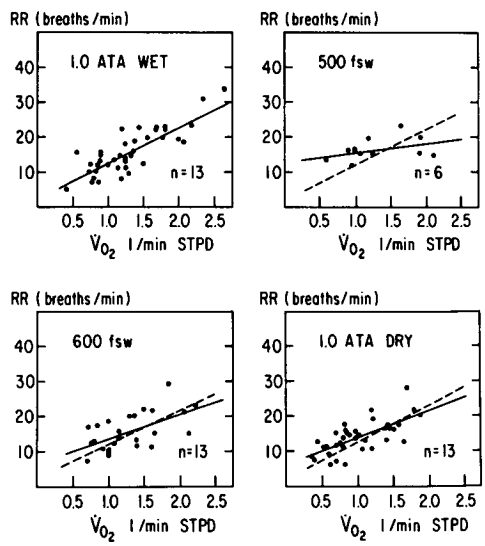

Fig. 4. Respiratory rate (RR) as a function of $\dot{V}O_2$ at 1 ATA wet (control), 500 and 600 fsw, and 1 ATA dry.

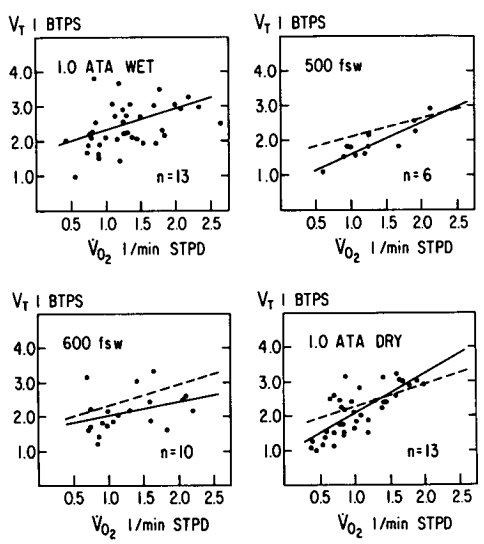

Fig. 5. Tidal volume (V_T) as a function of $\dot{V}O_2$ at 1 ATA wet (control), 500 and 600 fsw, and 1 ATA dry.

and 1.63 l/min STPD, respectively, and were subjectively considered very strenuous and far more heavy than the routine work performed in the field by these experienced divers. An interesting comparison is offered by the 0.90 l/min STPD of a previous study, in which divers at 500 fsw were allowed to choose arm exercise rates corresponding to those which they were accustomed to in the open sea (Fagraeus, unpublished observations). It is concluded therefore that the high work loads used in the present study were significantly heavier than those which are normally required in routine diving.

Heart Rate

There is general agreement that exposure to the hyperbaric environment is accompanied by a lower than normal heart rate response both to rest and leg exercise. Thus, an increase in inspired P_{O_2} has been shown to exert a significant effect (12,14,15,16,27), but other non-oxygen-dependent factors operating under increased ambient pressures also contribute to the decrease in heart rate at depth (12,13,14,15,16,17,19,29). The absence of any bradycardia at 500 and 600 fsw in the present study (Fig. 2) in the face of a high P_{O_2} (1.13–1.34 ATA) and substantially increased ambient pressures is therefore difficult to explain. Regardless of the cause, however, the unaltered heart rate response to immersed arm exercise from the surface down to 500 and 600 fsw may offer a simple and useful tool for estimating the diver's work load under water within that pressure range.

Using heart rate values recorded in the dry at 1 ATA for such estimations, as suggested by Weltman and Egstrom (31), would not seem feasible in view of the significant "immersion" bradycardia seen in our study at the surface upon transition from the dry to the wet environment (Fig. 2). Previous studies have shown that cold and wet environments can bring about a significant bradycardia, either secondary to peripheral vasoconstriction or through a direct reflex from cold receptors in the face (5,8,25). These effects were avoided in this study by using a protective dry suit with thermal underwear and an insulated helmet. The question then arises whether the present immersion bradycardia at 1 ATA could be related to changes in blood distribution, since it has been reported that during immersion the hydrostatic forces acting on the lower part of the body cause a central shift of blood from dependent veins (2,18,20). However, this central shift of blood causes a tachycardia and an increase in cardiac output during immersed leg exercise (10,18), and would not, therefore, explain the present immersion bradycardia during arm exercise at 1 ATA.

A possible explanation may instead be found in the special physiology associated with arm exercise per se. It is known that under normal conditions subjects have a higher heart rate during arm work than during leg work at comparable levels of oxygen uptake (3). This difference in circulatory adaption indicates a lower venous return, a higher relative work load and a higher sympathetic tone during arm exercise (6,30). If, however, at a given oxygen uptake the venous return to the heart is increased, e.g., by changing from the sitting to the supine position, cardiac output and stroke volume increase while heart rate decreases significantly (6,30). It seems probable then that the observed immersion bradycardia during arm exercise in water at 1 ATA in the present study can be explained as secondary to an increased central blood volume, in contrast to the tachycardia seen during immersed leg exercise.

VENTILATION

The observation that ventilatory response to heavy arm exercise was diminished at 500 and 600 fsw (Fig. 3) is in accordance with a review of previous exercise studies by Lanphier (22). It has been shown that an increase in P_{O_2} exerts a distinct retardant effect on exercise ventilation in the hyperbaric environment (12,13,14,21) and so does a higher than normal gas density (12,13,15,19,24,29). Recent studies in this laboratory indicate also that hydrostatic pressure per se or in combination with hyperbaric helium affects the respiratory regulation (26,28). Regardless of which factor predominates, in the present study the net effect at pressure is insufficient alveolar ventilation and consequent impeded CO_2 elimination. This may be reflected in the observation that most mixed expired P_{CO_2} values were close to or above 40 mmHg at the heavy work loads at 600 fsw as compared to values close to or below 30 mmHg under surface conditions.

If a reduction in lung volume secondary to a central shift of blood such as has been described in immersed subjects (1,7,9,20) did occur in the present study, some increase in airway resistance and a lower pulmonary ventilation would be expected in our subjects when exercising at 1 ATA during immersion compared to testing under similar but dry conditions. The unchanged ventilatory response observed in both surface conditions (Fig. 3), however, suggests that the responses to a larger central blood volume with compression of dependent lung units were modified by the breathing and gas collection systems. In fact, positive pressure breathing while in the immersed state has been reported to restore normal lung volumes and respiratory function (7,18,20). A pilot study of one of the divers showed an unchanged maximum voluntary ventilation (MVV) between dry and wet surface conditions, which would seem to indicate that positive pressure breathing mechanics may indeed have been involved in the present study.

Conclusions

We conclude that complete immersion at 1 ATA exerts a significant depressant effect on heart rate response to steady-state arm exercise, while increasing the pressure to 16 and 19 ATA, respectively, does not enhance this effect. Conversely, the ventilatory response is not affected by immersion per se, but decreases with increasing pressure. To what extent the protective suit and breathing equipment were responsible for the disparate effects of the wet hyperbaric environment on circulation and ventilation, respectively, has not been established and deserves further study.

ACKNOWLEDGMENTS

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PHYSIOLOGICAL STUDIES OF DIVERS WORKING AT DEPTHS TO 99 FSW IN THE OPEN SEA

Jeffrey Dwyer and Andrew A. Pilmanis

In the mid-1950's, Donald and Davidson (3) and Goff, Frasetto, and Specht (8) measured the oxygen consumption rates of divers working in the ocean at depths to 25 fsw. These studies and the *Cooperative Underwater Swimmer Project* of 1953 marked the beginning of undersea work physiology research, but this line of investigation has not been continued. Instead, many scientists began using hyperbaric chambers and shallow immersion tanks for diving physiology experiments involving work or exercise. The instrumentation advantages of chambers and immersion tanks, chamber depth capabilities, and the need for studies of mixed-gas at high pressures promoted a rapid shift from open-sea physiological research. However, recent advances in undersea science and technology have greatly expanded the diver's role in all phases of ocean exploration, exploitation, and research, and it has become obvious that little is known about the physiological basis of undersea work and the working diver's physical limitations. The importance of ensuring man's survival hundreds of feet beneath the sea and the race to extend depth limits commanded the attention of many scientists, and left only a few to study man as he worked in the ocean. Consequently, man's depth capability now extends to 2,000 fsw, while our knowledge of the physiology of diver work performance at relatively shallow depths remains far from complete. Our purpose therefore was to develop and utilize data acquisition systems to measure twelve physiological parameters under twelve work/depth conditions in the open sea.

One of our primary objectives was to measure the oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (R), and kilocalorie expenditure (kcal) during rest and work at depths to 99 fsw. It is well established that the $\dot{V}O_2$ during work performed in hyperbaric chambers is increased as the pressure is raised (2, 15, 18, 24, 25, 27). This is presumably due to a progressive increase in atmospheric density which increases the oxygen cost of breathing (7). A similar pattern was reported by Russell, McNeill, and Evonuk (23) for SCUBA divers at rest and during fin-swimming at moderate speed at depths to 66 fsw in a lake. However, their data were greatly influenced by extremely cold water which caused severe shivering during rest. We therefore attempted to measure the $\dot{V}O_2$ to determine if the usual relationship with depth would be found in shallow to moderately deep ocean dives, and also to measure the magnitude of the oxygen uptake difference at various depths. Other studies of oxygen consumption during fin-swimming in the ocean offered no further insight into $\dot{V}O_2$ patterns for different depths in the sea (8, 14). Also of interest was the actual oxygen consumption required for the performance of three specific work loads

imposed by a unique ergometer. It was anticipated that these data would enable a precise and meaningful interpretation of work performed on the ergometer in terms of energy requirements and equivalent land work stress.

A second objective was to measure the ventilation frequency (Vf), tidal volume (V_T), and pulmonary ventilation ($\dot{V}_E$) patterns of highly experienced divers. We were interested in changes which might occur in ventilation frequency and tidal volume and their possible effects on pulmonary ventilation as ambient pressure increased with greater depths. Previous studies have indicated that pressure-related reductions in Vf are frequently not compensated for by increases in V_T (12, 13, 25). As a result, the pulmonary ventilation and alveolar ventilation ($\dot{V}_A$) are reduced, leading to alveolar hypoventilation (5, 13). The only open-water study of ventilatory patterns which is available (23) supports these findings for chamber dives, but alveolar ventilation and alveolar partial pressure of carbon dioxide ($P_{A_{CO_2}}$) were not reported. Therefore, we also measured these parameters in an attempt to trace the development of carbon dioxide retention as work load and depth increased.

Our third objective was to examine the relationship between heart rate (HR) and oxygen consumption rate during underwater work at three depths. Weltman and Egstrom (28) concluded that the heart rate measured during underwater work could be used to characterize the physiological load in terms of surface equivalent work output. Land and underwater work loads of unknown dimensions were declared equal in cardiorespiratory stress solely on the basis of heart rate response. Other investigators found the heart rate an inaccurate index of the diver's work stress (15, 17). Regression lines for V_{O_2}-HR data were found to vary significantly with water temperature and pressure. We extended this line of investigation to moderately deep depths and very heavy work loads to determine the feasibility of using heart rate to assess energy expenditure and work stress in open-sea dives.

Methods

This investigation was conducted at the University of Southern California Santa Catalina Marine Biological Laboratory located on Santa Catalina Island, California. The data were collected at depths of 33, 66, and 99 fsw in water temperatures of 14.5, 13.0, and 11.0°C, respectively. Six highly experienced male SCUBA divers served as subjects. Each diver had at least five years of extensive diving experience and three were certified diving instructors. Vital statistics (means ± SD) describing the subjects follow: weight (kg), 75.45 ± 7.65; height (cm), 181.05 ± 8.29; BSA (m^2), 1.94 ± 0.13; and age (yr), 26 ± 0.14.

Each subject wore a standard diver's wet suit with gloves, boots, and hood. Weight belts were worn to achieve neutral buoyancy at the surface and a buoyancy compensator was used to compensate for changes in buoyancy as depth increased. The same style of fins (Jet Fin by Scubapro, Compton, Calif.) was used by all divers to eliminate wide variations in kick thrust due to fin size and configuration. The air supply was a self-contained system consisting of a standard SCUBA tank and a double-hose, two-stage regulator (U.S. Divers Co., Anaheim, Calif.). Filtered compressed air was the only breathing gas used.

Standardized underwater work loads were performed on a unique mobile ergometer described by Pilmanis, Henriksen, and Dwyer (21). The device is essentially a drag board which the diver holds in front of him as he swims, using a leg kick with the aid of fins. A drag indicator located on the ergometer continuously informed the diver of the resistance he was

working against. These data, together with measurements of the swim speed, were sufficient for the calculation of the amount of work performed in moving the ergometer through the water. However, the total work output could not be measured because the diver performed an unknown amount of additional work when moving his body and life-support equipment through the water. At any particular resistance on the ergometer, the swim speed was found to be constant. Therefore, work loads were reproductible and there was no need for external pacing to maintain a constant velocity. When swimming with the ergometer the diver works as he would if he were transporting a large tool or other resistive object to an underwater work site. Swims were performed at resistances of 1.5, 2.0, and 2.4 kg over a 50-meter course marked on the ocean floor in three locations with depths of 33, 66, and 99 fsw.

A compact, 11-channel underwater recording system developed by Pilmanis et al. (20) was used to obtain physiological data required for this investigation. The recorder was housed in a pressure-proof aluminum case attached to the diver's air tank. Measures of depth and water temperature were obtained by transducers mounted on the outside of the recorder housing. Heart rate was computed from the electrocardiogram recorded over precordial leads. The number of breaths taken per unit time (ventilation frequency) was determined from the signal of a thermistor inside the diver's mouthpiece.

Pulmonary ventilation was determined from the tank pressure differential which was monitored by a general pressure transducer built into the recording system. The volume of air removed from the tank over 1-minute periods was calculated in liters and corrected to STPD. The result was a measure of the inspired volume ($\dot{V}_I$) which was converted to expired volume ($\dot{V}_E$) by a factor derived by Dwyer (4). A depth correction factor and a BTPS factor were then applied to the data to obtain the conventional measure of pulmonary ventilation ($\dot{V}_{E_{BTPS}}$). The tidal volume was calculated by dividing $\dot{V}_E$ by Vf.

To determine oxygen consumption and carbon dioxide production it was necessary to obtain samples of mixed-exhaled gas. Sampling systems developed by other investigators (6, 17, 23, 26) were considered inadequate for our purposes so a new system was designed. Our system operated on the principle of vacuum sampling, and consisted of a U-shaped manifold which interrupted the exhaust hose of a double-hose regulator, and 10 evacuated steel cylinders which were connected to the manifold by O-ring-sealed valves. Unlike the apparatus designed by Loomis et al. (17), the diver's exhaled gas does not need to be vented to the surface, and in contrast to the systems of Foley et al. (6) and Russell et al. (23), gas sampling was not limited to one continuous sample. Our system allowed as many as 10 samples to be taken at several levels of metabolic activity and at varying depths during a single dive.

Three dive sites were located with depths of approximately 35, 70, and 103 fsw, so the divers could maintain the prescribed experimental depths by swimming a few feet off the bottom. At each depth physiological measurements were taken during the last minute of a 5-minute rest period and 3 4-minute work periods. The divers rested on their knees with their trunks at a slight forward angle. This position was maintained with minimal muscular involvement. In each 4-minute work period the divers swam against a resistance of 1.5, 2.0, or 2.4 kg so that each resistance was used at 33, 66, and 99 fsw. The rest period was followed by work at 1.5 kg at each depth. At 33 and 66 fsw the two remaining work loads were performed during a single dive to each depth. Due to the large air consumption rate of the divers at 99 fsw, the 2.0 and 2.4 kg work loads were performed during separate dives.

Results

Data for oxygen consumption, carbon dioxide production, respiratory exchange ratio, and kilocalorie expenditure are presented in Table I. In the resting condition the oxygen uptake was increased by 46 cc/min between 33 and 66 fsw, but essentially no change was found as the depth increased further to 99 fsw. Only two divers demonstrated a progressive increase in V_{O_2} over the full range of depths. Data showed that oxygen consumption for work at 2.4 kg progressively increased with depth, in the usual manner. The largest V_{O_2} for an individual diver was 3.89 l/min at 99 fsw. Under the 1.5 and 2.0 kg work conditions the oxygen uptake was highest at 66 fsw, but it was always higher at 99 fsw than at 33 fsw. This unusual pattern was found among the individual data for four divers at 1.5 kg and two at 2.0 kg. In the remaining cases the usual relationship between depth and V_{O_2} was found.

At rest the ventilation frequency showed small but progressive decreases as depth increased, but pulmonary ventilation and tidal volume were highest at 66 fsw (Table II). Individual data revealed a wide variation in pulmonary ventilation at 33 fsw. One diver respired 27.28 l/min while another required only 7.29 l/min. At the two remaining depths, however, the range was much smaller. Tidal volume and ventilation frequency showed little variation among the divers although one man did have a tendency to breathe at a higher frequency and lower V_T than the others at 33 and 99 fsw. During exercise at the two heaviest loads the ventilation frequency showed a tendency to decrease with depth but this pattern did not occur at 1.5 kg. Similar patterns were found in tidal volume and pulmonary ventilation at 1.5 and 2.0 kg, with the highest value occurring at 66 fsw and the lowest at 33 fsw. Under heavy work at 2.4 kg this pattern was reversed.

Alveolar ventilation and P_{CO_2} are presented in Table III. Alveolar ventilation was calculated by the formula: $\dot{V}_A = \dot{V}_E - \dot{V}_{DS}$. The dead space (DS) volume was computed by

TABLE I

OXYGEN CONSUMPTION, CARBON DIOXIDE PRODUCTION, RESPIRATORY EXCHANGE RATIO, AND KILOCALORIE EXPENDITURE DURING REST AND WORK AT 33, 66, AND 99 FSW

Condition	Depth, fsw	V_{O_2}, l/min, STPD		V_{CO_2}, l/min, STPD		V_{CO_2}/V_{O_2}		kcal/min/m²	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rest	33	0.385	0.177	0.322	0.130	0.84	0.04	0.986	0.44
	66	0.431	0.151	0.354	0.130	0.81	0.06	1.118	0.40
	99	0.429	0.173	0.356	0.141	0.82	0.03	1.103	0.43
1.5 kg	33	1.760	0.244	1.501	0.211	0.84	0.01	4.537	0.65
	66	2.370	0.344	2.128	0.333	0.89	0.06	6.209	1.02
	99	1.982	0.345	1.919	0.514	0.87	0.07	5.112	0.82
2.0 kg	33	2.539	0.245	2.257	0.171	0.96	0.03	6.535	0.57
	66	2.867	0.261	2.605	0.278	0.94	0.08	7.419	1.01
	99	2.715	0.281	2.474	0.321	0.90	0.05	7.024	1.02
2.4 kg	33	3.279	0.174	3.179	0.114	0.96	0.03	8.471	0.78
	66	3.325	0.256	3.253	0.206	0.94	0.08	8.587	0.92
	99	3.465	0.310	3.372	0.299	0.97	0.05	8.946	1.02

Values are means ± SD; $n = 6$.

TABLE II

PULMONARY VENTILATION, VENTILATION FREQUENCY, TIDAL VOLUME AND VENTILATION EQUIVALENT DURING REST AND 3 WORK LEVELS

Condition	Depth, fsw	V_E, l/min, BTPS		Vf, Breaths/min		V_T, l/Breath		Ventilation Equivalent (l V_E/l O_2)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rest	33	13.34	7.32	11.33	1.96	1.19	0.59	34,87	8.03
	66	15.67	4.51	10.50	1.64	1.49	0.43	38.74	15.74
	99	11.36	4.24	10.16	2.48	1.14	0.39	28.97	10.78
1.5 kg	33	37.35	10.12	21.50	4.53	1.77	0.48	21.08	4.28
	66	49.35	11.29	23.00	3.34	2.14	0.37	20.77	3.14
	99	43.47	11.19	23.83	9.60	1.92	0.51	22.27	5.99
2.0 kg	33	54.82	14.89	27.83	10.18	2.07	0.59	19.67	6.10
	66	63.94	8.76	25.50	5.12	2.55	0.35	22.51	4.13
	99	58.91	3.11	24.83	4.11	2.41	0.31	21.82	1.84
2.4 kg	33	78.54	10.74	30.00	6.22	1.67	0.61	24.03	3.72
	66	70.41	13.69	29.50	7.58	2.44	0.46	21.39	5.14
	99	70.90	8.87	27.66	4.47	2.58	0.12	20.69	4.03

Values are means ± SD; $n = 6$.

multiplying the divers' breathing rate times individual values established for respiratory dead space, according to the guidelines of Radford (22) and Asmussen and Nielsen (1), and the dead space of the mouthpiece. The alveolar P_{CO_2} was then computed from individual $\dot{V}_A$ and $\dot{V}_{CO_2}$ data according to the formula suggested by Lanphier (16).

Alveolar ventilation measurements generally follow an inverse pattern for $P_{A_{CO_2}}$. However, on an individual basis there were several different patterns for both parameters under each work condition over the three experimental depths. Many of the divers had abnormally low or high $P_{A_{CO_2}}$ during one or more work/depth conditions. There were several conditions in which the $P_{A_{CO_2}}$ exceeded 50 mmHg, but we could not identify any particular level of energy expenditure at 33 and 66 fsw beyond which the CO_2 retention occurred in a majority of the divers. At the deepest depth, however, four divers had alveolar CO_2 tensions which were higher at 2.4 kg than under the other two work loads. There was no consistent tendency for hypoventilation and elevated $P_{A_{CO_2}}$ at 1.5 and 2.0 kg as depth increased. However, at 2.4 kg five divers showed increases in $P_{A_{CO_2}}$ between 33 and 99 fsw, although two had their highest values at 66 fsw.

The highest $P_{A_{CO_2}}$ was found in *subject AP* at 33 fsw with the 1.5 kg work load. He did not report any symptoms or unusual feelings related to his carbon dioxide retention. However, immediately upon surfacing from dives to 99 fsw, *subjects JD* and *BJ* reported headaches. Their $P_{A_{CO_2}}$ values were 58.1 and 61.1 mmHg, respectively, following work at 2.4 kg. *Subject CS* also reported a headache upon surfacing from a dive to 99 fsw. His $P_{A_{CO_2}}$ was 57.8 mmHg during work at 1.5 kg.

As depth increased there was a progressive bradycardia during work at the two heaviest loads (Table IV). However, the heart rate at rest showed no meaningful relationship with depth. In fact, each diver displayed a different pattern over the three depths. A similar situa-

TABLE III

ALVEOLAR VENTILATION AND CARBON DIOXIDE TENSION DURING WORK AT 33, 66, AND 99 FSW

Condition	Subject	V_A, l/min, BTPS			P_{ACO_2}, mmHg		
		33	66	99	33	66	99
1.5 kg	*AP*	15.88	25.70	33.77	68.20	60.36	64.57
	BJ	33.02	47.05	32.85	44.13	42.64	49.56
	BK	43.19	42.86	48.32	32.22	42.44	28.80
	CS	36.60	58.11	30.68	37.39	39.71	57.81
	JD	28.45	36.41	41.96	49.49	48.69	47.89
	KW	20.34	36.91	21.96	51.27	42.28	43.30
	Mean	29.58	41.17	34.92	47.19	46.02	48.65
2.0 kg	*AP*	48.09	58.78	48.14	38.94	33.29	39.79
	BJ	40.99	62.48	47.60	53.57	40.91	40.40
	BK	63.61	61.30	51.29	31.05	32.20	37.88
	CS	44.52	51.20	53.53	45.08	47.34	49.08
	JD	48.15	41.94	50.11	38.71	54.43	43.25
	KW	23.51	53.06	49.62	51.08	43.19	44.97
	Mean	44.81	54.79	50.04	43.07	41.89	42.56
2.4 kg	*AP*	72.93	45.71	60.46	36.61	60.48	44.16
	BJ	65.26	61.69	44.92	41.78	51.20	61.11
	BK	80.26	73.34	67.75	34.26	36.83	41.08
	CS	56.46	41.46	58.28	51.82	66.61	56.54
	JD	56.94	57.77	54.65	48.11	48.09	58.09
	KW	60.60	64.70	62.66	43.68	41.34	44.67
	Mean	65.40	57.44	58.12	42.71	50.75	50.94

TABLE IV

HEART RATE AND OXYGEN-PULSE DURING REST AND 3 LEVELS OF WORK

Condition	Depth, fsw	Heart Rate, bpm		Oxygen-Pulse, cc O_2/beat	
		Mean	SD	Mean	SD
Rest	33	74.00	8.29	5.15	2.62
	66	68.83	9.68	6.40	2.53
	99	75.33	12.98	5.88	2.86
1.5 kg	33	134.50	19.01	13.16	1.43
	66	143.83	20.22	16.65	2.51
	99	138.83	18.70	14.45	2.97
2.0 kg	33	159.66	18.43	16.11	2.76
	66	155.66	13.23	18.49	1.94
	99	151.16	16.30	18.07	2.22
2.4 kg	33	172.16	11.56	19.10	1.31
	66	164.50	12.64	20.33	2.41
	99	163.50	14.88	21.33	2.63

Values are means ± SD; $n = 6$.

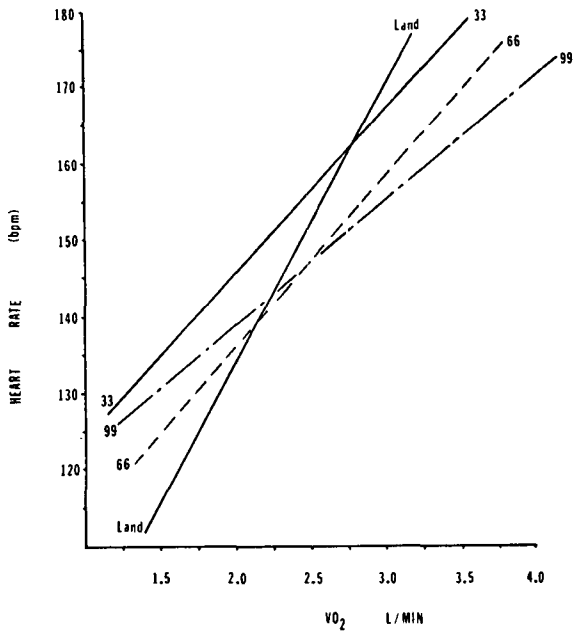

Fig. 1. Heart rate versus oxygen consumption during 3 levels of work on land and at three depths in the ocean. Land work loads were 540, 900, and 1260 kpm/min and the underwater work resistances were 1.5, 2.0, and 2.4 kg (n = 6).

tion was found at the 1.5 kg load but the mean values followed the same pattern as that for the oxygen consumption.

To examine the relationship between heart rate and oxygen consumption, mean values for these parameters were used to construct the curves for each depth presented in Fig. 1. For comparison, a curve for land work on a bicycle ergometer at sea level is included. At moderately high oxygen uptake rates and above, the heart rate is lower at any particular $\dot{V}O_2$ as the work situation progresses from land to 99 fsw. However, at low rates of oxygen uptake this relationship is nearly reversed. From Fig. 1 it is evident that underwater heart rates cannot be evaluated on the basis of the HR-$\dot{V}O_2$ relationship for work performed on land. Individual divers were studied by interpolating heart rates at 1.5, 2.0, 2.5, and 3.0 liters O_2/min from individual HR-$\dot{V}O_2$ curves drawn for land and underwater work. The data of two representative subjects are given in Table V. These data indicate that significant errors in estimated oxygen consumption and work stress would be made if heart rates measured underwater were evaluated in terms of the individual's land HR-VO_2.

Discussion

The resting oxygen consumption at 33 fsw was 38 cc/min greater than measurements made on land. Between 33 and 66 fsw, the difference was 46 cc/min but no increase occurred between the two deeper depths. In contrast, Russell et al. (23) found differences over 1-ATA intervals which exceeded 100 cc/min during an open-water dive. However, the cold stress was greater as the depth increased because wet-suit compression and shivering became severe at 3

TABLE V

INDIVIDUAL HEART RATES INTERPOLATED FOR 4 LEVELS OF OXYGEN CONSUMPTION

Subject	$\dot{V}O_2$, l/min	Heart Rate, bpm			
		Land	33 fsw	66 fsw	99 fsw
AP	1.5	103	101	97	108
	2.0	119	115	112	119
	2.5	137	130	127	130
	3.0	153	145	141	141
JD	1.5	96	122	111	114
	2.0	120	140	90	124
	2.5	144	155	120	135
	3.0	168	175	151	145

Data interpolated from regression lines drawn for land and underwater work.

ATA where the $\dot{V}O_2$ reached 755 cc/min. In the present study none of the divers felt cold or cool at 99 fsw. Soviet investigators (11) reported $\dot{V}O_2$ for a period of "relative calm" which exceeded 1.41 l/min. However, difficulties in maintaining positional stability apparently prevented complete relaxation in these experiments. In our experiments the resting position was maintained with minimal muscular involvement. Donald and Davidson (3) reported data which suggest that the amount of oxygen required for postural work under water is reduced due to the buoyant properties of the diving suit. Therefore, it is unlikely that our $\dot{V}O_2$ differences for land, 33 fsw, and 66 fsw reflect muscular effort required to maintain a fixed position in the sea.

The influence of increased ambient pressure on the resting $\dot{V}O_2$ is mediated through greater gas density which, in turn, increases the work of breathing (2) (16) (27). Studies with normoxic helium mixtures at high pressure (2) (25), gases more dense than air at sea level (7), and oxygen at moderate pressure (24) indicate that the oxygen cost of breathing is roughly 1.22 cc/l V_E/atm when air at sea level is the density reference expressed in atmospheres. With this figure the estimated oxygen cost of breathing between 33 and 66 fsw was 18.8 cc/min. Thus, the work of breathing probably accounted for less than one half of the "excess" oxygen uptake. The remainder may have been caused by a very mild cold effect. We have no explanation for the lack of a $\dot{V}O_2$ increase between 66 and 99 fsw. However, our data indicate that diving to greater depths in the ocean with conventional equipment does not invariably increase the oxygen consumption.

During work at 2.4 kg, the oxygen uptake showed a progressive increase with depth which conforms to the usual relationship between these parameters (2, 23, 25, 27). The differences in $\dot{V}O_2$ between depths may be sufficiently small to be wholly attributed to the effects of increased gas density and perhaps to lower water temperatures. However, because of the high P_{IO_2} it is reasonable to suspect that part of the "excess" oxygen uptake was used to reduce the contribution of anaerobic energy metabolism as suggested by Salzano et al. (24), Taunton et al. (27) and Bradley et al. (2). However, experimental evidence in support of this contention is not substantial. Taunton et al. (27) reported less disturbed blood pH during work

under hyperbaric conditions, which suggests reduced production of lactic acid, but Salzano et al. (24) found no difference in blood lactate following work at 1 and 2 ATA in 100% oxygen. In contrast, there is evidence for a substantial increase in anaerobic energy metabolism during work at 5 ATA in air (14). The excess $\dot{V}O_2$ of 700 cc/min found between 1 and 5 ATA certainly added to the aerobic energy production compared to that at 1 ATA, but the O_2 debt was still 55% higher. Therefore, it may be incorrect to assume that an excess $\dot{V}O_2$ represents an actual reduction of anaerobic energy metabolism compared to that in identical work conditions at 1 ATA.

The oxygen consumption at 1.5 and 2.0 kg followed an unusual pattern over the three experimental depths. It was highest at 66 fsw, always higher at 99 than 33 fsw, and differences between depths were generally in excess of 300 cc/min. Operational difficulties which tended to occur at 66 fsw appear to be responsible for this finding. Some divers overinflated the buoyancy compensator at this depth, under one or both work conditions, and swam with their feet above the depth plane. In other cases divers felt that they properly inflated the compensator but they swam with a "feet-down" attitude similar to that reported by Goff and his associates (9). One diver, swimming with an incorrect body position against 1.5 kg at 66 fsw, had a $\dot{V}O_2$ which was 1.035 l/min greater than at 33 fsw and .755 l/min larger than his rate at 99 fsw. These and other deviations in $\dot{V}O_2$ are similar to those reported previously for divers swimming in a moving water tank (9). In many cases swims were performed properly and buoyancy compensation was adequate. However, errors in swim attitude committed by a few of the divers under one or two conditions significantly influenced the mean $\dot{V}O_2$ figure at 66 fsw. These findings indicate that a diver's oxygen uptake at a specified work load may be significantly altered by factors other than pressure and cold. Slight changes in swim attitude, as well as efficiency and drag configuration, may increase the oxygen requirement by more than one liter.

Based on a regression line for oxygen consumption and land work output, the underwater work loads were approximately equivalent to 900, 1250, and 1600 kpm/min. It must be recognized that these are estimates of the equivalent land work stress. The actual physical work output was, of course, very much less than these figures would indicate, because of the inefficient nature of fin-swimming. However, subjective evaluations by the divers generally agreed with these estimates. Furthermore, five of the divers felt they could not have continued the heaviest load for an additional minute at any depth. After four minutes of 2.4 kg work at 99 fsw, these divers reported extreme fatigue and pain in the quadriceps muscles, and an acute awareness of respiratory impairment. At 33 fsw heat stress also contributed to the subjects' desire to stop work. In some cases the divers noticed headaches during the last minute of work, which perhaps signalled that the diver was approaching his physiological limits because of CO_2 retention. We feel certain that our subjects were working very close to their maximal aerobic power, but we only have comparable data for land work for *subject JD*. This diver reached 91% of his $\dot{V}O_{2\,max}$ at 99 fsw, but in so doing he developed an acute headache and a P_{ACO_2} of close to 60 mmHg. Our results indicate that work demanding a $\dot{V}O_2$ in excess of 3.0 l/min would require exceptional efforts from divers which could only be maintained for a few minutes.

Several investigators have found that ventilation frequency decreases and tidal volume increases as the ambient pressure is raised above sea level to as much as 31.3 ATA (2, 12, 13, 25). However, there is not complete agreement on the effects of increased pressure on

pulmonary ventilation. Bradley et al. (2) found no change in $\dot{V}_E$ during work in normoxic helium at 150 to 600 fsw. Others have found a slight reduction during rest at 99 fsw in air and moderate exercise at 1000 fsw in helium (25), and a 47% reduction during exhausting work at 6 ATA in air (5). The only comparable open-water study of ventilatory patterns found the classic patterns for moderate work as pressure increased from 1 to 3 ATA in air, including a 7.6% reduction in pulmonary ventilation (23). Unfortunately, our results are of no help in resolving this issue. We did find a progressive reduction in Vf at two work loads but the changes were small. The data for pulmonary ventilation and V_T at 1.5 and 2.0 kg follow the patterns found for oxygen uptake, and the same factors which altered the $\dot{V}_{O_2}$ probably influenced respiration as well. Under conditions of very heavy work (2.4 kg) the pulmonary ventilation was reduced between 33 and 66 fsw, but no change occurred between the two deeper depths. It is possible that the accumulation of acid products of metabolism at the heaviest work load may have counteracted any further depressive effect of gas density and high P_{IO_2} on respiration when compared to 66 fsw.

Jarrett (13) found that work loads requiring as little as 1 liter O_2/min resulted in consistent increases in P_{ACO_2} as the pressure was raised from 1 to 4 ATA. Furthermore, at constant pressures up to 4 ATA, the P_{ACO_2} progressively increased with the work load. Goff et al. (10) also found consistent elevations of end-tidal P_{CO_2} during swims in a moving water tank at 2 to 3 feet. On the basis of these studies and the greater resistance to breathing found in conventional underwater breathing apparatus, we expected CO_2 retention to occur in most of the subjects under each work/depth condition. Yet our results at 1.5 and 2.0 kg indicate no consistent tendency among the divers to hypoventilate and retain CO_2 to a greater degree as the depth increased. However, when the work load was raised to 2.4 kg, divers increased their P_{ACO_2} between 33 and 99 fsw, although two divers had their highest values at 66 fsw. Similarly, when depth was held constant we could not identify any particular level of energy expenditure at 33 and 66 fsw beyond which CO_2 retention occurred in a majority of the divers. At 99 fsw, however, four divers had higher P_{ACO_2} at 2.4 kg than they had under the other two work loads. It appears that our depth/energy expenditure threshold for CO_2 retention is much higher than would be expected on the basis of Jarrett's experiments in a hyperbaric chamber. But we should point out that the highest values for P_{ACO_2} occurred at 33 fsw under the lightest work load and significant CO_2 retention can occur under any work/depth condition.

We do not know why some of our divers were CO_2 retainers under the least difficult conditions and normal CO_2 eliminators at deeper depths and heavier work loads. Jarrett (13) was able to identify experienced and inexperienced divers on the basis of P_{ACO_2} and alveolar ventilation, and the former consistently retained CO_2 to a greater degree as depth and work load increased. Our divers were all highly experienced but they simply were not consistent from one dive to another. It is generally recognized that divers fall into one of two possible groups in relation to CO_2 elimination: they either eliminate CO_2 normally or retain it. Our data point to a third group consisting of those who hyperventilate and maintain low P_{ACO_2}. But the most perplexing characteristic of our divers is that they changed such classifications frequently in a manner which was often unrelated to depth or work load.

During rest the heart rate showed a tendency to decrease between the first two depths but it was highest at 99 fsw. Among the individual subjects the predominant trend was an increase in heart rate by as much as 14 beats/min between 33 and 99 fsw. These results do not agree with previous studies which found that increased pressure (2, 24, 25, 27), decreased

water temperature (18), or a combination of both (15) reduce the heart rate. However, our results generally agree with those of Russell et al. (23) who found a progressive increase in resting heart rate as pressure increased from 1 to 3 ATA during open-water dives. This atypical response was perhaps the result of a release of catecholamines induced by the intense cold. However, it is doubtful that this mechanism was responsible for our results since the divers did not feel cold even at 99 fsw. Soviet researchers (11) have suggested a "conditional-reflex reconstruction" of impending muscular activities to explain, in part, elevated physiological functions which they observed during rest in open-water dives. It is possible that a psychologically induced, prework intensification of the heart rate may have occurred in our study since rest was always immediately followed by work at 1.5 kg at all depths. As the rest-work situation progressed from 33 and 66 fsw, the anticipation of performing heavy exercise at a deep depth may have elevated the heart rate through sympathetic channels.

The heart rate at 1.5 kg showed no meaningful relationship with depth. However, the mean values did tend to follow the pattern for $\dot{V}O_2$, and heart rate was probably influenced by the same factors which affected $\dot{V}O_2$. A bradycardia was found for the two remaining work loads. It is not possible to define the mechanism of this phenomenon but water temperature was lower and P_{IO_2} progressively higher as depth increased. These factors have been implicated in heart rate reductions in several studies (18, 24, 25, 27, 29).

It may be of interest to note that some highly trained divers had resting heart rates near 50 bpm on land, between 78 and 84 bpm at 33 fsw, and 90 bpm at 99 fsw. It would appear that heart responses which are fairly typical at rest in hyperbaric chambers do not occur with equal regularity in open-water dives. Further, the highest heart rate recorded was 180 bpm at 99 fsw during extremely heavy work.

The HR-$\dot{V}O_2$ curves presented in Fig. 1 and the data for individual divers in Table V indicate that rather large differences in heart rate occur on land and at three depths in the sea when the oxygen uptake is held constant. At moderately high $\dot{V}O_2$ rates our results agree with those of Lally et al. (15) and Moore et al. (18) who found that heart rate decreased at a given $\dot{V}O_2$ as pressure increased and/or the water temperature decreased. However, at low $\dot{V}O_2$ rates we found this relationship to be nearly reversed. Whatever the relationship, our data indicate that land HR-$\dot{V}O_2$ curves should not be used to assess underwater energy expenditure, or equivalent land work stress, from heart rate data. As an example (Table V), at 3 liters O_2/min *subject AP* had a heart rate of 153 bpm on land and 141 bpm at 99 fsw. Using the land curve in Fig. 1, a heart rate of 141 bpm would be interpreted to reflect a $\dot{V}O_2$ of 2.2 l/min. Using the subject's HR-$\dot{V}O_2$ curve for land work, an error of 0.45 l/min was found. It appears that heart rate can only be used to monitor energy expenditure and work stress when the HR-$\dot{V}O_2$ relationship is known for the individual diver at particular working depths.

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INVESTIGATION OF A NEW BREATHING LIQUID

W. H. Matthews and J. A. Kylstra

The main obstacle which has prevented the practical application of liquid breathing to diving has been the inadequate removal of CO_2 through liquid-filled lungs. The elimination of CO_2 through liquid-filled lungs depends on three factors: the solubility of CO_2 in the breathing liquid; the partial pressure of CO_2 in the alveoli; and effective alveolar ventilation, which may be defined as the virtual volume of exhaled liquid in which the partial pressure of CO_2 is the same as in the arterial blood. If CO_2 elimination through liquid-filled lungs is inadequate, as evidenced by a greater than normal Pa_{CO_2}, either the effective alveolar ventilation or the solubility of CO_2 in the breathing fluid, or both, must be deficient and should be increased. Based on measurements of maximum flow of saline from human lungs, it has been estimated that the maximum alveolar ventilation in a liquid-breathing diver probably will not exceed 3 liters/min (1).

A resting diver, breathing saline, would require an effective alveolar ventilation of approximately 9 liters/min to maintain a normal Pa_{CO_2}. A resting diver, breathing FC-80 fluorocarbon, would require an effective alveolar ventilation of only 3 liters/min to maintain an arterial P_{CO_2} of 40 mmHg. However, for an active liquid-breathing diver to maintain a normal Pa_{CO_2}, the liquid must have a CO_2-carrying capacity which is even greater than that of FC-80 fluorocarbon. Addition of a substance to the breathing liquid which chemically combines with CO_2 might solve the problem.

A NaOH solution chemically combines with CO_2 to yield Na_2CO_3, but the lungs would be severely damaged by inhaling caustic NaOH unless direct contact with the tissues could be avoided. Fortunately, it is possible to make emulsions consisting of a continuous phase of fluorocarbon liquid in which small droplets of NaOH are suspended, surrounded by surfactant molecules. Such an emulsion combines the high oxygen solubility of fluorocarbon with the high CO_2-combining capacity of NaOH. The CO_2 can diffuse through the continuous fluorocarbon phase into the NaOH droplets, but the NaOH cannot diffuse through the water-immiscible fluorocarbon phase, and is therefore prevented from coming into contact with the alveolar wall.

Figure 1 shows the CO_2 content of air, saline, FC-80 fluorocarbon, and two different NaOH in FC-80 fluorocarbon emulsions at 37°C equilibrated with CO_2 at different partial pressures. The content of CO_2 in air, saline, and FC-80 fluorocarbon is equal to the Bunsen absorption coefficient (α) times the partial pressure of CO_2. The total CO_2 capacity of the emulsions is the sum of the amount of CO_2 physically dissolved in the FC-80 fluorocarbon

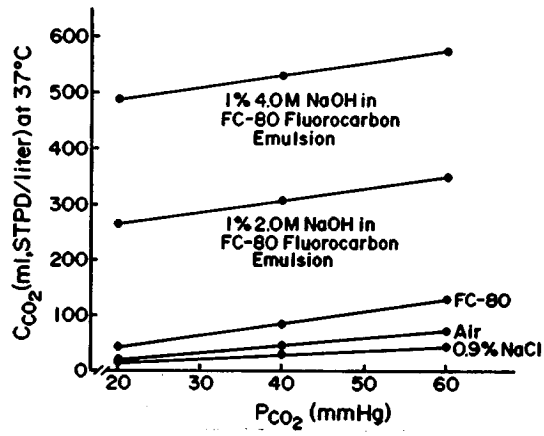

Fig. 1. CO_2 content of air, saline, FC-80 fluorocarbon, and 2 emulsions of different concentrations of NaOH at 37°C equilibrated with CO_2 at different partial pressures. FC-80 fluorocarbon solubility data from Sargent and Seffl (2). C_{CO_2} for 1% 2.0 M NaOH emulsion = $(P_{CO_2})(2.11)(.99) + 223$. C_{CO_2} for 1% 4.0 M NaOH emulsion = $(P_{CO_2})(2.11)(.99) + 446$.

($P_{CO_2} \times \alpha \times 0.99$) plus the amount of CO_2 which is bound chemically by the NaOH when Na_2CO_3 is formed.

Two questions need to be answered. First, how effective is a NaOH in fluorocarbon emulsion in removing CO_2 from spontaneously breathing, unanesthetized rats; second, does breathing this emulsion cause any damage to the lungs?

Methods

Emulsions of NaOH in FC-80 fluorocarbon were prepared with the surfactant L1478 (3M Company) by sonication with a Branson Model W185 Sonifier. The ingredients were placed in a beaker and circulated through a cooled continuous flow chamber attached to the horn of the sonifier. The chamber was cooled by circulating water which had been cooled to 5°C. The total volume of each batch prepared was approximately 3 liters. For a 1% 2.0 mole/liter (M) NaOH emulsion, the amount of each ingredient in a 3-liter batch was 2970 ml of FC-80, 30 ml of 2.0 M NaOH, and 12 g of L1478 (0.40 g L1478/ml of NaOH). The energy output from the sonifier horn was 50-60 watts, and the sonication time was approximately 4 hours. After use, the emulsion can be broken down and the FC-80 recovered by distillation, ethanol washing, washing with water, and separation by gravity.

The viscosity of the emulsions were measured with a Cannon-Fenske Viscometer suspended in a water bath maintained at 37°C. The emulsion sample was placed in the viscometer and allowed to warm in the water bath for 15 minutes before any measurements were made. Three measurements were made on each sample and the average kinematic viscosity was computed. The absolute viscosity was calculated from this value and the known density of the emulsion at 37°C.

The approximate size of the NaOH droplets in the emulsion was estimated by pressure filtration through Millipore filters of different pore sizes.

Male rats weighing approximately 300 g were anesthetized with ether. A cannula was placed in the femoral artery and a thermistor probe inserted in the rectum of the rat. The animal was attached to a plexiglass plate, and the plate assembly was placed in a clear plastic chamber as seen in Fig. 2. The animal chamber was placed in a large hyperbaric chamber and pressurized to either 4 or 5 ATA. Ten minutes prior to and during compression, the rat breathed 100% O_2. Once the pressure was achieved, hyperbarically oxygenated liquid, either the emulsion or the FC-80, was transferred from a small pressurized liquid container to the animal chamber. Oxygen had been bubbled through the liquid in the chamber at 4 or 5 ATA for several hours prior to the experiment to ensure that the liquid was saturated with oxygen at the pressure of the particular experiment.

In one series of experiments, the liquid was maintained at room temperature. Consequently, the rectal temperature of the rats fell to approximately 30 °C after they had been submerged for 30 minutes in the liquid. In another series of experiments, the liquid was maintained at 36 °C in a water bath so that the rectal temperature of the rats remained at 37 °C. All blood gas measurements were made with suitable electrodes (Instrumentation Laboratory) maintained at 37 °C in the hyperbaric chamber at pressure. The blood gas measurements from the hypothermic rats were corrected for the temperature difference.

After having been submerged for 15 or 30 minutes, the rats were removed from the liquid, the liquid was drained from their lungs by gravity, and they were placed in a 100% O_2 environment in which they remained overnight.

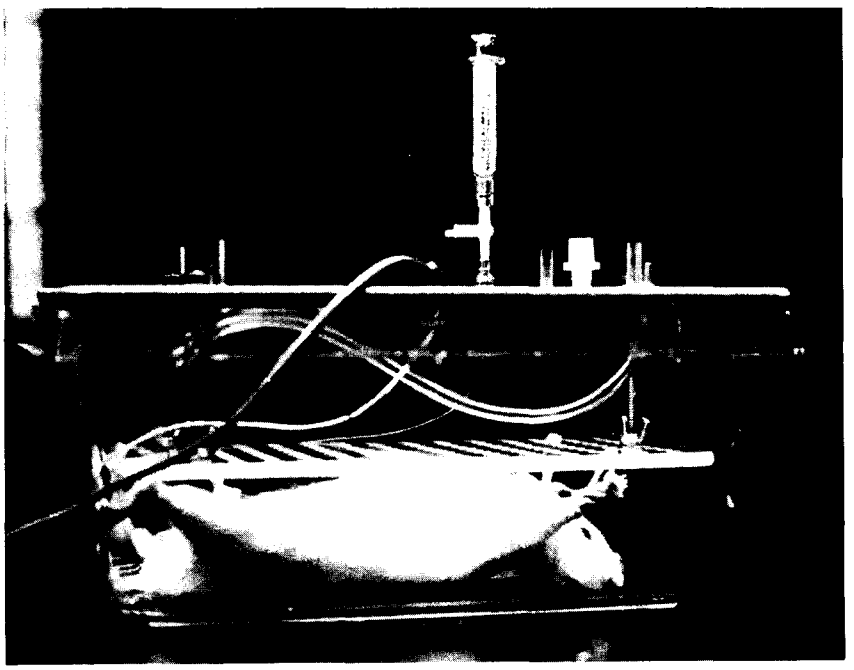

Fig. 2. Rat attached to plexiglass plate and suspended in plastic animal chamber. Thermistor probe is inserted in rectum of rat and cannula is inserted in femoral artery.

Results and Discussion

Emulsion Characteristics

The absolute viscosity of the 1% (by volume) 2.0 M NaOH in FC-80 fluorocarbon emulsion was 1.17 centipoise, which is nearly equal to the absolute viscosity of 1.12 centipoise for FC-80 alone (3). The approximate diameter of the NaOH droplets in the emulsion was 0.45 microns.

Hypothermic Liquid-Breathing Rats

Figure 3 shows the change in the mean arterial P_{O_2} and mean rectal temperature with time in the hypothermic liquid-breathing rats. This series of experiments was conducted at 4 ATA and the average inspired P_{O_2} was 2960 mmHg. This was sufficient to keep the rats well oxygenated. The mean arterial O_2 tension increased with time during the 30 minutes of liquid breathing. This was probably due to the decrease in metabolic rate resulting from the decreasing body temperature of the rats.

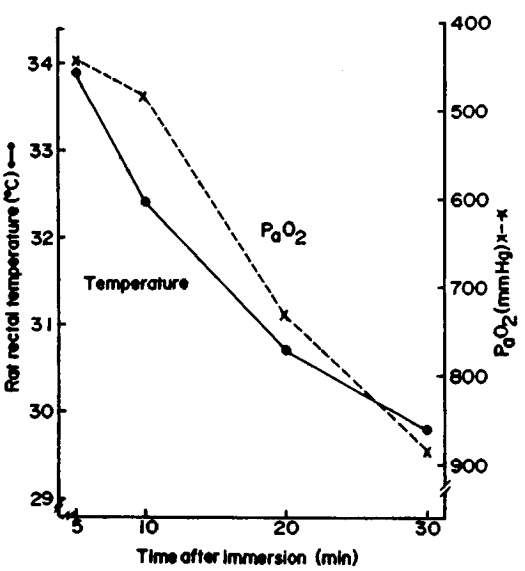

Fig. 3. Mean rectal temperature and arterial O_2 tensions in 29 rats breathing FC-80 fluorocarbon (n = 14) and 1% 2.0 M NaOH in FC-80 fluorocarbon emulsion (n = 15) for 30 minutes.

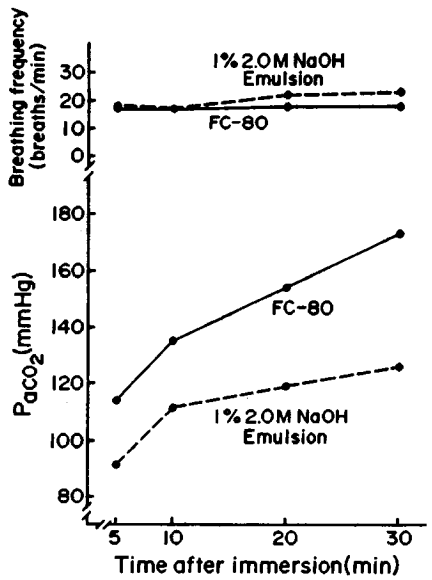

Fig. 4. Mean breathing frequencies and Pa_{CO_2} in hypothermic rats 5, 10, 20, and 30 minutes after breathing FC-80 fluorocarbon (n = 14) and 1% 2.0 M NaOH in FC-80 fluorocarbon emulsion (n = 15). There was no significant difference in mean breathing frequencies of 2 groups ($P > 0.05$). Differences between mean Pa_{CO_2} levels of 2 groups at 10, 20, and 30 min are statistically significant ($P < 0.05$).

Figure 4 shows the change in arterial P_{CO_2} and respiratory rate with time in the two groups of hypothermic rats. The mean Pa_{CO_2} in the emulsion-breathing rats was significantly lower ($P < 0.05$) after 10, 20 and 30 minutes of liquid breathing than the mean Pa_{CO_2} in the FC-80-breathing rats. The mean respiratory frequencies of both groups were not significantly different from each other during the immersion period ($P > 0.05$). There was no significant increase in the breathing frequencies during the immersion period in either group even though the Pa_{CO_2} levels in both groups increased dramatically during the same period. The lower arterial CO_2 tensions in the emulsion-breathing rats clearly reflect the increased CO_2-combining capacity caused by the presence of NaOH droplets in the fluorocarbon.

All of the rats in both groups survived until they were killed for pathologic examination of the lungs, which took place at different times up to four weeks after the liquid breathing.

Normothermic Liquid-Breathing Rats

In the next series of experiments, the breathing liquid was prewarmed to 36 °C so that the rats would remain normothermic. The same procedure was followed as described above, with several exceptions: the rats were pressurized to 5 ATA instead of 4; they breathed the liquid for 15 minutes instead of 30 minutes; arterial blood samples were taken at 5, 10, and 15 minutes, and the NaOH concentration of the emulsion was varied from 1% 4.0 M NaOH in FC-80 fluorocarbon to 2% 4.0 M NaOH in FC-80 fluorocarbon.

The average rectal temperature in all three groups of normothermic rats remained at 37 °C during the 15 minutes of liquid breathing. The arterial O_2 tensions in these rats remained well above 100 mmHg and did not increase during the 15-minute liquid-breathing period, in contrast to the pattern seen in the hypothermic liquid-breathing rats. This supports the view that the increase in arterial O_2 tension with time in the hypothermic rats was due to the decrease in metabolic rate caused by the decrease in body temperature during the 30-minute liquid-breathing period.

The changes in mean arterial CO_2 tension with time are shown in Fig. 5. The rats breathing the FC-80 had the highest Pa_{CO_2} at the end of 15 minutes, while the rats breathing the 2% 4.0 M NaOH emulsion had the lowest. The rats breathing the weaker 1% 4.0 M NaOH emulsions had intermediate Pa_{CO_2} levels.

As seen in Fig. 6, the normothermic rats increased their breathing frequency concomitant with increasing arterial CO_2 tensions during the 15-minute liquid-breathing period. The breathing frequencies in the hypothermic rats remained constant even though the arterial CO_2 tensions were increasing during the same period. This indicates that the CO_2 response in the hypothermic rats was abolished, at least as far as breathing frequency was concerned.

Figure 6 also reveals that normothermic emulsion-breathing rats had a greater breathing frequency increase per mmHg increase in arterial CO_2 than did normothermic FC-80-breathing rats. This indicates that at higher arterial CO_2 tensions, the CO_2 response of the normothermic FC-80-breathing rats was somewhat attenuated, at least as far as breathing frequency was concerned.

The rats which breathed the stronger emulsions survived overnight, but died when removed from the O_2, suggesting that breathing these stronger emulsions resulted in lung damage. This may be due to the fact that the more concentrated emulsions are not as stable as the 1% 2.0 M NaOH emulsions and that some of the NaOH may have come in contact with the alveolar walls.

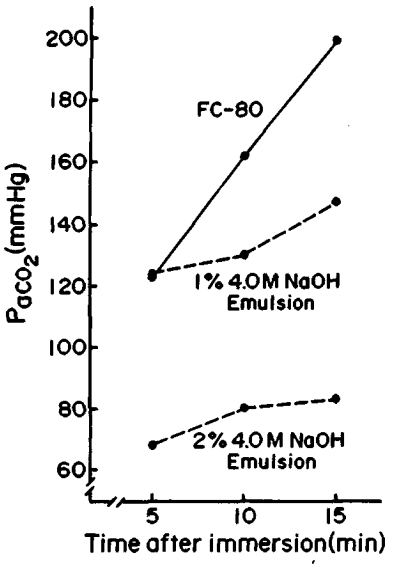

Fig. 5. Mean Pa_{CO_2} in normothermic rats 5, 10, and 15 min after breathing 3 different liquids. Mean values are from 3 rats in each group.

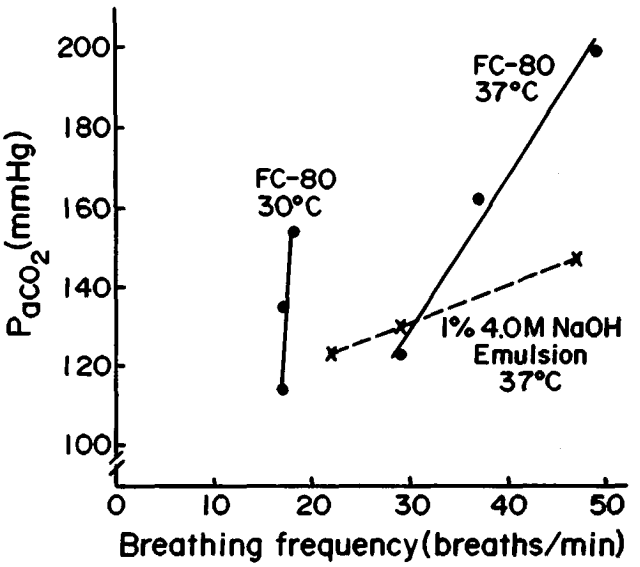

Fig. 6. Breathing frequency versus Pa_{CO_2} in hypothermic FC-80 breathing rats (rectal temperature = 30–32°C) and normothermic (rectal temperature = 37°C) rats breathing FC-80 fluorocarbon and 1% 4.0 M NaOH in FC-80 fluorocarbon emulsion.

Histological Findings

On microscopic examination the lungs from rats which had breathed FC-80 30 days before being killed appeared normal. Lungs from rats which had breathed the 1% 2.0 M NaOH emulsions three hours before being killed also appeared normal. Eight days after breathing these emulsions the only pathological findings consisted of some macrophages in the alveoli near the alveolar ducts. Thirty days after breathing the emulsion, the lungs contained a greater number of macrophages in the alveoli and there was some perivascular edema. There were also lymphocytes in some alveoli.

A lung from one rat that had breathed the stronger 1% 4.0 M NaOH emulsion one day before being killed contained both polymorphonuclear leukocytes and fibrin in the alveoli near the alveolar ducts. This indicates that this stronger emulsion may have caused some damage to the capillaries and would account for the fact that rats which breathed these emulsions died as soon as they were removed from the oxygen environment.

Conclusion

The results of this study indicate that emulsions consisting of NaOH droplets suspended in FC-80 fluorocarbon can be used as breathing liquids without causing damage to lung tissue, and that these emulsions remove more CO_2 per unit volume from liquid-breathing rats than does FC-80 alone. The more concentrated NaOH emulsions used in this study were not as stable as the 1% 2.0 M NaOH emulsions, and did cause some lung damage.

A diver breathing a 1% 2.0 M NaOH emulsion at 37 °C at an effective alveolar ventilation of 3 liters/min with a respiratory frequency of 3 breaths/min could perform work requiring an oxygen consumption of 1150 ml STPD/min without incurring a rise in Pa_{CO_2} above 40 mmHg. The partial pressure of oxygen in the inspired emulsion would not need to be higher than 700 mmHg.

ACKNOWLEDGMENTS

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PART III. RESPIRATORY-PULMONARY COMPETENCE AT HIGH AMBIENT PRESSURES IN ATMOSPHERE AND WATER, AT REST AND WORK

DISCUSSION

C. M. Hesser, Chairman

Dr. Hong: Dr. Winsborough, did you see a bradycardia during your experiment?

Dr. Winsborough: Yes, there certainly was a bradycardia at heavy exercise.

Dr. Hong: What about the resting heart rate?

Dr. Winsborough: The resting heart rate was slightly raised.

Dr. Hong: Was it increased from the beginning?

Dr. Winsborough: Yes.

Dr. Hong: You did not find the usual hyperbaric bradycardia reported by many other people.

Dr. Winsborough: We did at exercise, but at rest our subjects certainly did not show any bradycardia.

Dr. Hong: You made a comment on tolerance exercise; did you measure the maximal oxygen uptake as such?

Dr. Winsborough: No, we didn't.

Dr. Hong: What would you predict on the basis of the cardiorespiratory measurements you made? The cardiac output at a given O_2 consumption was maintained very well, if it was not actually higher in the hyperbaric environment. Also, did you maintain the P_{O_2} level at 250 meters at 0.3 atmospheres?

Dr. Winsborough: No, it was 0.4 atmospheres.

Dr. Hong: We have done something very similar. We did measure the cardiac output and the resting heart rate during exercise but we did not measure $\dot{V}_A/\dot{Q}$ distribution. We did, however, get into a rather systematic analysis of cardiorespiratory functions during our saturation diving three months ago to a depth of 18.6 ATA with 17 days of bottom time. We measured both resting and exercise heart rates, cardiac output and maximum oxygen uptake. We noticed that the heart rate did indeed decrease during the first two or three days of the stay at 18.6 ATA, but then this bradycardia mysteriously disappeared. I think it is important to realize that we do not have to live with this bradycardia in hyperbaric environments. It is only a temporary phenomenon. I talked with Dr. John Salzano of Duke, and he has also reported a similar disappearance of this bradycardia in a dive to 1,000 fsw. In his case, the bradycardia disappeared in about 72 hours. Dr. Nakayama reported yesterday that in a 7-day dive to 200 feet this bradycardia tended to disappear towards the end of one week. Evidently this bradycardia is a temporary thing, and is depth-dependent. The deeper the dive, the shorter the duration of the bradycardia. We have to realize this is a new set of data. The question is, why does this resting bradycardia disappear? The heart rate during exercise is maintained at a low level, so evidently we have to deal with two different levels or types of regulation of the heart rate. One deals with resting heart rate regulation, the other with heart rate regulation during exercise. Some of us will have to find out why there is a difference.

My second point concerns exercise capacity. We measured the maximal oxygen uptake at 18.6 ATA first while we maintained the level of P_{O_2} at 0.3 atmosphere. In that case we found a slight (about 5%) increase in the maximal O_2 uptake in all four subjects. Since P_{O_2} was at 0.3 atmosphere, which is higher than usual, we did another experiment at a normoxic level. With a P_{O_2} of 0.2 atmosphere, the maximal O_2 uptake at 18.6 ATA was identical to that at 1 ATA air.

Dr. Hody: This is addressed to Dr. Dwyer. First I would like to thank you for presenting a tremendous amount of material very clearly and concisely. I have two questions, one simple, one more involved. The simple one is, how long were the experiments, what was the duration?

Dr. Dwyer: Resting periods lasted 5 minutes, and work loads lasted 4 minutes. Data were collected during the last minute.

Dr. Hody: Were they repetitive? Did you do more than one in one session?

Dr. Dwyer: At each depth the subjects descended to the bottom, went through the rest period, and then performed the light work load. At 66 feet the subjects returned on other days, and performed the two heavier loads consecutively with a 2-minute rest in between. But at 99 feet, because of high air consumption, the two heavy work loads were performed on separate dives; there were sometimes as many as 3 weeks between dives because of weather and sea conditions, or subject unavailability.

Dr. Hody: My second question is about your electrocardiographic observations, which you didn't have time to go into detail about. In particular, I noticed that the rates that you reached were very high; I noticed one as high as 170. In somewhat analogous situations where heart rates are driven very high, for example in free fall parachuting, the very high heart rates may precipitate arrhythmias of a dangerous nature in people who have never had such experiences before, people who have otherwise normal hearts under any other kind of testing. I would be very interested to know if you looked for and observed any such arrhythmias, and also whether anyone has postulated that some of the mysterious deaths that occur during sport diving may be the result of exercise-induced tachycardia and arrhythmias of this kind?

Dr. Dwyer: I didn't examine the ECG traces for arrhythmias in particular. I did find an occasional PVC from time to time. We did have heart rates higher than 180 beats per minute in some cases at 33 feet and 99 feet. I plan to go back over each trace by 10-second intervals and look for arrhythmias. Regarding cases of deaths among SCUBA divers, I can only speculate, but I would suspect a diver who is in very good physical condition shouldn't have much trouble working at from 2 to about 3 liters of oxygen per minute at moderate depths.

Dr. Hody: I understand this has been very hotly pursued in free fall parachuting because of a number of unaccounted for cardiac arrests.

Dr. Dwyer: We could answer that question if we put multiple leads on the subject. We have six channels on our recorder that are not used at this time, and it would be easy to get six standard leads.

Dr. Kuehn: This is a question for Dr. Matthews. Have you considered the use of other chemicals less caustic than sodium hydroxide in your emulsions, calcium hydroxide for example?

Dr. Matthews: That is an obvious thing to pursue. We were looking for a super CO_2 sink, and that is the first thing that came to mind.

Dr. Salzano: I would like to correct the record on a comment made by Dr. Hong. We recently observed bradycardia in divers exposed to a simulated depth of 148 fsw or 5.5 ATA. The bradycardia disappeared, however, after 24 hours of exposure to this pressure. In 1969, in a dive to 1,000 fsw, bradycardia persisted through 72 hours of bottom time. I would like to comment on Dr. Linnarsson's finding. We too have recently found an increase in the $P_{.1}$ or P_{100} at a depth of 148 fsw in a heliox dive with a Po_2 equivalent to air at 1 ATA and with a gas density the same as in air at 1 ATA. Since this is not expected to produce narcosis, I don't believe we need to invoke an effect similar to that of nitrous oxide. We may be dealing with a pressure effect.

Dr. Linnarsson: Yes, it may also have been an effect of pressure per se.

Dr. Madsen: A question for Dr. Matthews. The experiments you did with low-strength sodium hydroxide solutions were of very short duration, 15 to 30 minutes as compared to previously published experiments with pure FC-80. I would like to know how your rats would behave in longer experiments?

Dr. Matthews: At the P_{IO_2}'s we were working at they usually died, especially the 37° rats. The hyperthermic rats went for 30 minutes. We probably could have gone longer but we only needed the P_{ACO_2} levels.

Dr. Vorosmarti: I have a comment to address to Dr. Dwyer. He has done a lot of work trying to get some data underwater, but I am not surprised that he has had a huge variation in his results, now that I have heard the duration of times for exercise and rest. I don't think that at the work loads you were working at you had any chance at all of reaching a steady state. I realize that this is probably a technical problem. I think you are measuring a lot of transients which give you odd results, particularly since you are doing them on different days. The other thing is, divers do normally have much higher heart rates, particularly when they get in the water. But I would like to emphasize the fact that even well-trained athletes can be found to have a fair number of PVC's and cardiac arrhythmias when they are racing. I wouldn't want to speculate about whether this causes deaths in sport divers; it may be a cause, but I wouldn't get too alarmed about it.

Dr. Dwyer: The incidence of PVC's in highly trained athletes is about 30%. Regarding our heavy work load, I feel we were quite close to the $Vo_{2\ max}$. We have comparable land data only on subject *J.D.*, who is myself. I was working at 91% of my $Vo_{2\ max}$ after 3 minutes at 99 feet. I don't believe I could have gone another minute longer.

Dr. Saltzman: I would like to compliment the speakers for a series of clear and excellent presentations. Dr. Dwyer seems to have made a breakthrough. To obtain values of that quality at depth is a feat indeed. My question is directed to Dr. Winsborough. There are many complexities in these so-called noninvasive methods for

FRUCTUS, X. and J.C. Rostain.
HPNS: a clinical study of 30 cases.
In: Shilling, C.W. and M.W. Beckett, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology, p. 3-8. Bethesda, Md., Federation of American Societies for Experimental Biology, 1978.

Copy conclusions; p. 7. (Authors' conclusions)

* HPNS O-25/ compression rate S-54/ depth limit P-56/ France GN-4:/ proceedings GN-9

measuring systemic blood flow, or pulmonary blood flow, as she termed it. These require validation even at 1 atmosphere. Under hyperbaric conditions, it is important to try to verify these convenient and very desirable methods by methodologies not involving gas going in and out of a lung. Do you have data that validate these measurements?

Dr. Winsborough: No, we haven't validated the pressure.

Dr. Hills: I would like to ask Dr. Matthews a question about some work he was doing two years ago. In those days I think you had the aqueous phase as the continuous phase, but now I think you are using the fluorocarbon as the continuous phase. But there is quite a contact angle between an aqueous medium and your FC-80. Isn't this a great problem in your changeover from air breathing to FC-80 breathing and vice versa, because if you kept the aqueous phase as a continuous phase you wouldn't have a contact angle.

Dr. Matthews: I'm not sure I even understand that. We tried THAM in fluorocarbon emulsions with the continuous phase being the THAM. We tried that on dogs and were having a lot of problems with electrolyte shifts and THAM getting into the bloodstream. Also, you have to go to higher pressures to get the same O_2 content, because we were using 30% fluorocarbon instead of 90% fluorocarbon, as we were using here.

Dr. Hills: If you put oil into an airway, it gives you a plug, whereas otherwise you don't have it. This plug gives you an actual resistance because you have sort of a hysteresis in contact angle which I would think would make it very difficult changing over in your terminal airways to pure liquid from air and vice versa. Maybe this is why your rats died on coming back to air.

Dr. Matthews: The hypothermic rats had no trouble. All of the pure FC-80 breathing rats and all the emulsion-breathing rats lived when they came back, and that was 30 minutes.

Unidentified: Dr. Dwyer, regarding the $P_{A_{CO_2}}$ that was high for an experienced diver working in 33 feet of water, did you see any correlations between ventilation rate and $P_{A_{CO_2}}$? Was there any sort of skip breathing or any slowing?

Dr. Dwyer: No, there wasn't. There wasn't any skip breathing, which is normally defined as a diver breathing about 3 to 6 times per minute. Diver *A.P.* was breathing about 13 times per minute, which isn't unusually slow for him. His tidal volume was close to 3 liters. I tried to find correlations among all the subjects with mean values or decreases in ventilation frequency and alveolar ventilation and increases in alveolar CO_2. These are very hard to find because of the very large variations, so you have to go on a subject-by-subject basis, and even under those conditions you don't see the very nice clear-cut situations that have been found in hyperbaric chambers.

Dr. Flynn: I would like to ask Dr. Linnarsson about the occlusion technique. As I understand it, the negative pressure generated in the mouthpiece is due to a small volumetric expansion of the thorax in response to the central respiratory drive. Under pressure, if the thorax were to expand by the same volume, the pressure would diminish more than at the surface, and the diminution would be in proportion to the absolute pressure. You have shown that in fact this is true in a hyperbaric situation with nitrogen, and Dr. Salzano has shown that it is true with helium. My question is whether or not the greater diminution under pressure is not an artifact rather than an indicator of central respiratory stimulation.

Dr. Linnarsson: The pressure drop in the airways was of a magnitude of 5–10 cmH_2O, 0.1 second after the onset of an occluded inspiration. The corresponding volume changes of the airways will be about 0.5 % at 1.3 ATA and about 0.1% at 6 ATA. We do not feel that the difference between those small volume changes has influenced our results.

Dr. Flynn: My point is this. Let's say that you are at 1 atmosphere, and you occlude the airway and then double the lung volume, as a gross example. The mouthpiece pressure would drop to a half atmosphere. If you did the same thing at 6 atmospheres, the pressure would drop to 3 atmospheres. The absolute change is 3 atmospheres at the deeper depth and only half an atmosphere at the surface. Now if we reduce the magnitude of this example to the very small increment in lung volume which actually occurs when the airway is occluded, it would seem to me that the pressure drop, although much smaller, would still be greater the deeper you went and this effect would not be related to gas density, but simply to the pressure. I guess it really depends on whether the respiratory system is operating as a volume-driven or pressure-driven system. If the normal unoccluded response to a given level of CO_2 stimulation at depth would have been a tidal volume similar to that on the surface, you would expect the muscles at depth to contract initially during occlusion with the greater strength necessary to produce that volume. Under these circumstances, the greater pressure drop at depth would be artifactual. On the other hand, if the response to CO_2 stimulation is the generation of a certain pressure, then what you say would be true. The greater diminution of mouthpiece pressure at depth would be indicative of central respiratory stimulation.

Dr. Linnarsson: I believe that we are dealing with a system which responds in terms of pressure rather than in terms of volume during the initial 100 msec of an occluded inspiration.

Dr. Webb: Dr. Dwyer, again, a very nice piece of work. The apparatus you used was very impressive and in-

genious. My question has to do with one of the measurements. How accurately were you able to resolve gas volume from a pressure measurement in a SCUBA tank?

Dr. Dwyer: Of course, we couldn't validate our measurements underwater; it is difficult to take a Douglas bag down to 99 feet. But in the laboratory, I connected a couple of divers to our apparatus and fed the gas into a Douglas bag. With subjects at rest, the differences in pulmonary ventilation calculated from tank pressure and measured from the Douglas bag were no greater than 100 cc, which is extremely accurate.

Dr. Webb: I don't know if you did this sort of measurement or not. We are familiar with studies of swimmers' speed versus oxygen uptake without any particular load except the drag of the tanks or whatever. Did you have any data like that?

Dr. Dwyer: We found that at the 1.5 kg work load divers swam at 21.5 meters per minute. This swim speed normally requires about 1.5 liters of oxygen per minute when swimming in the open sea. We were a little bit higher than that. We found with a heavy load our divers were swimming at 24 meters per minute and this was very constant. The research diver who swims along with the subject has an added advantage: he does not have to swim at a very high speed even though the subject is working at a very high V_{O_2}.

Dr. Wissler: My question for Dr. Dwyer concerns his measurement of the expired CO_2 partial pressure. The dead space of the mouthpiece added to the physiological dead space could have been large enough to cause rather large variations in the CO_2 partial pressure of gas flowing past the sampling ports. Perhaps some samples were collected from the end-tidal portion of the exhaled gas, while others were collected from gas in the dead space, and this could account for the large variation in CO_2 partial pressures that was observed.

Dr. Dwyer: Are you asking if the dead space of the mouthpiece was excessively large? It was about 100 cc.

Dr. Wissler: Did you take continuous samples in the laboratory rather than in the water and observe the variations in CO_2 partial pressure of the sampling ports?

Dr. Dwyer: No, I didn't do that in the laboratory. Those vacuum cylinders will only work with a pressure difference. I could have taken in a sample in the laboratory, but I couldn't have gotten it out again because it would have been in the cylinder at 1 ATA.

Dr. Morrison: Dr. Linnarsson, I understand you were looking for a drop in P_{O_I} values due to the anesthetic effect of the nitrogen. In fact, you saw an increase in the P_{O_I} value. Would you like to comment on what may have caused the increase, as opposed to the decrease, which you actually saw?

Dr. Linnarsson: We can offer no simple explanation of why respiratory drive should be enhanced during exposure to hyperbaric N_2 despite a simultaneous ventilatory depression. I suggested in my presentation that the enhanced respiratory response to CO_2 that has been observed during nitrous oxide inhalation might be a related phenomenon. Dr. Fagraeus has suggested that a tendency to hyperventilate, although offset by the increased gas density, might be due to an excitation of the CNS similar to that observed during the induction of general anesthesia. As Dr. Salzano pointed out earlier today, pressure per se must also be considered as a possible causative agent.

Part IV. TOXICITY OF RESPIRATORY GASES

PULMONARY FUNCTION DURING SHALLOW HABITAT AIR DIVES (SHAD I, II, III)

J. H. Dougherty, Jr., R. L. Frayre, D. A. Miller and K. E. Schaefer

A large percentage of diving work is carried out at relatively shallow depths; compressed air diving has the advantages of being less complex and less expensive than nitrogen-oxygen and other mixed-gas diving. This paper concentrates on pulmonary function during a series of Shallow Habitat Air Dives (SHAD I, II, and III). These dives have been a progressive, systematic attempt to probe man's limits for long-term air diving with an operationally practical excursion protocol. The measurements were made before, during, and after four human saturation air dives in a hyberbaric chamber, following extensive animal studies (20). Figure 1

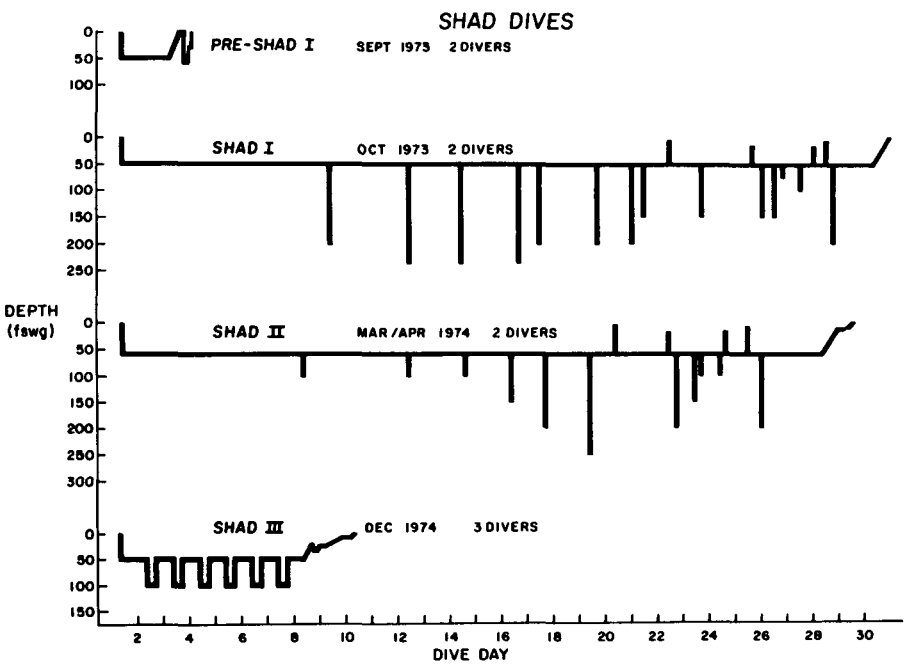

Fig. 1. Pressure profiles of 4 Shallow Habitat Air Dives (SHAD).

shows some pressure-depth-oxygen relationships in the SHAD dive series. Pre-SHAD I had a saturation time of two days, with a decompression period of 9½ hours. SHAD I (50 fsw air saturation) and SHAD II (60 fsw air saturation) had bottom times of 29 and 27 days, respectively, and brief excursions to pressures of from 5 to 250 fsw. No hard physical work was carried out during the excursions. SHAD III had a saturation depth of 50 fsw for 7 days, with daily 8-hour excursions to 100 fsw; hard physical work was performed on a bicycle ergometer and with dumbbells and handgrips. The diving profiles and an overview of the numerous biomedical and behavioral investigations carried out during these dives are reported in greater detail elsewhere (1).

During the SHAD dives, subjects were exposed to moderately elevated partial pressures of O_2 at saturation depths of 0.53 to 0.59 atmospheres absolute (ATA). The maximum excursion P_{O_2} was 1.79 ATA.

The oxygen exposure at 60 fsw (0.59 ATA) is close to the concentration that most oxygen tents and nasal catheters provide. From a scientific point of view exclusively, an exposure to a constant P_{O_2} without excursions would have been desirable. However, since it is unlikely that any actual diving operation will ever require that all work be done at exactly the saturation depth, this project was designed to simulate an operational situation and excursions were therefore performed.

Materials and Methods

Pulmonary monitoring, which served both investigative and medical safety functions, was carried out with a "Wedge" spirometer system, consisting of a Med-Science Electronics Model 370 spirometer and power supply-amplifier unit, and a Model 580 Pulmo Digitizer unit. A Tektronix Model 503 oscilloscope was connected in parallel with the Med-Science equipment. The oscilloscope allowed the investigator to monitor chest position during the respiratory cycle, and to give instructions and coaxing at the proper moment. The spirometer Wedge or bellows was inside the chamber (11,12). Both subject and investigator had communication head sets with boom microphones; the subject moved the microphone away from his mouth during the actual tests.

Data for the following six parameters were obtained at each sampling period: (1) forced vital capacity (FVC), in liters; (2) forced expiratory volume in 1 second (FEV_1), in liters; (3) forced expiratory volume in 2 seconds (FEV_2), in liters; (4) peak expiratory flow rate (PEFR), in liters/second; (5) peak inspiratory flow rate (PIFR), in liters/second; and (6) maximum voluntary ventilation (MVV), in liters/minute.

All tests were run in duplicate and the higher of two data which appeared to be valid was used. If the investigator felt that a value indicated a submaximal effort, an additional maneuver was performed.

Training was carried out and control data were obtained during a period of two weeks prior to the actual dives. Predive control data were obtained on 7 to 14 occasions; values were obtained at approximately 0800, 1200, 1800, and 2200 hours. In almost all instances, tests were run before meals and after subjects had been upright for some time. The subjects were told that the pulmonary function testing served a primary medical monitoring function in addition to being valuable as scientific data. This explanation presumably increased the subjects' motivation; they all showed a competitive interest in their values throughout the dives.

The physical characteristics of the 9 male diver-subjects are shown in Table I. None of the subjects was grossly overweight or underweight.

Results

During the initial 2-day test dive (Pre-SHAD I) no significant changes in pulmonary function were observed at the 50-fsw saturation or at 40 fsw during decompression. Both divers who participated in pre-SHAD I developed decompression sickness and were treated with 100% oxygen (USN Treatment Table 6). Both suffered from substernal burning and inspiratory pain; one also had a "tingling" of the lips.

A summary of changes in FVC found during SHAD I, II, and III is presented in Table II. Decreases in FVC greater than two standard deviations (SD) from the control are listed. In SHAD I, *subject B* had a reduction in FVC of this magnitude on 6 of 94 sampling periods; the largest reduction was a 9.7% decrease. Other decreased values which showed a less than 2-SD reduction clustered around these low values on *days 2, 3* and *17.* The low values on *days 23* and *28*, by contrast, were isolated. Only 2 of 94 values recorded for *subject W* were reduced as much as 2 SD. One of these reached a 12.0% decrement, and both were clustered with other reduced FVC values. During SHAD II, none of the 85 values recorded for *subject F* and only 1 of 85 values recorded for *subject S* were more than 2 SD below the predive control means. The occurrence of any decrement was therefore unusual.

During SHAD III, *subject M* showed decreases in FVC greater than 2 SD below control on 14 of 37 sampling periods; the greatest decrease was 9.7%. Most decreased values followed the last three excursions (*dive days 5, 6* and *7*). *Subject O* only once demonstrated a decrease greater than 2 SD in 34 recorded values. *Subject P* had three such decreases in 35 recordings, all following O_2 therapy for decompression sickness. Other low values showing decreases of less than 2 SD also occurred during this period. Following O_2 therapy, *subject P* had a decrease of 23.7% from his predive control FVC mean. His FVC had increased up to this point, and it is probably more realistic to use a new reference point of 4.99 liters, which is the mean of 12 values on *dive days 6, 7* and *8*, prior to O_2 treatment. This would indicate a decrease of 27.2% in FVC following the O_2 therapy.

A plot of FVC values vs. time for 2 subjects, *M* and *O*, who did not receive 100% O_2 as a treatment for decompression sickness in SHAD III is shown in Fig. 2. Both subjects showed

TABLE I
VITAL STATISTICS OF SHAD SUBJECTS

Dive	Subject	Age, yr	Height, inches
Pre-SHAD I	*A*	31	72
Checkout Dive	*T*	36	71
SHAD I	*B*	28	72
	W	26	69
SHAD II	*F*	35	69.5
	S	24	70
SHAD III	*M*	37	71
	O	26	78
	P	42	72

TABLE II

FORCED VITAL CAPACITY MEASUREMENTS SIGNIFICANTLY BELOW CONTROL MEAN IN SHAD DIVES

	Subject	FVC, liters	ΔFVC, liters	% Change from control
SHAD I	*B* (6 of 94 sampling periods)			
	Control (Mean ± SD)	5.80 ± 0.17		—
	Dive Day			
	2		−0.36	−6.2%
	3		−0.40	−6.9%
	17		−0.56	−9.7%
	17		−0.42	−7.2%
	23		−0.43	−7.4%
	28		−0.39	−6.7%
	Postdive (Mean ± SD)	5.98 ± 0.09	+0.18	+3.1%
	W (2 of 94 sampling periods)			
	Control (Mean ± SD)	6.52 ± 0.32		
	Dive Day			
	4		−0.78	−12.0
	5		−0.67	−10.3
	Postdive (Mean ± SD)	6.54 ± 0.12	+0.02	+ 0.3
SHAD II	*F* (0 of 85 sampling periods)			
	S (1 of 85 sampling periods)			
	Control (Mean ± SD)	4.95 ± 0.07		
	Dive Day			
	4		−0.25	−5.1
	Postdive (Mean ± SD)	5.41 ± 0.14	+0.46	+9.3
SHAD III	*M* (14 of 37 sampling periods)			
	Control (Mean ± SD)	4.96 ± 0.10		
	Dive day			
	2		−0.23	−4.6
	5		−0.33	−6.7
			−0.36	−7.3
	6		−0.48	−9.7
			−0.32	−6.5
			−0.26	−5.2
			−0.30	−6.0
	7		−0.45	−9.1
			−0.22	−4.4
			−0.34	−6.9
			−0.36	−7.3
	8		−0.25	−5.0
	9		−0.23	−4.6
	Postdive (Mean ± SD)	4.79 ± 0.16	−0.17	−3.4
	Postdive, day 6 (Mean ± SD)	4.48	−0.48	−9.7
	O (1 of 34 sampling periods)			
	Control (Mean ± SD)	7.08 ± 0.22		
	Dive day			
	5		−0.45	−6.4
	Postdive (Mean ± SD)	7.50 ± 0.12	+0.42	+5.9
	P (3 of 35 sampling periods)			
	Control (Mean ± SD)	4.77 ± 0.24		
	Dive Day			
	O_2 − 7		−0.49	−10.2(−14.2)*
	8		−1.13	−23.7(−27.1)*
			−0.60	−12.6(−16.4)*
	Postdive (Mean ± SD)	5.04 ± 0.15	+0.27	+5.7
	Late-Dive Mean ± SD	4.99 ± 0.16	+0.22	+4.6

* % Decrease from late-dive mean established on *days 6, 7,* and *8* prior to O_2 treatment.

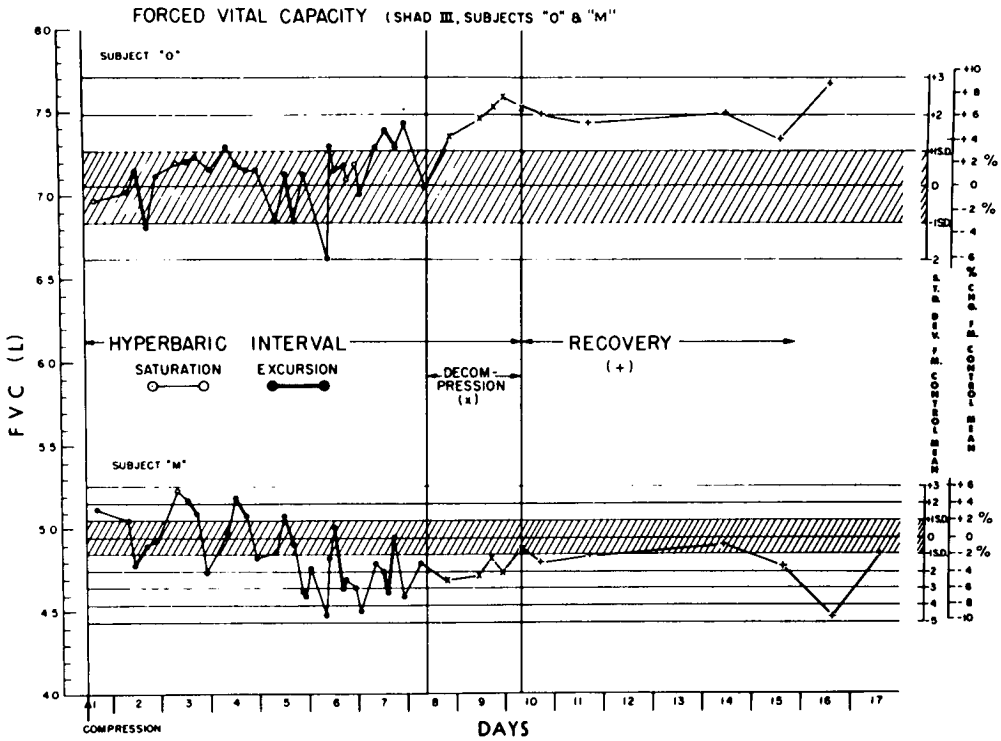

Fig. 2. Effect of SHAD III air saturation-excursion profile on forced vital capacity (*subjects O* and *M*). Dive and postdive data shown for 2 subjects not treated with 100% O_2 during decompression. Predive control mean for FVC is horizontal line in center of shaded area representing ± 1 SD from mean.

greater variability during the dive than before or after the dive. It should be noted that *subject M* was the only one of 7 subjects to show a decrease between predive and postdive FVC means. The other 6 men had increases, 4 of which were statistically significant. Both *M* and *O* show minimum values which are considerably below the control level on *day 6*. Two subjects show an increase from the late-dive to the postdive period; the FVC of *subject O* went up markedly.

Subject M showed a progressive fall in daily FVC averages beginning with *excursion 2*, reaching 3 SD below control (−6%) after *excursion 4*. This highly significant decrement is indicative of pulmonary O_2 toxicity, beginning with *excursion 4*. *M*'s subjective chest discomfort increased at those times when FVC decreases were greatest. Beginning with *excursion 2*, FVC cycles can be seen in this subject, with daily high values shortly after reaching 100 fsw (Fig. 2).

These cycles were found only in the subject who exhibited chest symptoms and a declining FVC during the dive when ambient P_{O_2} increased to 0.83 ATA. Immediately upon commencing decompression, this variability greatly decreased and regular cycles in FVC were no longer evident. Because hyperoxia can cause FVC decreases, these FVC cycles were investigated further.

To calculate the average daily cycle, it was necessary to factor out the concurrent down-

ward trend in FVC. This was done for each cycle by taking the FVC at approximately 1200 hours (early excursion) as a reference point and subtracting the measurements at 1800, 2200, 0800, and 1200 hours the next day (early subsequent excursion) from it. To avoid overemphasizing the times of depressed FVC, a single mean value was calculated for each of the time periods for each cycle when repeated measurements were made at closer time intervals. In Fig. 3 the resulting ΔFVC's are plotted vs. time of day for these 5 excursions, with the daily cycle of the P_{O_2} and P_{total}. A regular daily cycle of significant (paired *t*-test) FVC decreases beginning with the early excursion value is shown, reaching a daily minimum FVC 430 ml below the reference point at 0100 (6 hours postexcursion) and essentially recovering by the beginning of the next excursion.

Even though the daily FVC cycles ceased when daily 8-hour excursions to 100 fsw stopped, (see Fig. 2), the possible contribution of circadian rhythms could not be completely discounted. Hence, a circadian follow-up study was performed on this subject 7 months postdive, to look for evidence of circadian FVC cycles under steady-state conditions while the subject was breathing air at 1 ATA. To provide a comparison with the dive data, pulmonary function tests were performed for 5 days at the same times as those performed during the dive. The overall mean FVC (4.89 ± 06 liter) was not significantly different from predive

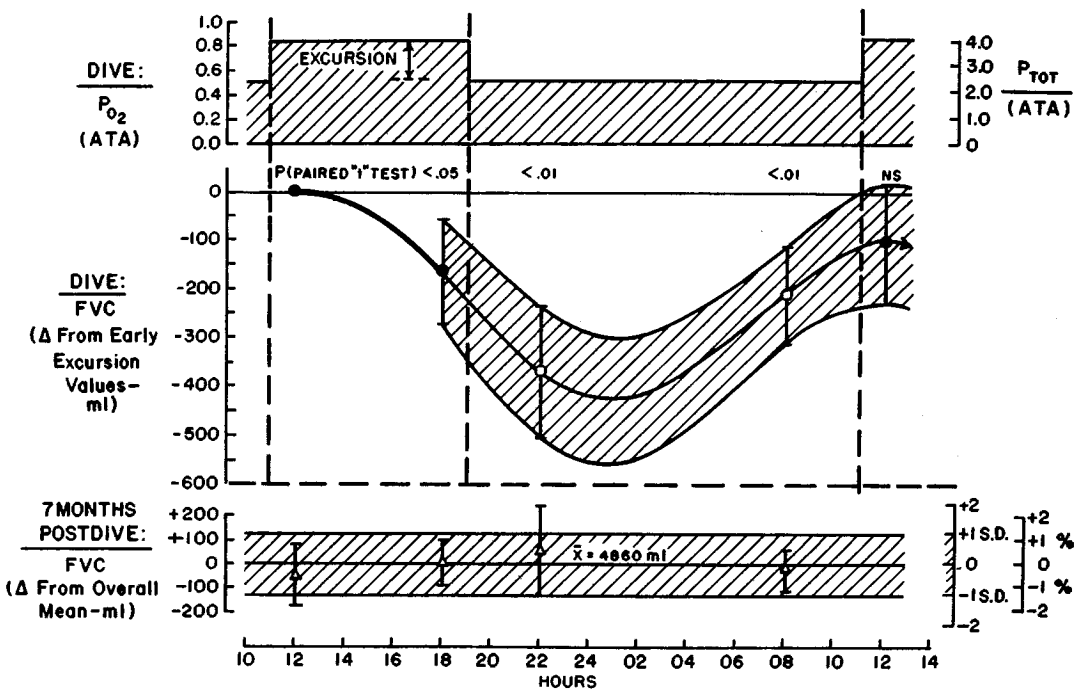

Fig. 3. Time course of forced vital capacity in *subject M.* Excursion-related decrement is shown. For each excursion, from 2 to 6 mean values were obtained for 4 points: late excursion, postexcursion evening, next morning, and early in subsequent excursion. These were subtracted from respective early excursion values, and measured standard deviations were calculated and plotted at corresponding mean time from early excursion FVC. Curves were fitted by eye. Bottom portion of figure shows points representing means and SD's of separate determinations made on 5 days at 4 sampling periods (0800, 1200, 1800, 2200 hours). Daily schedules of P_{O_2} and P_{tot} are shown at top of figure.

control (4.96 ± 0.10 liter). Because these data appeared to represent a single population, the overall mean of these 20 measurements and this mean ± 1 SD are indicated by the shaded area in Fig. 2. The individual means and standard deviations were plotted for correlation with the dive data as ΔFVC from this overall mean. In this subject circadian cycles of FVC were not observed during the hours between 0800 and 2200, when the measurements were made. Similarly, the absence of cycles was noted for FEV_1, FEV_2, PEFR, and PIFR. Hence, the FVC cycling during the dive is not attributed to circadian rhythms, and environmental factors are implicated.

During the decompression phase of SHAD III, *subject P* experienced knee pain; at 18 fsw the subject was recompressed to 28 fsw. Failure to obtain relief resulted in the decision to administer 100% O_2 for 10 minutes, followed by 5 minutes of air. This cycle was repeated 3 additional times, resulting in a total of 40 minutes exposure to 1.85 ATA of O_2. Figure 4 shows the FVC values for *subject P.* Predive and late-dive means and standard deviations are presented at the left. They are followed by individual values measured during the saturation-excursion dives. The FVC decreased to 4.28 liters within 1.5 hours following O_2 therapy, and recovered to 4.96 liters by 14 hours, only to fall more dramatically after 22 hours, reaching the lowest point of 3.64 liters. After that time, recovery was progressive.

Table III summarizes pulmonary function data with statistical analysis of changes between predive control means and postdive recovery means. Two main trends are apparent. *Subject M,* who showed indications of pulmonary O_2 toxicity, had a decrement in all eight para-

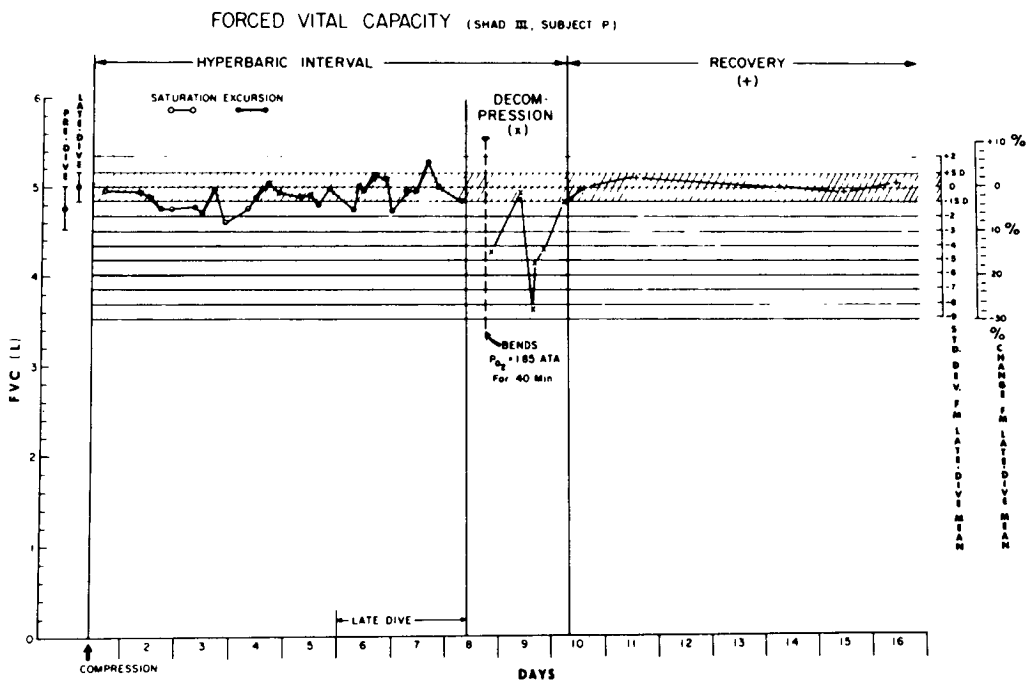

Fig. 4. Effect of SHAD III air saturation-excursion profile and superimposed 40 minutes of acute hyperoxia on FVC (*subject P*). Dive and postdive data are for 1 subject treated with 100% O_2 during decompression for "bends." Shaded area for evaluation of effect of acute hyperoxia is late-dive mean ± 1 SD.

TABLE III

PREDIVE CONTROL AND POSTDIVE VALUES OF PULMONARY FUNCTION TEST FOR SHAD DIVES

SHAD Dive No.	Subject	FVC (liters)			MVV (liters/min)			PEFR (liters/sec)			PIFR (liters/sec)		
		Control	Post	% Change	Control	Post	% Change	Control	Post	% Change	Control	Post	% Change
I	*B*	5.80 ±0.17	5.98 ±0.09	+3.1**	160 ±12	158 ±9	−2	11.45 ±0.85	11.13 ±0.55	−0.32	8.79 ±2.14	9.21 ±0.61	+0.42
	W	6.52 ±0.32	6.54 ±0.12	+0.3	133 ±12	112 ±7	−21†	12.07 ±0.63	11.15 ±0.82	−0.92*	8.58 ±0.57	9.53 ±0.66	+0.95**
II	*F*	4.23 ±0.14	4.48 ±0.05	+5.9†	139 ±18	153 ±8	+14	9.49 ±0.52	9.39 ±0.22	−0.10	6.87 ±0.64	7.66 ±0.57	+0.79*
	S	4.95 ±0.07	5.41 ±0.14	+9.3†	190 ±11	188 ±7	−2	10.52 ±0.71	10.66 ±0.59	+0.14	9.72 ±0.59	10.22 ±0.47	+0.50
III	*M*	4.96 ±0.10	4.79 ±0.16	−3.4	232 ±10	219 ±13	−13	14.59 ±0.44	14.18 ±0.32	−0.41	12.09 ±0.67	10.68 ±0.46	−1.41**
	O	7.08 ±0.22	7.50 ±0.12	+5.9**	177 ±8	201 ±9	+24	11.53 ±0.45	11.20 ±0.36	−0.33	11.44 ±0.69	11.43 ±0.79	−0.01
	P	4.77 ±0.24	5.04 ±0.15	+5.7	179 ±11	186 ±7	+7	13.16 ±1.01	14.20 ±0.60	+1.04	7.16 ±1.14	7.94 ±0.67	+0.78
		FEV_1 (liters)			FEV_1/FVC (%)			FEV_2 (liters)			FEV_2/FEV (%)		
		Control	Post	% Change	Control	Post	% Change	Control	Post	% Change	Control	Post	% Change
I	*B*	4.89 ±0.23	4.81 ±0.12	−1.6	84.1 ±2.4	80.8 ±1.8	−3.9**	5.24 ±0.17	5.32 ±0.12	+1.5	90.4 ±1.2	89.0 ±1.8	−1.4
	W	4.76 ±0.22	4.72 ±0.17	−0.8	73.0 ±2.3	72.3 ±2.0	−1.0	5.62 ±0.23	5.55 ±0.15	−1.2	86.1 ±1.7	85.0 ±1.4	−1.1
II	*F*	3.21 ±0.15	3.48 ±0.10	+8.4†	76.1 ±1.8	77.4 ±2.2	+1.7	3.74 ±0.14	4.01 ±0.08	+7.2†	88.5 ±1.4	89.6 ±1.4	+1.1
	S	3.94 ±0.11	4.42 ±0.14	+12.2†	79.7 ±1.9	81.8 ±2.3	+2.6	4.55 ±0.07	5.19 ±0.14	+14.1†	92.1 ±0.9	95.9 ±1.5	+3.8†
III	*M*	4.24 ±0.11	4.06 ±0.11	−4.2*	85.4 ±2.0	85.2 ±1.5	−0.2	4.66 ±0.12	4.43 ±0.13	−4.9**	94.0 ±1.5	92.7 ±1.4	−1.3
	O	4.53 ±0.18	4.76 ±0.13	+5.1*	63.9 ±1.5	63.6 ±0.9	−0.5	5.81 ±0.20	6.09 ±0.13	+4.8*	82.1 ±1.6	81.2 ±0.4	−0.9
	P	3.72 ±0.14	3.87 ±0.07	+4.0*	78.3 ±2.4	76.6 ±1.5	−2.2	4.20 ±0.15	4.38 ±0.09	+4.3*	88.1 ±2.2	86.8 ±2.2	−1.3

Data are means ± SD; *$P < 0.05$; **$P < 0.01$; †$P < 0.001$.

meters. The FEV_1, FEV_2 and PIFR decreases were statistically significant at the 5% level; the postdive FVC mean decrease approached significance. The second major trend is the increase in FVC of six of the seven subjects, by a statistically significant amount in four of them. Thus, all six subjects who did not show any persistent signs and symptoms of pulmonary O_2 toxicity had an increase in FVC. Both SHAD II divers had an increase in seven of eight parameters. *Subject F* showed a slight decrease in PIFR and *subject S* in MVV; neither was statistically significant.

Discussion

SHAD I (50 fsw saturation) had brief excursions to depths of 5 to 235 fsw. The various excursion depths were accomplished in a fairly random order. Exercise, which was performed on a bicycle ergometer, was irregular and infrequent; it was done primarily to test the exercise tolerance of each subject. Exercise was always performed at the saturation depth. Because of the decrease in exercise tolerance observed in SHAD I, daily exercise was performed during SHAD II, always at the 60-fsw saturation depth. The fairly short excursions were carried out to progressively deeper depths. One can only speculate whether the pattern of excursion dives used in SHAD II provided any adaptive protection. The literature gives some indication that an "adaptive" tolerance as a result of pre-exposure to increased levels of O_2 exists (2, 10, 16, 22, 23). Conversely, it has also been shown that a previous exposure to hypoxia gives some protection against later hyperoxia (4–7). A protective effect resulting from an intermittent lowering of O_2 levels during a hyperoxic exposure has been demonstrated (7, 17, 21, 24, 25). It is obvious that this procedure reduces the cumulative oxygen dose and also allows time for recovery and repair during the periods of lower P_{O_2}. However, the majority of evidence shows that increased exposure times and concentrations of oxygen cause progressive impairment. SHAD I allowed 8 days of saturation for subjects to obtain biological stabilization before the excursion program started; SHAD II allowed 7 days, and SHAD III 1 day. SHAD III, with a 50-fsw saturation, had an exercise program which was always conducted at the 100-fsw excursion depth. The cumulative oxygen dose in unit pulmonary toxicity dose (UPTD) (26), a concept which takes both increased partial pressure and time into account, was increased in the following order: Pre-SHAD I < SHAD I < SHAD III < SHAD II. However, the UPTD at the beginning of decompression after 7 days in SHAD III was much greater than after 7 days in SHAD I or SHAD II. This is discussed in greater detail elsewhere (1).

Apparently, individual variation in susceptibility to the effects of increased O_2 during air saturation diving exists. This was shown most clearly by the problems which *subject M* encountered in SHAD III. His two fellow subjects were exposed to exactly the same conditions, but showed no untoward lung effects, as indicated by these tests prior to the 100% O_2 treatment for *subject P.* The SHAD I subjects showed some early transient effects and the SHAD II subjects did not; yet the SHAD II subjects had been exposed to a higher cumulative oxygen dose than had the SHAD I subjects. It is apparent that SHAD III conditions slightly exceeded the pulmonary threshold for O_2 exposure for some individuals, because one of three subjects showed a decrement in pulmonary function. Those subjects who did not have decreases in pulmonary function due to O_2 toxicity appear to have had improved pulmonary function during shallow habitat air saturation diving. This was presumably due to an increase in the strength of the respiratory accessory muscles caused by breathing gas of in-

creased density. There may be other explanations since previous diving studies have shown both increases and decreases in FVC following long-term breathing of gases of increased density (3, 11–15, 18, 19, 27). The spirometer was calibrated before and after the dives and checked within 1%; therefore equipment variability does not provide an explanation for the changes.

Clark and Lambertsen's earlier predictions (8) indicated a maximum safe long-term O_2 concentration of 0.6 ATA. They later revised this to 0.5 ATA (9). The SHAD I subjects had mild, transient FVC decreases when exposed to an average residence O_2 concentration of 0.51 ATA during the early saturation phase of this dive. The results of SHAD II, with saturation at 60 fsw (average residence O_2 concentration = 0.57 ATA) and with deeper excursions, would indicate that for these two subjects, the earlier prediction of 0.6 ATA is more nearly correct than the conservative revision.

Daily FVC cycles were seen in one subject during the saturation-excursion portion of SHAD III, concurrent with daily P_{O_2} cycles (16 hours of O_2 = 0.53 ATA; 8 hours of P_{O_2} = 0.83 ATA). These FVC cycles are attributed to hyperoxia, for the following reasons:

(1) Declining FVC, FVC cycles, and chest symptoms were all found in the same subject.
(2) FVC decreases and chest symptoms are well-known early effects of chronic hyperoxia.
(3) The degree of hyperoxia (0.83 ATA) is of a magnitude where pulmonary symptoms might be expected.
(4) FVC cycles were found only in the presence of P_{O_2} cycles and ceased following the last excursion to 100 feet.
(5) Other cyclic environmental influences present (P_{total}, P_{N_2}, relative humidity) have not been reported to cause FVC decrements in normal individuals.

For these reasons the FVC cycles were attributed to the daily 8-hour increases in P_{O_2} from 0.53 to 0.83 ATA which are associated with excursions to 100 fsw.

Breathing 100% O_2 as a treatment for decompression sickness during the decompression phase of the SHAD dives was associated with subjective symptoms of O_2 toxicity in three of three cases. Pulmonary function changes were observed in the case for which tests were performed following 100% O_2 exposure. This oxygen treatment was used after exposure periods of 2 to 7 days of hyperoxia during the SHAD dives. Oxygen therapy for decompression sickness following a SHAD-type dive should be used only after very careful evaluation of the alternatives. The high probability of triggering pulmonary oxygen toxicity in individuals with an existing degree of damage due to oxygen toxicity would rarely, if ever, make this the treatment of choice.

Consideration should be given to having divers monitor their own FVC's during operational SHAD-type dives. Divers could be trained to make an approximate measurement of FVC on an inexpensive spirometer, and report the results to topside for evaluation. Though this would not be precise, this procedure would provide reasonably objective evidence on which to base appropriate action before changes became dangerously large and/or permanent.

Most of our subjects showed no significant decrement in the parameters measured as a result of the SHAD dives, other than the density-related flow effects. Only one of seven SHAD divers had pulmonary problems of sufficient magnitude, duration, and consistency to warrant significant concern. Six of the seven had no persistent indication of pulmonary O_2

toxicity, and tests of the seventh eventually showed return to control values. This type of air saturation diving shows promise; however, extension of these limits should be evaluated cautiously.

ACKNOWLEDGMENT

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SOME CARDIORESPIRATORY EFFECTS OF OXYGEN TOXICITY

S. M. Gošović and A. I. Radović

Exposure of living organisms to oxygen at pressures higher than 0.6 ATA provokes, sooner or later, either specific or nonspecific responses in the cardiorespiratory, central nervous, endocrine or other systems (1, 6–9, 12, 15, 18, 22). Numerous studies have established that exposure to oxygen induces bradycardia. Others have demonstrated that neurotoxic convulsions are preceded by an acceleration in the heart rate (4, 11, 14, 17). This study was undertaken to determine whether changes occurring in some cardiorespiratory parameters of subjects exposed to oxygen under pressure can be used for the prompt detection of convulsive attacks.

Material and Methods

Experiments were performed with 16 accomplished divers, aged 23 to 35, who were exposed 60 times to oxygen under pressure in a recompression chamber. After the air pressure in the recompression chamber was raised to 4.0 ATA, the subject under observation was connected to pure oxygen coming from a Lar III-Draeger closed-circuit SCUBA. In the course of the experiment, the oxygen percentage in the breathing bag was checked on the Orsat-Fischer apparatus: first, in the fifth minute, and, from then on, at regular 15-minute intervals. The average oxygen percentage was 95.23 ± 0.26, equivalent to 3.8 ATA oxygen pressure, and the average CO_2 percentage was below 0.03 percent, equivalent to 0.23 mmHg.

In each individual experiment, the ECG, cardiotachogram, pneumotachogram and electromyogram of the muscular orbicularis oris were continuously recorded on an 8-channel Beckman Dynograph RN biomedical recorder. Normally, recordings were continued for a period of 20 minutes following exposure to oxygen. In the case of neurotoxic convulsions, however, recordings were kept until the initial heart rate value was approached, for a maximum of 180 minutes.

The data obtained were processed using standard statistical procedures. Histographic analysis of the instantaneous heart rate was made by applying a modified form of the Parin-Baevski method (16).

Results

Exposure to oxygen lasted from 10 to 140 minutes, with an average of 65.2 minutes.

Exposure was interrupted, as a rule, when at least two simultaneous neurotoxic prodromal signs or symptoms appeared. The main signs and symptoms for which oxygen exposure was interrupted were: intensive nausea and gagging (47.3% of the cases); strong fascicular twitchings (42.1%); coughing and choking (18.4%); and pallor, stupor, vertigo and progressive narrowing of the visual field and amblyopia (7.89%). Generalized convulsions occurred in four cases. In one of these, convulsions came on unexpectedly, without any critical preconvulsive signs. In the other three cases, oxygen seizures began from 5 to 70 seconds after the subject was taken off oxygen.

In all experiments, a heart rate decrease was noted during compression and when breathing air at 4.0 ATA. With the application of oxygen, bradycardia was further accentuated. The average fall in the heart rate during oxygen breathing was 15.9%, and the maximum was 24.0%. No significant changes were observed in the average respiratory rate, although respiratory rate did accelerate somewhat in the final phase of exposure to oxygen (Fig. 1).

A decrease in heart rate on the cardiotachographic traces was accompanied by a fall in respiratory oscillations in heart rate. These respiratory oscillations became increasingly pronounced as exposure progressed, eventually resulting in totally aplanated cardiotachographic traces (Fig. 2). Progressive bradycardia was characterized by symmetric histographic curves of the instantaneous heart rate which were shifted to the left, indicating vagotony (Fig. 3). Relative bradycardia persisted up to 20 minutes following exposure to oxygen. The bradycardia accompanied by low respiratory oscillations in the heart rate was occasionally replaced, during manifestations of prodromal signs and symptoms, by a cyclic acceleration in the heart rate, and also by faster, deeper and less regular breathing. In the course of these cardiorespiratory disturbances, pallor, fascicular-muscular twitchings, perspiration, nausea and gagging were pronounced. These cardiorespiratory disturbances were characterized by atypical, irregular, aplanated, asymmetric and often bimodal histographic curves which were

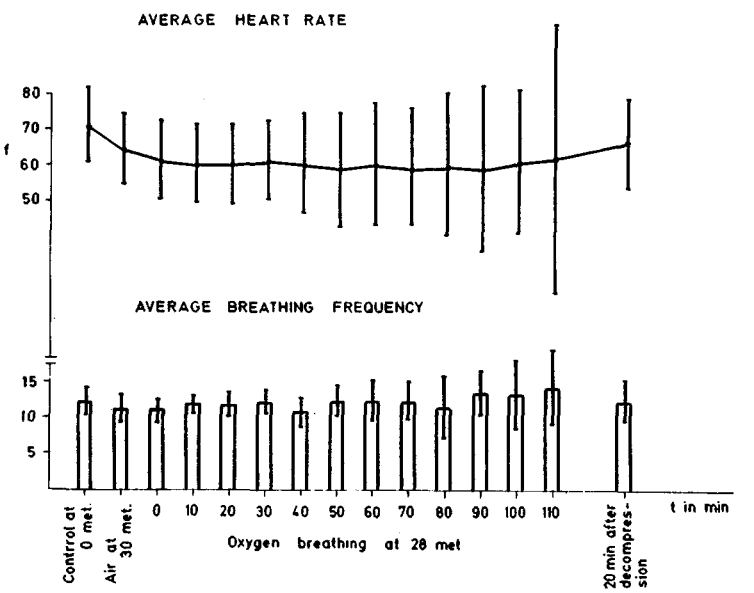


Fig. 1. Average heart rate and breathing frequency.

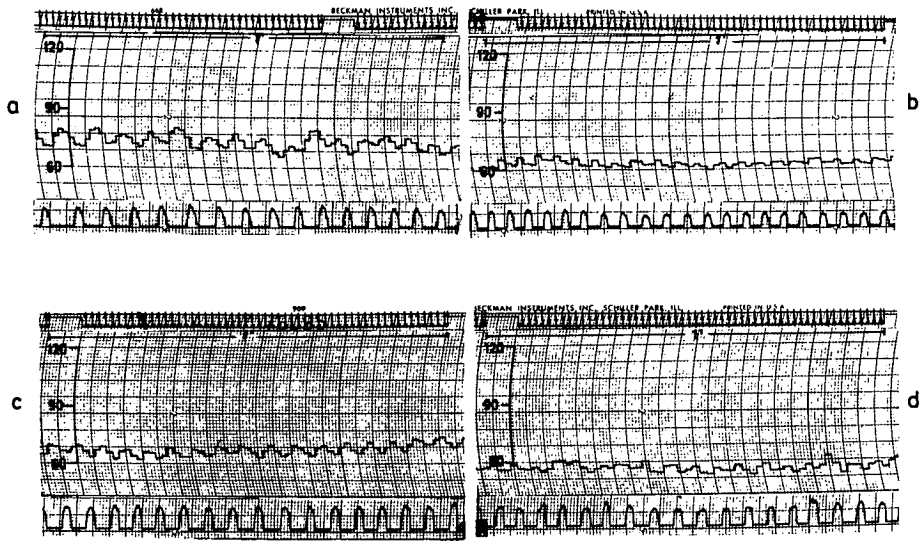


Fig. 2. Heart rate. (a) At 1 ATA, air; (b) after O_2 breathing at 3.8 ATA; (c) at 4 ATA, air; (d) during O_2 breathing at 3.8 ATA.

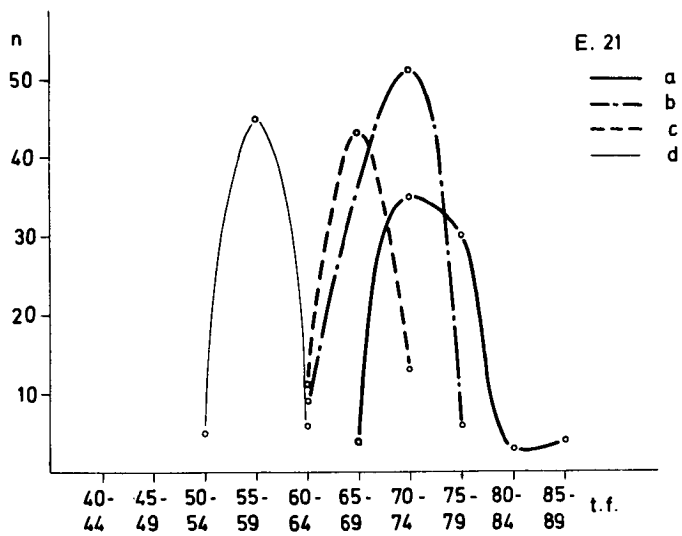


Fig. 3. Instantaneous heart rate histographic curves. (a) At 1 ATA, air; (b) after O_2 breathing at 3.8 ATA; (c) at 4 ATA, air; (d) during O_2 breathing at 3.8 ATA.

shifted to the right, indicating sympathicotonia (Fig. 4). In most cases, these disturbances were transitory and were followed by a restabilization of cardiorespiratory functions (Figs. 5 and 6). In some instances, initial disorders were followed by a short stabilization, which was then replaced by new disorders (Figs. 7 and 8).

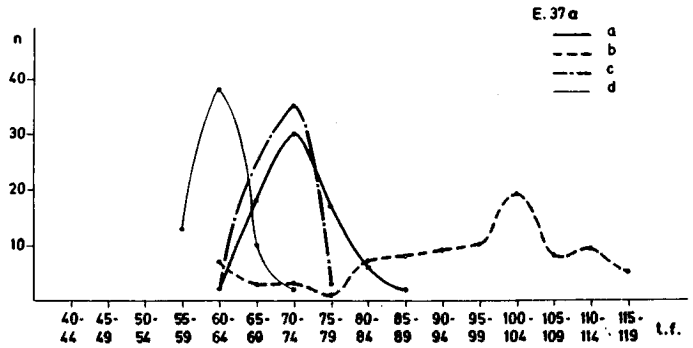
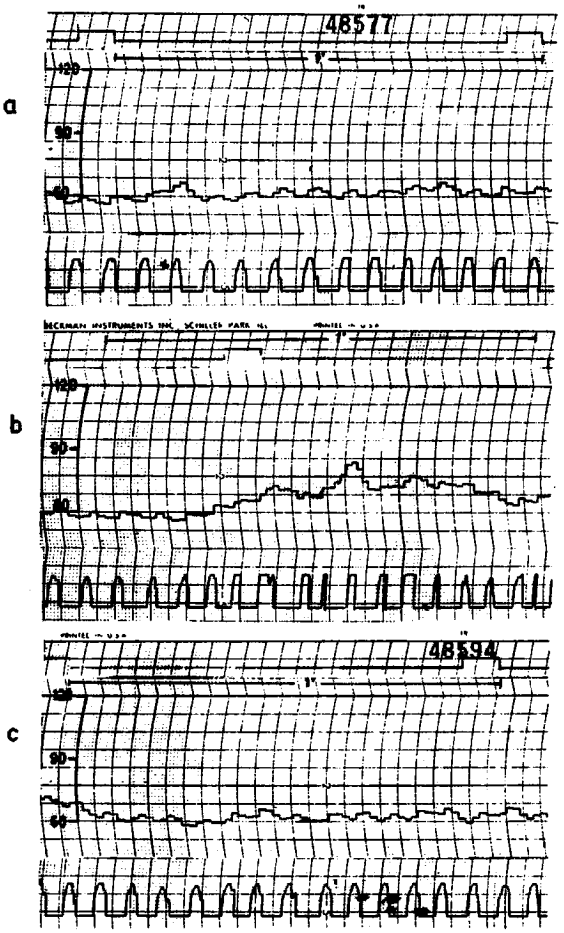
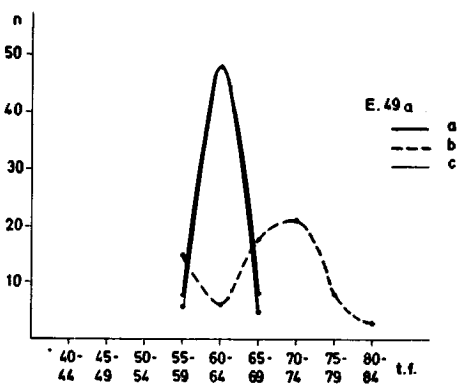
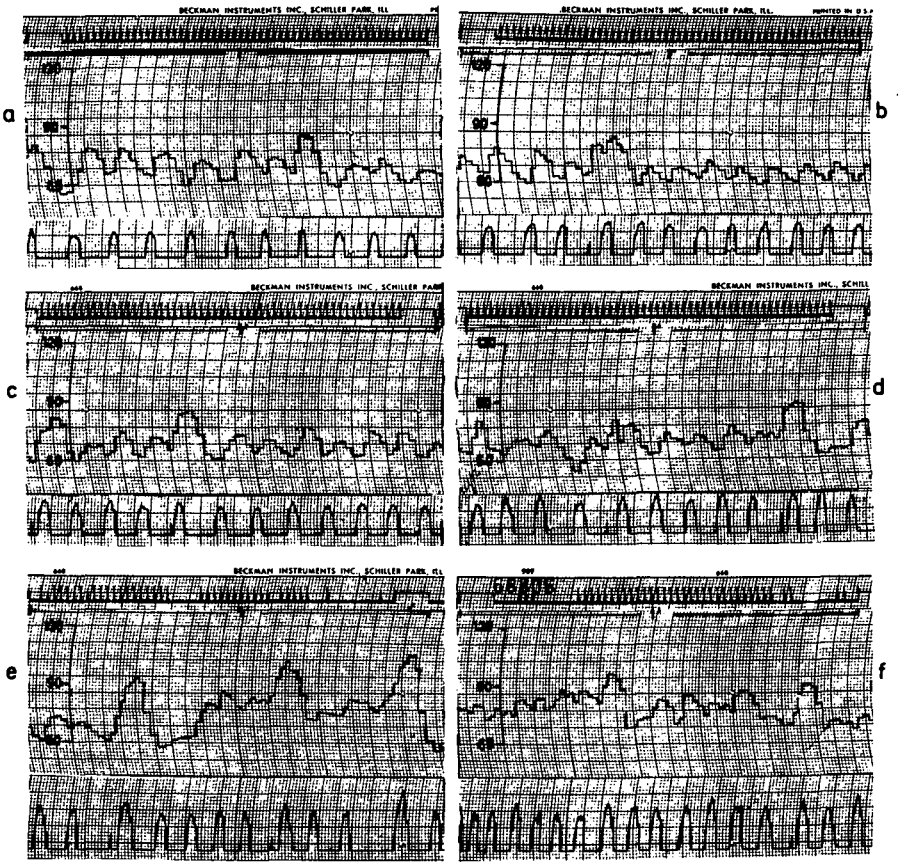


Fig. 4. Histographic curves. Normal cardiotachograms (a, c, d); during cardiorespiratory crisis (b).





Figs. 5, 6. Normal cardiotachographic traces at *min 88* (a) and *111* (c) of oxygen breathing and cardiorespiratory crisis at *min 100* (b).



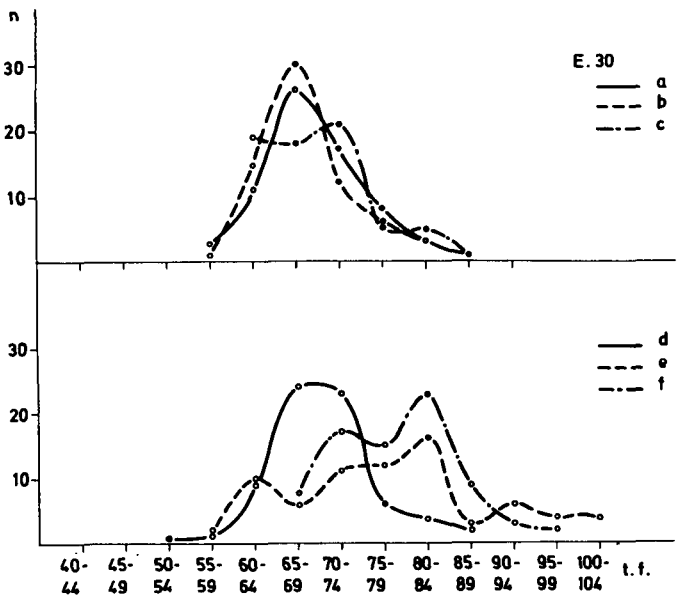


Fig. 7. Characteristic cardiotachographic curves for unstable states. (a) 1 ATA, air; (b) 4 ATA, air; (c), (d), (e), (f) during O_2 breathing at 3.8 ATA at *minute 20, 90, 100,* and *107,* respectively.

Fig. 8. Parameters are identical to those of Fig. 7.

In 80% of the experiments, the interruption of exposure to oxygen was not preceded by any notable acceleration in the heart rate, not even in the case of generalized convulsive attacks (Fig. 9). In 20% of the experiments, an insignificant acceleration in the heart rate was observed 6 to 90 seconds prior to the interruption of oxygen exposure. This type of response was regularly accompanied by intensive nausea and gagging, coughing, and movements to remove the mouthpiece; this response was usually manifested by the same subjects.

Generalized convulsions were regularly accompanied by tachycardia. However, when convulsive attacks ended, the heart rate was found to be near the initial level. Six to 12 minutes after convulsions would start, a sudden onset of characteristic tachycardia, of from 110 to 130 beats per minute, was observed in all 4 cases. This type of tachycardia lasted from 130 to 180 minutes (Fig. 10). In this phase, cardiotachographic traces showed no respiratory oscillations in the heart rate. Histographical curves of the instantaneous heart rate were high, extremely narrow, symmetric and shifted to the right (Fig. 11).

Discussion

Breathing oxygen at normal pressure and, in particular, at pressures higher than 1.0 ATA causes a decrease in the heart rate (9, 11, 13, 18, 20, 21). Similar to the results of Zinoveva, these experiments have also demonstrated that a relative acceleration in the average heart rate may occur in the final stage of oxygen exposure (21). Nevertheless, bradycardia persists throughout these exposures. Although the phenomenon itself has been known, its origin still remains vague.

In a review of works on cardiovascular changes caused by hyperbaric oxygen, Stadie et al. have stated that bradycardia is of vagal origin (18). Brue thought it was of reflex origin, caused by the stimulation of baroreceptors through raised arterial pressure (5). Observations made by Bean and Rotschafer support the view that bradycardia is of vagal origin, since

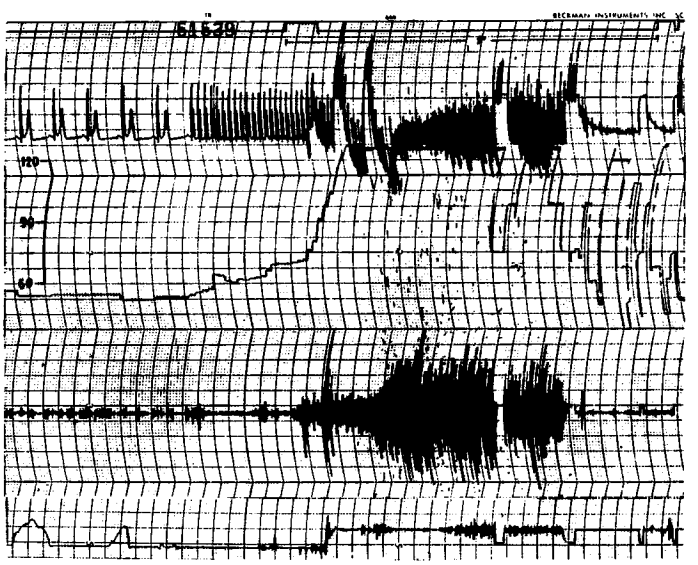


Fig. 9. Absence of tachycardia before convulsive attack.

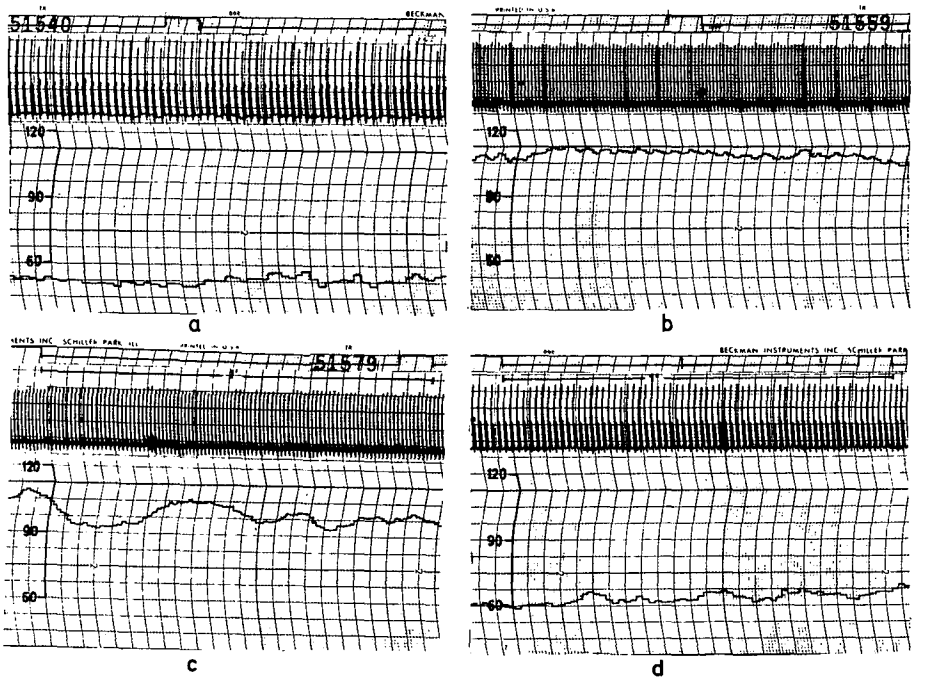


Fig. 10. Cardiotachograms after convulsive attack. (a) 5 minutes; (b) 21 minutes; (c) 50 minutes; (d) 150 minutes.

tachycardia which follows vagotomy does not essentially change when the vagotomized animal is exposed to oxygen at high pressures (1). Histographic analyses of the instantaneous heart rate show the curves to be shifted to the left, which also points to the vagal origin of bradycardia. The decrease or disappearance of respiratory oscillations in the heart rate is a further indication of the absence of sympathetic influences and the prevalence of the parasympathetic autonomic nervous system. Our findings suggest that the bradycardia brought on by breathing hyperbaric oxygen is probably complex in origin. The heart rate decreases during breathing of oxygen at normal pressure, as it does during breathing of air at high pressures. Bradycardia could be a result of the combined influence of both compression and oxygen.

Occasional and transitory accelerations in the heart rate, observed on our cardiotachographic traces, cannot be directly compared with the results obtained by other authors who have not used the same technique. This phenomenon was accompanied by irregular breathing and an aggravation of both the subjective and objective condition of the subjects. We believe disorders in the regulation of cardiorespiratory functions are probably involved in instances such as these, and are of central origin. In any case, it is evident that transitory cardiorespiratory crises occur particularly in the final stage of exposure to neurotoxic "doses" of oxygen, and that they are accompanied with prodromal signs of oxygen toxicity. Behnke and his co-workers wrote in 1935 that an increase of arterial blood pressure accompanies other signs of oxygen toxicity, including pallor and an accelerated heart rate (2).

It is widely believed that the onset of generalized neurotoxic convulsions is preceded by

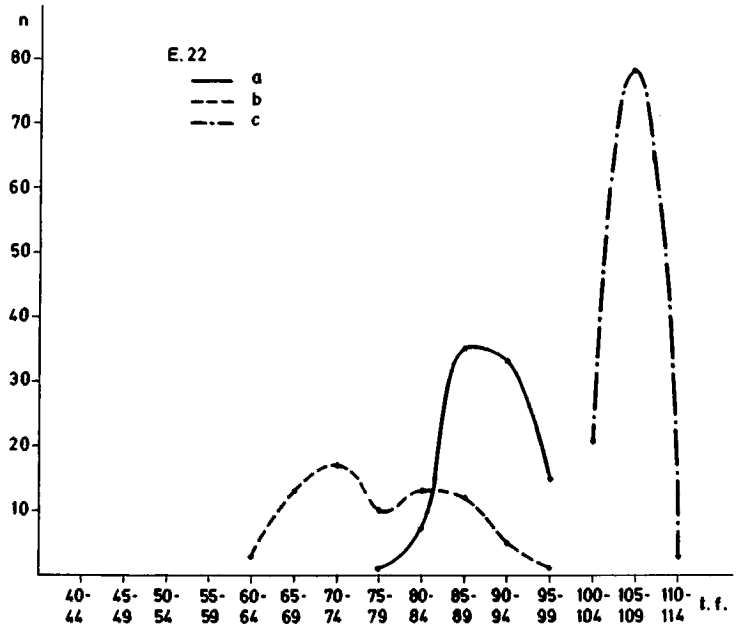


Fig. 11. Histographic curves. (a) Stable phase; (b) cardiorespiratory crisis; (c) postconvulsive tachycardia.

tachycardia (3, 4, 14, 17). Broussolle and Orsetti categorically claim that acceleration in heart rate precedes convulsive attacks by 2 to 3 minutes (4). Perimond-Trouchet and Rispe are of the opinion that tachycardia only occasionally precedes the onset of generalized convulsions (17). In 80% of our experiments, even in the case of generalized convulsions, no typical acceleration in heart rate was noted. A transitory acceleration in the heart rate was observed in about 20% of the cases, along with critical preconvulsive signs and symptoms. This type of tachycardia was regularly observed in the same subjects. Consequently, we believe that tachycardia cannot be considered a typical warning sign of oncoming convulsive attacks. Tachycardia could be a manifestation of cardiorespiratory crises which are not followed by restabilization, or could be provoked by the subjects' motor excitement caused by choking, gagging and the effort to remove the mouthpiece.

It is difficult to explain the origin of prolonged tachycardia which appears a few minutes after cessation of generalized convulsions. At this state there are no respiratory oscillations in heart rate on the cardiotachographic traces, and histographic analysis indicates a total predominance of the sympathetic nervous system. The pathogenesis of this phenomenon still remains vague. No similar phenomenon was observed in subjects not affected by convulsions, although their heart rates were continuously recorded for a 20-minute period following oxygen exposure. In any case, it seems that disorders which follow oxygen seizures last far longer than could be suspected on the basis of the clinical picture alone—that is, on the basis of cessation of convulsions. It is also impossible to explain why this type of tachycardia appears for only a few minutes after the end of convulsions, when the subject is again feeling well.

Summary

The experiments reported in this study lead to the following conclusions.

1) Exposure to oxygen at a pressure of 3.8 ATA induces progressive bradycardia with a maximum average heart rate fall of 24%. Histographic analysis of the instantaneous heart rate is indicative of bradycardia's vagal origin.

2) Periodic transitory cardiorespiratory disturbances occur at the time prodromal neurotoxic signs and symptoms of oxygen toxicity appear. These crises are manifested in a transitory acceleration in heart rate, and changes in the rhythm, amplitude and frequency of breathing rate.

3) Cardiorespiratory crises are accompanied by an intensification of prodromal oxygen neurotoxic manifestations.

4) These crises are followed by longer or shorter stabilizations in cardiorespiratory functions, other successive crises, or sudden onset of generalized convulsions.

5) The findings of this study do not support the view that tachycardia precedes convulsive attacks. Consequently, tachycardia cannot be taken as a characteristic preconvulsive sign.

6) The postconvulsive phase is followed by a prolonged and characteristic tachycardia. In such instances, histographic analysis of the instantaneous heart rate is indicative of the total predominance of the sympathetic autonomic nervous system. The pathogenesis of postconvulsive tachycardia remains unclear.

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EFFECT OF HIGH-PRESSURE OXYGEN ON CELLULAR FINE STRUCTURE, AMMONIA AND GLUTAMATE METABOLISM. INFLUENCE OF LITHIUM PRETREATMENT.

E. W. Banister, N. M. G. Bhakthan, N. Radhakrishnan and A. K. Singh

It is well established that nervous activity is accompanied by ammonia liberation and that the rate of this process is correlated with the intensity of nervous activity (20, 25). Thus the ammonia level in the brain of animals convulsed by a variety of causes, including drugs (19), electric shock (22), anoxia (22), and oxygen at high pressure (OHP) (10), is significantly elevated. Separate reports on changes in fine structure attendant upon oxygen convulsive states have been made on brain and other vital tissues (3, 18, 24), and previous reports (5, 7) have attempted to relate these fine structural changes to a developing ammonia toxicity. This report is concerned with investigating this concept by analyzing the biochemical and fine structural correlates of preconvulsive and convulsive states induced in animals by OHP after premedication with lithium. Lithium is an inorganic drug (26) with ammonia chelating properties (11, 17) demonstrated to be effective against OHP toxicity (21).

Materials and Methods

Animals and Treatment Groups

Groups of five male Sprague-Dawley rats were each assigned to one of six treatments as follows:

(1) A control group, no oxygen exposure, no lithium medication;
(2) An untreated OHP group, exposed to oxygen at 6 ATA and convulsed;
(3) A lithium group premedicated 15 minutes prior to OHP, and oxygen-exposed for a period equal to the time to convulsion of the untreated group (group 2);
(4) A lithium group premedicated 15 minutes prior to OHP and exposed to oxygen at 6 ATA until convulsion;
(5) A lithium group premedicated 24 hours prior to OHP at 6 ATA and oxygen-exposed until convulsion;
(6) A lithium group premedicated 24 hours prior to OHP supplemented with a pre-OHP 15 minute 'booster' injection and exposed to oxygen until convulsion;

Animals were maintained on a diet of standard lab chow and water ad libitum, and were

fasted for 14 hours immediately prior to the experiment. Lithium was administered in a single dose of 9.4 mmol/kg intraperitoneally (ip) at the time indicated prior to OHP exposure.

Chamber Operation

A small animal chamber of about 100-liter capacity was used for hyperbaric oxygenation of the animals. The two ends of the chamber were made of thick Plexiglas for easy viewing of the interior. Animals were exposed singly to their particular experimental condition. Before pressurization, after the animal was placed in the chamber, 100% O_2 was flushed through at a flow rate of 5 liter/min for 5 minutes, after which pressurization with pure oxygen to 72.5 psig over a period of 6 to 8 minutes occurred. Gas flow was maintained at the 1-atmosphere equivalent of 5 liter/min to minimize CO_2 accumulation. Decompression after oxygenation took place over an 11-minute period which included stops at 40 psig (5 minutes), 30 psig (1 minute) and decompression from 15 psig to the surface over a 3-minute period.

Blood and Brain Tissue Amino-Acid Assays

All animals, including the control group, were lightly anesthetized with ether and the abdominal cavity immediately opened. The animals were killed by exsanguination by withdrawing the maximum blood obtainable with a needle and syringe from the bifurcation of the abdominal aorta.

Blood

Serum was separated from the blood by centrifugation after allowing clotting; an equal amount of citrate buffer was added and the solution was kept at room temperature for 30 minutes. Protein was precipitated with 80% ethanol and free amino acids were extracted twice. The alcohol was removed from the final extract by evaporation on a water bath at 50°C and 0.05–0.1 ml of the residue was used for analysis by an amino acid analyzer using the procedure of Benson et al. (6).

Brain

After exsanguination, the brain was quickly removed, weighed and kept cold. It was homogenized, after removal of small pieces for electron microscopy, in 5 ml of phosphate buffer (pH 7.5). The homogenate was centrifuged for 15 minutes (2000 *G*) and the supernatant removed. It was then deproteinized, and amino acids were extracted twice with 80% ethanol, and the residue finally obtained by evaporating off the ethanol on a water bath at 50°C. For analysis by an amino acid analyzer (Beckman Instruments), 0.05–0.1 ml of residue was used.

BLOOD AND BRAIN LITHIUM ASSAY

Blood

Serum (250 μl) from the exsanguination procedure was pipetted into a centrifuge tube containing 5.0 ml trichloroacetic acid (TCA). After mixing and standing 10 minutes, the sample was centrifuged and the supernatant measured in a flame photometer against standard lithium

solutions containing 0 and 2 mEq/liter lithium, respectively, with 140 mEq/liter sodium, 4 mEq/liter potassium and 5 mEq/liter calcium (1).

Brain

Brain tissue was weighed, homogenized with a known volume of TCA, and allowed to stand for 10 minutes, after which the homogenate was centrifuged. The supernatant was used for lithium estimation in the flame photometer.

Electron Microscopy

After anesthetizing the animal with ether, and exsanguination, very small pieces of tissue were removed from: (1) left belly of the gastrocnemius muscle; (2) left lobe of the liver; (3) right cerebral hemisphere; and (4) papillary muscle from the left ventricle.

Immediately after removal, the tissues were fixed in 3% glutaraldehyde at pH 7.4 in 0.1 molar sodium cacodylate. The tissues were then diced and after 2 hours postfixed with 2% osmium tetroxide in 0.1 molar cacodylate buffer for another 2 hours. All fixation was carried out at 4 °C with utmost care. The samples were dehydrated by upgrading through an ethanol water gradient, and flat embedded in Epon 812. Thick sections were cut by glass knives, stained with toluidine blue and examined with a light microscope. Thin sections were cut with diamond knives on a Rheikert OMU2 ultramicrotome, stained with uranyl acetate and lead citrate and examined in a RCA EMU-3H transmission electron microscope.

Quantification of Electron Microscopy

Five blocks were prepared and sectioned from every tissue sample which was flat embedded. Five grids with 4 or 5 sections were prepared from every block. The grids were scanned initially at low magnification, and suitable areas were examined in detail. Tissue was examined from comparable locations for all the experimental conditions. Areas of sections which appeared to show fixation defects such as insufficient polymerization (causing softness of tissue which will expand and break under the electron beam) and possible damage due to dicing (broken cells, areas where cytoplasmic inclusions appeared in intercellular space) were discarded and only those fine structural changes that appeared to be due to the experimental conditions were examined in detail and used for interpretation (7).

Results

Blood-Brain Lithium Distribution

Table I shows lithium levels in rat brain and blood tissue at various times after injection with or without exposure to OHP and confirms previous work by others (21).

Lithium and Blood Metabolites

Significantly lower lithium concentrations existed in the blood of animals that were lithium-premedicated 15 minutes prior to OHP and oxygen-exposed for a time equal to that normally producing convulsions in untreated animals than in control animals an equal time after

TABLE I

BRAIN AND BLOOD LITHIUM (Li) CONCENTRATIONS IN ARTERIAL BLOOD OF RATS EXPOSED TO OHP AT DIFFERENT INTERVALS AFTER LITHIUM INJECTION

Tissue	15-min Control (no Li)	60-min Control (no Li)	2-hr Control (no Li)	24-hr Control (no Li)	24-hr, 15-min Control (no Li)	Li-treated 15 min before normal OHP exposure	Li-treated 15 min before OHP, convulsed	Li-treated 24-hr before OHP, convulsed	Li-treated 24 hr and at 15 min before OHP, convulsed
Brain	1.0 ± 0.8	2.74 ± 0.22	4.7 ± 1.38	7.12 ± 0.90	7.56 ± 1.01	4.81 ± 0.48	10.0 ± 1.33	9.58 ± 0.28	9.06 ± 1.19
Blood	21.6 ± 4.6	20.08 ± 1.05	12.64 ± 1.94	2.40 ± 0.53	23.34 ± 3.76	9.50 ± 0.39	9.86 ± 0.67	2.26 ± 0.41	21.24 ± 2.39

Data are means ± SE, for concentrations in mEq/kg and mEq/liter. Experimental animals, $n = 5$.

lithium injection that were not exposed (9.50 compared to 20.8 mEq/liter) (Table II). At the same time, arterial blood ammonia, arginine and urea concentrations were significantly lower than in any other experimental condition, and were almost equal to control levels in animals unexposed to oxygen.

Significantly greater arterial blood glutamate concentrations existed in lithium-protected animals than in normal unprotected convulsed rats. Concomitantly, compared to the normal unprotected convulsed group, the group premedicated with lithium 15 minutes prior to OHP and oxygen-exposed for an equivalent time showed significantly lower blood glutamine levels. The group lithium-premedicated 24 hours prior to OHP and then convulsed showed significantly higher blood glutamine levels. Time to convulsion was significantly shortened in animals where the lithium brain-blood distribution profile showed high levels of lithium in the brain from the onset of oxygen exposure regardless of the level of lithium in the blood.

TABLE II

METABOLITES IN BRAIN OF RATS EXPOSED TO O_2 AT 6 ATA, AND AMMONIA, AMINO ACID, AND LITHIUM (Li) CONCENTRATIONS IN BRAIN OF RATS AFTER LITHIUM INJECTION

	Control (no Li, no OHP)	No Li, O_2 convulsed	Li-treated 15 min before normal OHP exposure	Li-treated 15 min before OHP, convulsed	Li-treated 24 hr before OHP, convulsed	Li-treated 24 hr and at 15 min before OHP, convulsed
Exposure Time	—	50 ± 10	50	115 ± 9	30	30
Lithium	—	—	4.8 ± 0.48	10.0 ± 1.32	9.58 ± 0.28	9.06 ± 1.19
Ammonia	5.96 ± 0.30	18.94 ± 1.34	10.14 ± 0.82	22.2 ± 1.61	12.20 ± 1.07	17.66 ± 1.20
GABA	1.17 ± 0.12	0.79 ± 0.10	1.04 ± 0.12	0.94 ± 0.12	0.72 ± 0.08	0.76 ± 0.12
Glutamate	9.92 ± 1.30	5.82 ± 0.63	8.36 ± 0.53	6.18 ± 0.54	3.34 ± 1.94	4.92 ± 0.85
Glutamine	0.92 ± 0.34	2.98 ± 0.25	1.04 ± 0.08	3.78 ± 0.81	5.3 ± 0.32	3.22 ± 0.36
Aspartate	3.92 ± 0.38	3.88 ± 0.58	3.84 ± 0.67	2.48 ± 0.51	3.60 ± 0.51	3.80 ± 0.25
Arginine	0.10 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	0.11 ± 0.02	0.10 ± 0.02	0.09 ± 0.03
Lysine	0.10 ± 0.02	0.16 ± 0.03	0.18 ± 0.02	0.14 ± 0.02	0.11 ± 0.02	0.12 ± 0.02

Data are means ± SE, for time in minutes to convulsion, or for concentrations of ammonia (μg/g), amino acid (μmol/g), and lithium (mEq/kg). Experimental animals, $n = 5$.

Lithium and Brain Metabolites

Brain ammonia concentration in the group lithium-premedicated 15 minutes prior to OHP and then exposed for a time equivalent to that causing convulsion in unprotected animals was significantly lower than in any other experimental group whether lithium-protected or not (Table III). The attendant glutamate level was significantly higher and glutamine significantly lower. Although brain ammonia in animals lithium-premedicated 24 hours prior to OHP and then oxygen-convulsed was significantly greater than that for animals lithium-premedicated 15 minutes prior to OHP and then exposed to oxygen for a time equal to that for convulsed, untreated animals, it was significantly lower than for any other group; at the same time, this group's glutamate levels were significantly lower than those of any other group, and glutamine levels were significantly higher. Aspartate concentrations in animals lithium-premedicated 15 minutes prior to OHP and then oxygen-exposed until convulsion were significantly lower than for any other group, but brain arginine was unchanged by any treatment. Lysine concentration in the brain was significantly depleted from the control level in every group except in the group lithium-premedicated 15 minutes prior to OHP and then exposed to oxygen for the length of time required to cause untreated animals to convulse.

ELECTRON MICROSCOPY

Examination of the tissues from gastrocnemius muscle, heart, brain, and liver of unprotected rats exposed to oxygen under pressure confirmed the findings of an earlier report (7). Therefore these observations will not be described in detail; this description will concentrate on changes observed after lithium pretreatment at various times prior to oxygen exposure.

There were no significant structural changes in the gastrocnemius muscle in any experimental condition. In papillary muscles, apart from slight swelling of mitochondria and dilation of sarcotubular systems, the most conspicuous change was along the intercalated disc.

TABLE III

METABOLITES IN ARTERIAL BLOOD OF RATS EXPOSED TO O_2 AT 6 ATA, AND AMMONIA, AMINO ACID, AND LITHIUM (LI) CONCENTRATIONS IN BLOOD OF RATS AFTER LITHIUM INJECTION

	Control (no Li, no OHP)	No Li, O_2 convulsed	Li-treated 15 min before normal OHP exposure	Li-treated 15 min before OHP, convulsed	Li-treated 24 hr before OHP, convulsed	Li-treated 24 hr and at 15 min before OHP, convulsed
Exposure Time	—	50 ± 10	50	115 ± 9	30	30
Lithium	—	—	9.50 ± 0.39	9.86 ± 0.67	2.26 ± 0.41	21.24 ± 2.39
Ammonia	2.12 ± 0.30	6.82 ± 0.86	3.64 ± 0.44	8.59 ± 0.54	7.72 ± 0.95	7.58 ± 0.89
Glutamate	3.5 ± 0.62	2.1 ± 1.42	3.18 ± 0.89	3.44 ± 0.54	3.02 ± 0.33	3.10 ± 0.83
Glutamine	16.98 ± 0.73	22.66 ± 4.09	19.40 ± 3.07	22.7 ± 2.14	25.62 ± 1.72	21.32 ± 2.49
Aspartate	1.58 ± 0.38	2.0 ± 0.39	1.84 ± 0.42	1.50 ± 0.34	1.50 ± 0.32	1.48 ± 0.30
Arginine	1.8 ± 0.29	3.64 ± 0.51	2.5 ± 0.40	4.40 ± 0.46	4.18 ± 0.27	4.26 ± 0.34
Urea	30.6 ± 4.75	51.92 ± 6.45	30.22 ± 2.25	61.72 ± 2.39	60.02 ± 1.01	60.56 ± 1.72

Data are means ± SE for time in minutes to convulsion, or for concentrations of ammonia (μg/ml), amino acid (μmol/100 ml), and lithium (mEq/liter). Experimental animals, $n = 5$.

Under normal conditions in longitudinal sections, the opposing plasma membrane at the intercalated disc can be identified as two parallel, dense lines that closely follow a sinuous course separated for the most part by a 150–200 Å intercellular cleft (Fig. 1). In animals exposed to oxygen after lithium premedication 15 minutes prior to OHP and then oxygen-exposed for the same period of time which produced convulsions in untreated rats, there was no noticeable difference at the intercalated disc. In those animals which were convulsed after lithium premedication at other times prior to OHP, i.e., 24 hours, 24 hours with a 15-minute 'booster,' and a 15-minute group which needed to be exposed for twice the normal period, there was similar separation of the intercalated disc along most of its entire course in several regions (Figs. 2 and 3). Similar separation can be achieved in vitro if the heart is allowed to beat in a calcium-free perfusion solution (16).

Liver

Liver from rats subjected to pretreatment with lithium 15 minutes before oxygen exposure had slight mitochondrial swelling but no dilation of the reticulum (Fig. 4). In animals injected with lithium 24 hours before OHP, the hepatocytes had extensive cytoplasmic alterations, although the severity of these alterations varied from cell to cell. In addition to mitochondrial swelling, the cisternae of both endoplasmic reticula were dilated (Fig. 5). More severe alteration occurred in animals exposed to oxygen 24 hours after lithium injection. In tissue from these animals, even though the mitochondria appeared less affected, the cisternae of the endoplasmic reticulum were vacuolated and had very few ribosomes attached to the membrane (Fig. 6). There were occasionally small, osmophilic whorls of lamellae in other sections, though Fig. 6 does not show these. There appeared to be more lipid droplets in the hepatocytes of animals lithium-premedicated 24 hours before exposure than in those from other experimental conditions. The number of peroxisomes and lysosomes in these liver cells from the 24-hour group also increased. However, the hepatocytes from rats convulsed after lithium pretreatment 15 minutes before OHP had condensed mitochondria and condensed endoplasmic reticulum (Fig. 7). Most of the glycogen store was depleted, and in cells closer to sinusoids there were many lipid droplets. The striking difference in hepatocyte structure among rats pretreated with lithium 24 hours, and 24 hours and 15 minutes, respectively, and those pretreated with lithium only 15 minutes before oxygen exposure indicates either that the protective nature of lithium pretreatment is of short duration (15 minutes as opposed to 24 hours) or that the high levels of lithium in the brain 24 hours later predisposed such brain tissue to dysfunction under OHP stress.

Brain

As in the case of other tissues, the most severe cytoplasmic alteration was evident in the neurons of rats exposed to oxygen after pretreatment with lithium 24 hours before. The normally functional motor neuron has a well-developed granular endoplasmic reticulum as well as free ribosomes (Nissl substance) in polysomal configurations (Fig. 8). There was little structural difference between neurons from control animals and neurons from animals premedicated with lithium 15 minutes before OHP exposure for a time sufficient to cause convulsions in untreated animals, except that the premedicated group had a few swollen mitochondria (Fig. 9). As in other tissues, the degree of alteration varied from region to region in different sections from the same animal, and also among animals. However, in rats pre-

Fig. 1. Longitudinal section through part of papillary muscle fiber from control rat. Note intact intercalated disc **(ID)**. **M**, mitochondria. ×12800.

Fig. 2. Papillary muscle from rat heart exposed to OHP 24 hours after lithium medication. Separation of intercalated disc **(ID)** is obvious. **F**, myofibrils. ×12800.

Fig. 3. Section (as in Fig. 2) magnified to indicate greater separation of **ID** where myofibrils are attached. **L**, lipid. ×22000.

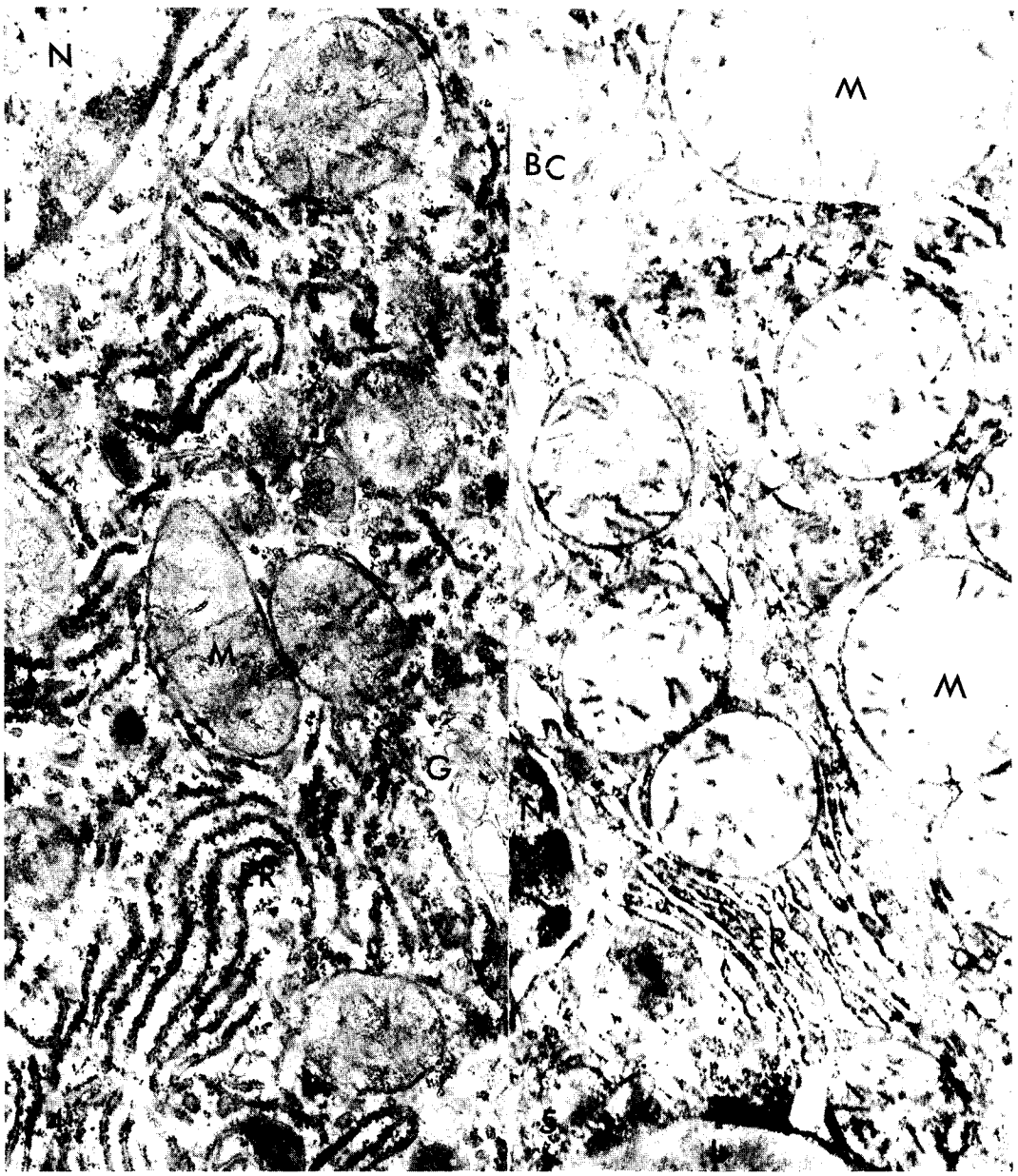

Fig. 4. Portion of hepatocyte showing normal configuration of mitochondria (**M**) and endoplasmic reticulum (**ER**). **G**, Golgi complex; **N**, nucleus; **P** peroxisome. ×16400.

Fig. 5. Portion of hepatocyte from rat injected with lithium 24 hours before killing. Both smooth and granular reticulum (**ER**) are dilated. Mitochondria (**M**) also appear swollen. **BC**, bile canaliculus; **L**, lipid; **N**, nucleus. ×16400.

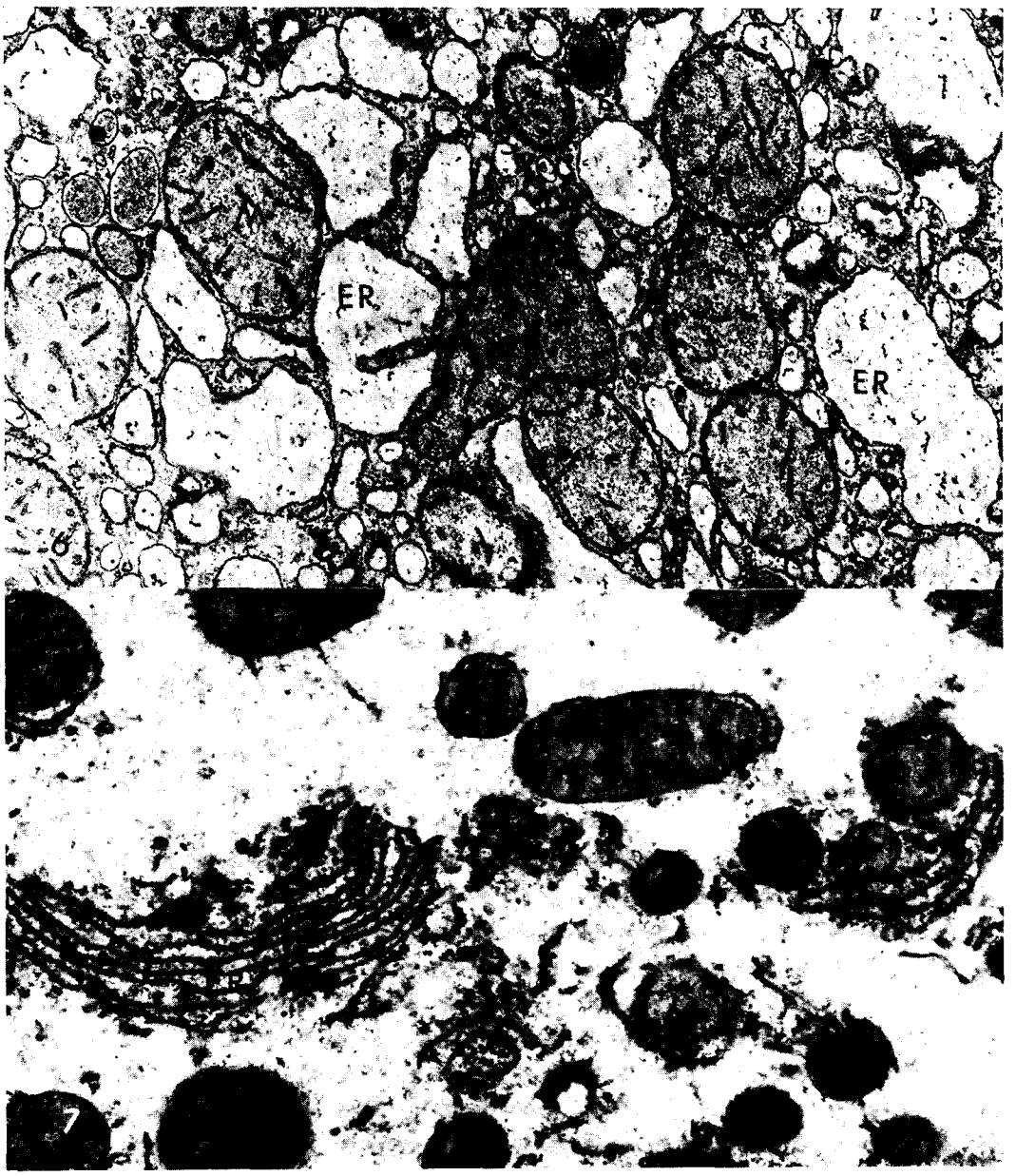

Fig. 6. Region of liver cell from rat premedicated with lithium 24 hours before oxygen exposure. Note highly vacuolated endoplasmic reticulum (**ER**). **M**, mitochondria; **P**, peroxisome. ×16400.

Fig. 7. Portion of liver cell from rat left to convulse 15 minutes after lithium injection. Both mitochondria (**M**) and endoplasmic reticulum (**ER**) appear to be condensed. Electron lucent space is where glycogen was stored. ×16400.

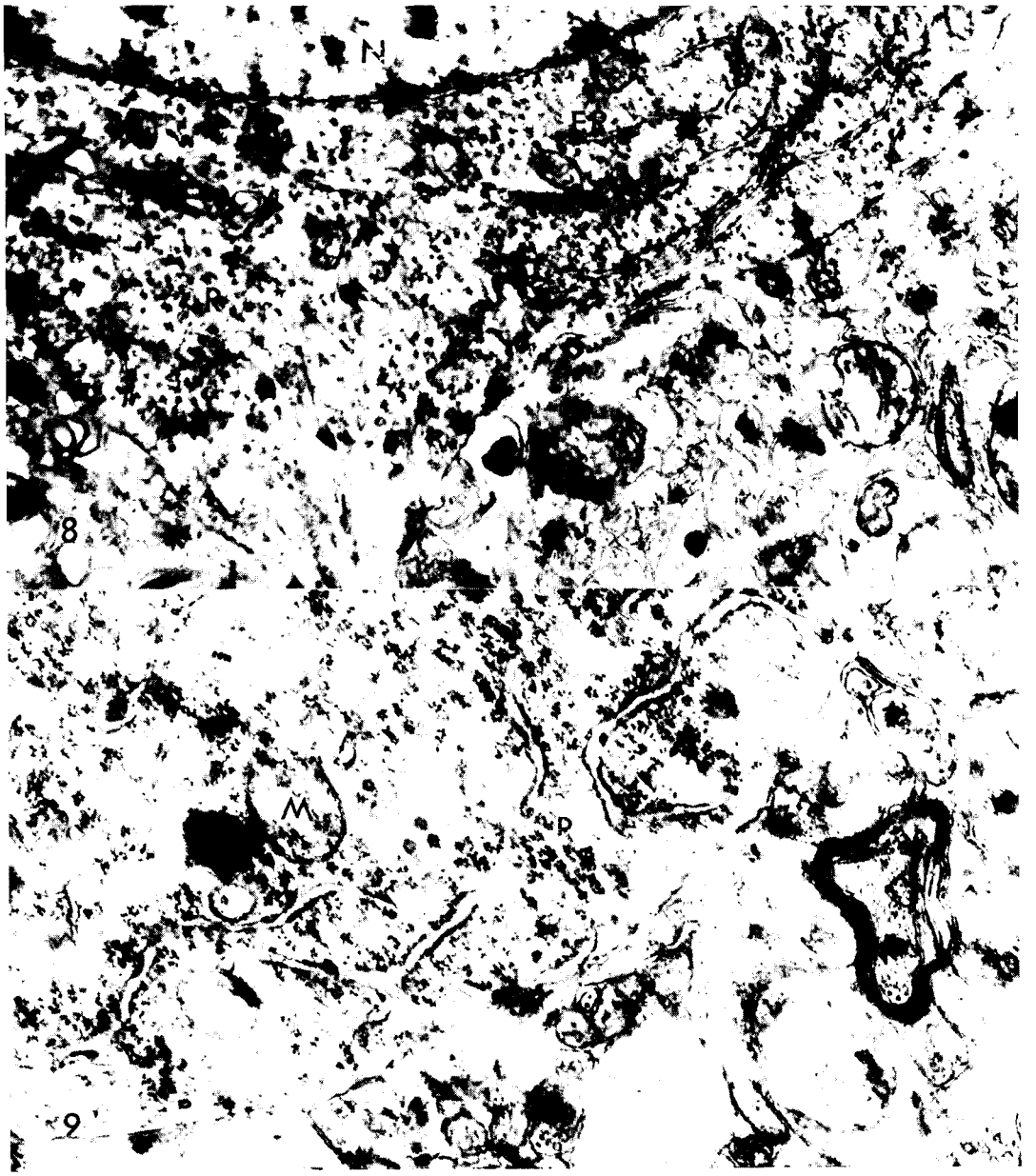

Fig. 8. Portion of normal motor neuron showing highly developed granular endoplasmic reticulum (**ER**) and numerous free ribosomes (**R**). **N**, nucleus. ×16400.

Fig. 9. Portion of neuron from rat exposed to OHP 15 minutes after lithium medication but not allowed to convulse. Little damage to cytoplasmic organelles. **M**, mitochondria; **R**, ribosomes. ×16400.

treated with lithium 24 hours before OHP, or those pretreated 24 hours in advance who received an additional booster 15 minutes before exposure, samples showed dilation of the Golgi complex (Fig. 10), formation of dense lysosome-like granules near the Golgi complex (Fig. 11), and loss of Nissl substance, which indicate disruption of endoplasmic reticulum and lack of ribosomes (Figs. 12, 13). In several such severely altered neurons, the neuronal membrane appeared to be discontinuous (Fig. 14). The axonal process and presynaptic terminals contained fewer vesicles and in several cases damaged mitochondria. Mitochondrial damage was more evident in large axonal processes with large mitochondria (Fig. 15), while small axonal processes with smaller mitochondria appeared unaffected. As observed previously, (7) edematous conditions prevailed in the endothelial cells of the capillaries, in addition to disruption of the astrocyte process around the capillaries in the brain of rats convulsed by OHP exposure 24 hours after lithium pretreatment.

Discussion

OHP exposure per se has been shown to induce a wide variety of fine structural damage in mammals, including alterations in striated muscles (8). These alterations lead to necrotic conditions if animals are forced to breathe OHP for long periods. These necrotic changes include mitochondrial damage leading to osmophilic whorls, sarcotubular vacuolation and deranged Z-lines. In the short-term experiments of this investigation, significant changes from the normal architecture of striated muscle were observed but they were not of the same magnitude as those described by Caulfield (8). Swelling of mitochondria in several tissues, even after cacodylate buffer fixation, indicates that physiological events are indeed occurring inside and outside the mitochondria which may lead to their conformational change. These events include increased blood lactate concentration, change of pH, and hypoxia, leading to ischemic conditions in the heart (2, 12). Accumulation of metabolic inhibitors either induced under experimental conditions or as an effect of catabolism may also alter the mitochondrial morphology, as suggested by Laiho et al. (14), where inhibition of adenosine 5′-triphosphate (ATP) synthesis or induced cell membrane permeability may cause swelling or condensation of mitochondria.

Various metal compounds are toxic to humans and animals at greater than physiologic concentrations. Although lithium compounds are used clinically in treating various psychological disorders (26), their effects on the fine structure of vital organs such as the brain and liver are not well known. It is obvious from this investigation that within 24 hours after a single injection of lithium, without OHP exposure, there are significant cytoplasmic alterations in the hepatocytes and the brain neuron. The alterations in the liver may be due as much to a change in membrane permeability as to the mammalian liver function of detoxifying the binding of deleterious substances, whether artificially introduced or naturally produced during physiological functions.

Hoffman et al. (9) reported deterioration of endoplasmic reticulum and increased vacuolation in hepatocytes after acute cadmium administration. It may be that lithium produces just such an effect in the liver, and at the time (24 hours after injection) when brain lithium levels are high, the toxic effect of lithium on the neuron also outweighs its protective ammonia-complexing activity. Since fine structural alterations are common to hepatocytes and neurons in animals premedicated with lithium 24 hours before OHP exposure and are absent in nor-

Fig. 10. Part of an astrocyte showing dilated Golgi complex (G) and swollen mitochondria, while mitochondria (M) in synapse appear unaffected in rat injected with lithium 24 hours before. N, nucleus. ×16400.

Fig. 11. Part of neuron from rat brain premedicated with lithium 24 hours before. Note as in Fig. 11 dilation of Golgi complex (G) and disruption of mitochondria (M). Ribosomes (R) and reticulum appear unaffected. ×16400.

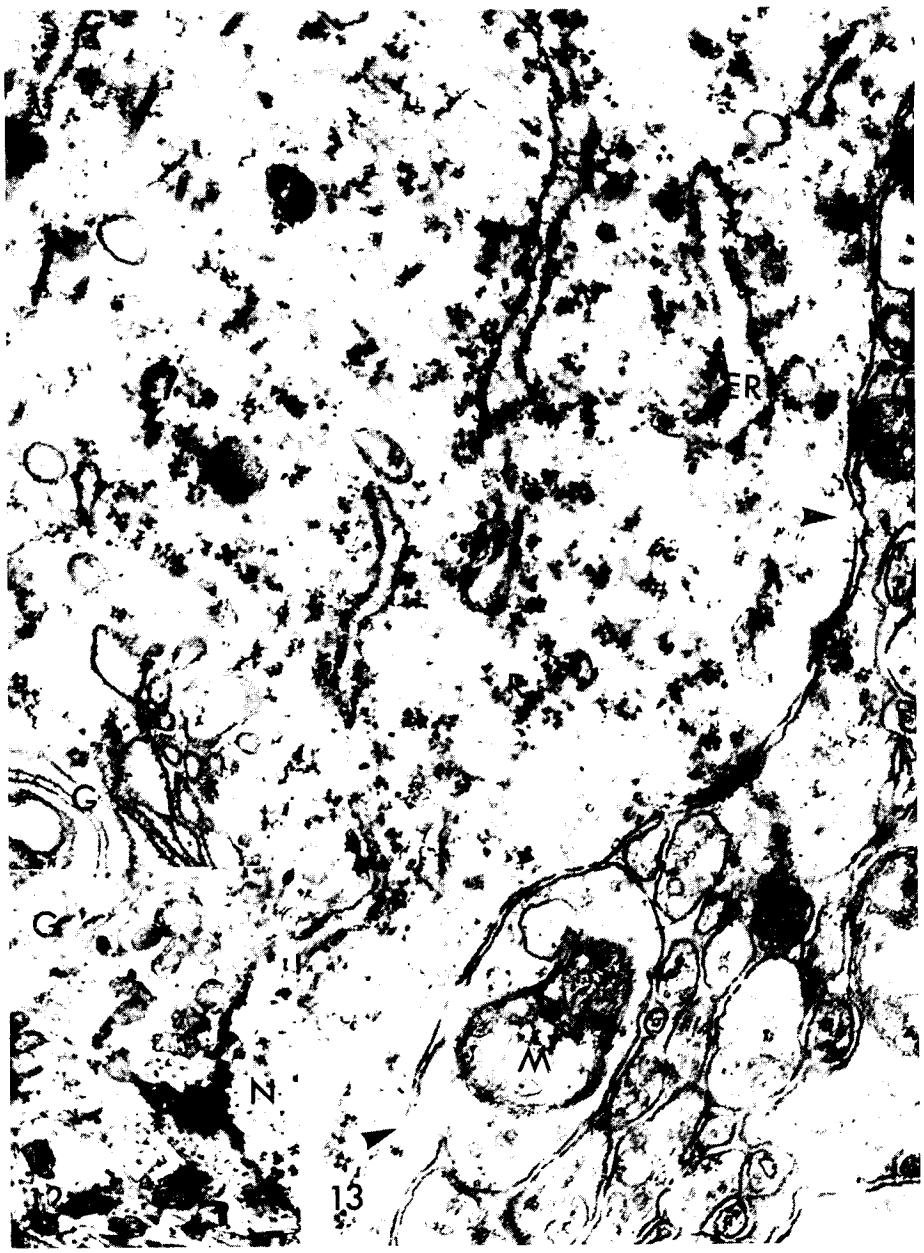

Fig. 12. (Inset) Portion of neuron from rat exposed to OHP 24 hours after lithium premedication. Severe disruption of reticulum and disintegration of ribosomes, swollen mitochondria and Golgi complex (**G**). **N**, nucleus. ×7200.

Fig. 13. Portion of neuron similar to Fig. 12 magnified to show extent of disruption in cytoplasmic organelles. Both Golgi complex (**G**) and endoplasmic reticulum are dilated. Abundance of free ribosomes (**R**) is no longer evident. Note discontinuity in cell membrane of neuron around body (arrows). **M**, mitochondria. ×33000.

Fig. 14. Similar to Fig. 13, neuron with severely altered reticulum (ER) and mitochondria (M). Number of free ribosomes is very few. Again shows discontinuity of neuronal membrane near an astrocyte process which itself has broken membrane (arrows). ×33000.

Fig. 15. Section passing through an axon (**A**) from rat exposed to OHP 24 hours after lithium injection showing the damaged mitochondria (**M**) while smaller mitochondria in smaller processes (**m**) are unaffected. **mt**, microtubules. × 16400.

mally exposed rats premedicated with lithium 15 minutes before OHP exposure, it may be that the faster convulsion rate in the group pretreated with lithium 24 hours before exposure is caused by the inability of these animals' tissues to adapt to the stress of OHP while continuing to perform their detoxifying function. OHP exposure tolerance in rats pretreated with lithium 15 minutes before exposure indicates that the lithium protective mechanism may be found in the blood rather than the brain.

The high content of potassium in cerebral neurons is a function of the state of polarization of the cells and can only be maintained by metabolism (13). A group of amino acids, including glutamate, glutamine, γ-amino butyrate and aspartate, which have been referred to as the glutamate system (20), plays a significant role in this metabolism. Both glutamate and aspartate are excitatory to neurons, while γ-amino butyrate is inhibitory. It has been proposed that the excitatory effect of glutamate occurs through its triggering action on the entry of sodium into the neuron, followed by an outward flux of potassium, which results in depolarization (13).

The biochemical events which produce convulsive activity and the accompanying fine structural changes in vital organs of rats exposed to OHP and lithium are undoubtedly related to the relative predominance of lithium, which provides on the one hand an ammonia-chelating activity with its attendant buffering of rising blood and brain ammonia levels, and on the other hand an obvious toxic effect when it is present in high concentrations in the brain. If we assume that conditions which threaten the constancy of the brain glutamate system jeopardize integrated brain function, several relationships may be perceived in this study:

(1) If the liver fails to detoxify the blood of ammonia, the brain is presented with the formidable load of rising blood and brain ammonia.

(2) Ammonium ions are considered to have a basic role in a neuron-glial interaction, which emphasizes the buffering role of the glia for neuronal glutamate and potassium during excitation, the conversion of glutamate in the glia via ammonium to glutamine, and rapid return to the neuron. In the face of high concentrations of ammonium, glial glutamine synthetase activity may be great enough to cause a serious depletion of glial ATP and the loss of the glia's ability to concentrate potassium or glutamate against a concentration gradient. This would lead to increased extraneuronal concentrations of both potassium and glutamate, resulting in extensive, disturbed neuronal activity (20).

(3) Secondary to glutamate depletion, the action of γ-amino butyrate, which has an important inhibitory role in the brain, would also be weakened (13).

(4) High cerebral ammonia might possibly compromise the activity of the tricarboxylic acid cycle by reductive amination of α-oxoglutamate which would have serious energy repercussions (23).

(5) Glutathione, which plays an important protective role against free oxygen radicals and metal cations through its reductive SH group, is an integral component of the γ-glutamyl cycle (15), iinportant in amino acid transport across membranes. Several regions of the brain have γ-glutamyl cycle activity. If the adequacy of glutathione for this role is compromised by the added stress of oxygen-free radical removal, then glutamate may be furnished from other sources, i.e., reductive amination of α-oxoglutamate or hydrolysis of glutamine. A diminished amino acid transport, resulting either from OHP or lithium toxicity, would explain the increased sensitivity of animals to convulsion when the level of lithium in the brain is high and combined with the absence of ribosomal activity in the neuron. This

study provides compelling evidence that ammonia plays a key role in the development of convulsions through OHP exposure. When lithium distribution is high in the blood and low in the brain, it exerts a more powerful protective action against rising blood and brain ammonia, probably through its chelating propensity, evidenced by the maintenance of high concentrations of glutamate system substrates both in brain and blood. The operation of the Krebs-Henseleit cycle for urea formation is shown by high blood urea, arginine and aspartate, and need not be employed in animals pretreated with lithium 15 minutes before OHP exposure who are exposed for periods of time which, under other conditions, would markedly enhance ammonia elimination via urea.

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CONSUMPTION COAGULOPATHY INDUCTION BY HYPERBARIC OXYGEN AND ENHANCEMENT BY LEAD ACETATE

L. A. Kiesow, S. Shapiro, B. F. Lindsley and J. W. Bless

Recent reports from our laboratory (3, 4) have shown that a single injection of a lead salt, such as lead acetate ($PbAc_2$), can enhance oxygen toxicity. This enhancement resulted in a marked reduction of the survival time of rats when exposed to hyperbaric oxygen, but it failed to produce any significant changes in several physiological and biochemical parameters. Blood gases, white cell counts, serum electrolytes, and several serum enzyme activities were the parameters that had been studied in these reports, with negative results. However, recent observations on the mechanism of sensitization of animals to bacterial endotoxins by lead acetate (5) led us to re-examine the relationship between lead ions and oxygen with respect to the enhancement of oxygen toxicity. It now appears that increased oxygen partial pressures result in a gradual induction of coagulation changes and that the rate of this induction is greatly enhanced by lead acetate, leading to an activation of the intrinsic coagulation pathway with consumptive coagulopathy, and to disseminated intravascular coagulation (DIC).

Methods

Femoral venous catheterization was performed, with the aid of an incision, on male Sprague-Dawley rats under light ether anesthesia. With the exception of those studies in which rats were compared with chicks, the rats always weighed 200 ± 20 grams. Lead acetate or sodium acetate (NaAc), dissolved in deionized water, was given as a single iv injection. The dose of lead acetate used was normally 100 mg/kg of body weight, unless otherwise specified. In the normobaric experiments, groups of eight animals were placed in specially designed chambers, and either 100% oxygen or air was allowed to flow through the chambers at a constant flow rate of 1 liter per minute. The temperature was maintained at 23° ± 2°C, and the relative humidity was kept at 50%. The animals had free access to food and water.

In hyperbaric oxygen exposures in this study, groups of animals were placed in a small lucite animal chamber (Bethlehem). Soda lime served as the carbon dioxide scrubber. The chamber was flushed for 5 minutes with pure oxygen at 1 ATA and then pressurized to 4 ATA of oxygen over a 3-minute period. After pressurization the chamber was continuously vented with oxygen at an initial flow rate of 50 liters per minute for 5 minutes and at a continuous flow rate of 1 liter of oxygen per minute. At the end of the experiments, the animals

were decompressed at a rate of 33 feet per minute and were immediately removed from the chambers and kept under 1 ATA oxygen until blood was sampled.

Eleven-day old, white Leghorn chicks, weighing 70-90 grams (obtained from Truslow Farms), were used one week following delivery. Their weight range at this time was 120 ± 20 grams.

Blood was drawn by cardiac puncture under light ether anesthesia by using siliconized needles and plastic syringes. Introduction of tissue thromboplastin was carefully avoided and, in addition, the blood sampled initially was discarded.

All coagulation assays were performed using the Clotek™ System (Hyland). Prothrombin times (PT) and activated thromboplastin times (APTT) were determined by standard techniques. Fibrin monomers were assayed by the protamine sulfate test (6), and fibrin degradation products were assayed by latex agglutination using the Thrombo-Wellcotest® (Wellcome). The assays for Factors XII, VIII, VII and V were carried out by using factor-deficient human plasma (Dade, Sera-Tec). Citrated, pooled rat plasma was used to develop standard curves for the factor activities in rats. Owrens veronal buffer, pH 7.35, served in all plasma dilutions.

Results

The exposure of rats injected with sodium acetate to 4 ATA of oxygen results in prolongation of both PT and APTT values (Table I). This increase, although apparent after a short exposure time of only 30 minutes, becomes statistically significant after 60 minutes of exposure. However, none of the animals in any of these experimental groups exhibited detectable levels of fibrin monomers (FM) or fibrin degradation products (FDP). In addition, a single injection of lead acetate ($PbAc_2$) given 18 hours prior to the exposure to hyperbaric oxygen greatly enhances the prolongation in PT and APTT values at both 30- and 60-minute exposure times ($P < 0.001$). Furthermore, lead treatment results in the appearance of fibrin monomers in more than 50% of the animals in the 30-minute and in almost 90% of the animals in the 60-minute exposure time groups. Fibrin degradation products can also be detec-

TABLE I

EFFECT OF LEAD ACETATE AND 4 ATA O_2 ON PROTHROMBIN TIME, ACTIVATED PARTIAL THROMBOPLASTIN TIME, FIBRIN MONOMERS, AND FIBRIN DEGRADATION PRODUCTS IN RATS

Experimental Group	Exposure Time, min	PT, sec	APTT, sec	FM, + or −	FDP, μg/ml
Control (no treatment)	0	20.7 ± 3	40.6 ± 5	− (0)	0 (0)
NaAc	30	26.0 ± 3	60.6 ± 7	− (0)	0 (0)
NaAc	60	35.5 ± 4	100.6 ± 7	− (0)	0 (0)
$PbAc_2$	30	66.0 ± 3	171.3 ± 6	+ (56)	>40 (100)
$PbAc_2$	60	119.2 ± 4	271.6 ± 7	+ (86)	>40 (100)

Experimental animals per group, $n = 24$. Percentages of animals showing positive FM and FDP responses given in parentheses. PT and APTT values are means ± SD. Absolute survival time of sodium acetate group = 265 ± 76 min and for lead acetate group 146 ± 35 min.

ted with levels exceeding 40 μg/ml at both exposure times and they are demonstrable in all animals of the lead acetate groups.

Since these results suggested that an activation of the coagulation system occurs in rats as an early response to an exposure to hyperbaric oxygen, the activities of coagulation Factors XII, VIII, VII and V were also determined. A progressive decrease in all factor activities with time (Fig. 1) was found to be the result of an exposure of sodium-acetate-injected rats to 4 ATA of oxygen. In addition, a significant enhancement of this decrease in factor activities was caused by the administration of lead acetate, which could be clearly observed at both 30 and 60 minutes of exposure. Therefore, the data presented in Fig. 2 confirm an activation of the intrinsic coagulation system caused by hyperbaric oxygen alone; furthermore, the rate of this activation is greatly accelerated by treatment with lead acetate, and leads to a rapid onset of consumption coagulopathy.

Exposure of rats to 100% oxygen at only 1 ATA results in a slower but significant increase of PT and APTT values after 27 hours of exposure. In addition, some decreased coagulation factor activities can be observed and fibrin monomers become apparent (Table II). A single injection of lead acetate, however, while having little effect on PT and APTT values or factor activities in animals exposed to air for 23 hours, does produce significant increases in PT and APTT values in animals exposed to 1 ATA of oxygen for 18 hours. Furthermore, the activities of coagulation Factors XII, VIII, VII and V appear to be drastically decreased, and the number of animals showing fibrin monomers increases to almost 75% of the survivors. It should be noted that animals of the sodium acetate-oxygen group were within approximately 50 hours of their median survival time, while animals of the lead acetate-oxygen

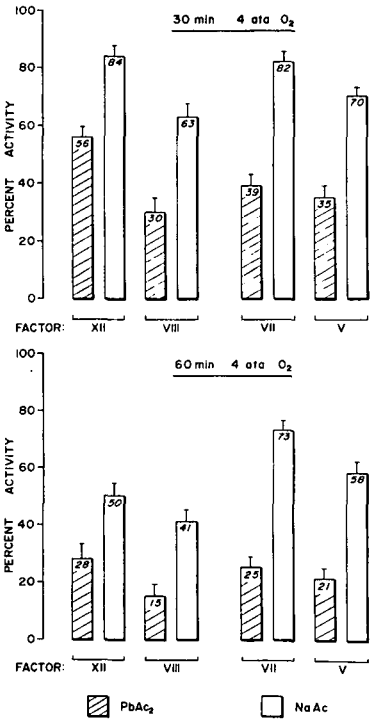

Fig. 1. Coagulation factor activities in rats exposed to 4 ATA O_2 for 30- and 60-min time periods and effect of lead acetate.

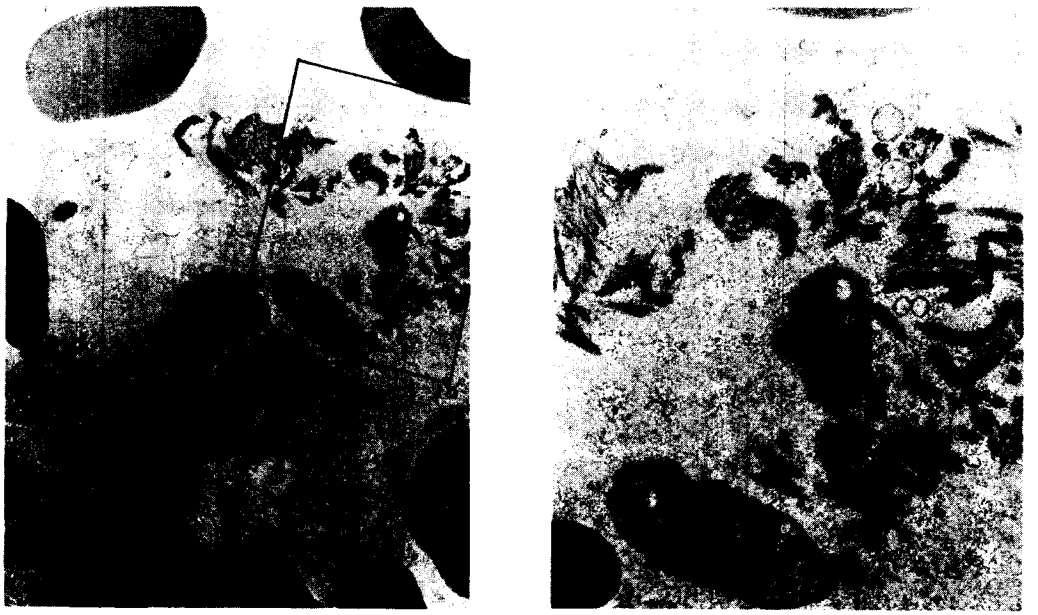

Fig. 2. Electron micrographs of ultra-thin sections of lungs of rats exposed to 100% O_2 at 1 ATA for 20 hr following treatment with $PbAc_2$. Magnifications are: **A**, 17,500×; **B**, 70,000×.

group were within 10 hours of their median survival times. This resulted in the death of animals in both such groups, with a distinctly higher death rate in the lead acetate-oxygen group.

Since these results again indicated that consumption coagulopathy was induced and en-

TABLE II

EFFECT OF LEAD ACETATE AND 1 ATA O_2 ON PROTHROMBIN TIME, ACTIVATED PARTIAL THROMBOPLASTIN TIME, COAGULATION FACTOR ACTIVITIES AND FIBRIN MONOMERS IN RATS

Experimental Group	No. of Survivors/No. of Animals	Exposure Time, hr	PT, sec	APTT, sec	Factor Activity (%)				FM No. of Positives
					XII	VIII	VII	V	
NaAc - Air	35/35	34	21.5 ± 3	31.8 ± 5	100	98	100	100	0
$PbAc_2$ - Air	29/29	23	23.2 ± 3	34.4 ± 5	98	100	100	100	0
NaAc - O_2	38/45	27	49.1 ± 6	64.3 ± 7	78	61	68	92	11
$PbAc_2$ - O_2	40/65	18	98.7 ± 8	187.3 ± 9	43	12	36	32	29

All coagulation factor activities are reported as mean percent values based on standard activity curves developed for rat plasma with factor-deficient human plasmas. Standard deviations of all factor activity values are < ±9%. Median survival time of sodium acetate-oxygen group = 79 ± 16 hr, and for lead acetate-oxygen group 29 ± 5 hr, which resulted in death of some animals in both groups.

hanced by lead acetate, leading to DIC, animals of both groups were killed after their exposure to 1 ATA of oxygen for 20 hours. Samples of lung, heart, brain, kidney and liver were taken for histological examination of ultra-thin sections by electron microscopy. While indications of DIC could only be detected occasionally in the sodium acetate group at this time, it was observed quite regularly in the lead acetate group. Lungs and kidneys were particularly affected; a typical electron micrograph of such alterations is shown in Fig. 2, which depicts the thin section of the lungs from an animal of the lead acetate group at two different magnifications (17,500×; 70,000×). The typical picture of DIC with intravascular depositions of fibrin that are associated with platelets and red cells can be seen, thus indicating the formation of microthrombi.

These combined results consistently suggested that an induction of consumption coagulopathy was caused by hyperbaric oxygen at rates which depended on the oxygen partial pressure and which could be enhanced by lead acetate. Since chickens are naturally deficient in several coagulation factors (2), including Factor XII (Hageman Factor), it was possible to test our hypothesis further. The survival rates and the median survival times of chicks that were injected with sodium acetate or lead acetate and then subjected to 1 ATA of oxygen were determined and compared with those of rats treated similarly. Table III shows that all chicks survived exposure within the 10-day observation period regardless of whether they were injected with lead or sodium acetate. The rats, however, showed a median survival time of 128 hours when injected with sodium acetate; their survival time decreased to 49 hours with a single injection of 23 mg of lead acetate per kg of body weight. Apparently, a dose of lead acetate capable of enhancing the induction of consumptive coagulopathy in the rat also shortens this animal's survival time. The same dose, however, is without effect in a coagulation-factor-deficient animal (e.g., chicken) where an activation of the intrinsic clotting system by oxygen and lead ions becomes impossible. Furthermore, it should be noted that chicks survived exposure to 1 ATA of oxygen for more than 240 hours, at which time the experiment was terminated.

Chronic respiratory disease (CRD), also known as murine pneumonia, appears to affect

TABLE III

EFFECTS OF LEAD ACETATE ON SURVIVAL TIME OF CHICKS AND RATS IN 1 ATA O_2

Experimental Group	No. of Survivors/No. of Animals, days										Median Survival Time
	1	2	3	4	5	6	7	8	9	10	
Chicks injected with:											
$PbAc_2$	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	> 240 hr
NaAc	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	> 240 hr
Rats injected with:											
$PbAc_2$	6/20	9/20	13/20	18/20	20/20	—	—	—	—	—	49 ± 5 hr
NaAc	0/20	0/20	1/20	2/20	8/20	14/20	18/20	20/20	—	—	128 ± 11 hr

Median survival time was determined in 3 groups of 6 animals each. Average weight range of all animals was 120 ± 20 g. Dose of lead acetate $PbAc_2$ was 23 mg/kg; NaAc was equimolar with $PbAc_2$ based on Ac.

all rat colonies periodically, and its milder forms often remain unnoticed. However, the disease is accompanied by dramatic alterations of the coagulation system (Kiesow and McKee, unpublished observations); in fact, the prolongation of the APTT values and the comsumption of coagulation factor activities, such as Factor XII, can serve as an index to the progression of the disease. In light of these changes in the coagulation system, groups of normal and CRD-diseased male Sprague-Dawley rats were exposed to 1 ATA of oxygen. The results are shown in Table IV. This table shows the significant prolongation of APTT values, frequent lack of clot formation, and reduction of Factor XII activities which result from the infection of rats with CRD. It also shows the survival rates and the median survival times of normal rats and compares them with those of rats suffering from CRD. It is clear that both survival rates and median survival times of CRD-infected rats have significantly increased over those of normal, healthy rats. In other words, the CRD-affected rat responds to an exposure to hyperbaric oxygen very much like the coagulation-factor-deficient chicken. The fact that some rats of the diseased group died, while none of the chickens did, may be explained by the varying degrees of severity of the disease in randomly selected groups of animals.

Discussion

It appears that hyperbaric oxygen is capable of activating the intrinsic coagulation pathway, leading to a slowly progressing induction of consumption coagulopathy and DIC. Also, lead acetate appears to be capable of enhancing the rates of induction of consumptive coagulopathy, thereby acting synergistically with hyperbaric oxygen. Furthermore, a dose-response relationship between the partial pressure of oxygen and the time required for the onset of consumption coagulopathy exists. This dose-response relationship applies to untreated animals as well as to those animals treated with lead acetate. These observations could be confirmed in a somewhat independent manner by using animals, like the chicken, which are naturally deficient in certain coagulation factors and are, therefore, unable to respond with an activation of the intrinsic coagulation pathway. Such animals are remarkably resistant to the lethal effects of hyperbaric oxygen and, also, do not respond to an enhancing dose of lead acetate. Even in an animal that is normally affected by hyperbaric oxygen, the degree of resistance to oxygen toxicity is considerably increased when a disease like CRD deprives the animal of coagulation factors which are essential for a functional intrinsic coagulation pathway.

TABLE IV

SURVIVAL RATES AND MEDIAN SURVIVAL TIMES OF NORMAL AND CRD-DISEASED RATS IN 1 ATA O_2

Experimental Group	APTT, sec	Factor XII Activity, %	No. of Survivors/No. of Animals, days							Median Survival Time, hr
			Days							
			2	4	6	8	10	12	14	
Normal Sprague-Dawley rats	35.5 ± 6	100 ± 5	0/20	3/20	16/20	20/20	—	—	—	116 ± 12
CRD Sprague-Dawley rats	>200	<16	0/20	0/20	0/20	1/20	2/20	2/20	4/20	>336

CRD = chronic respiratory disease or murine pneumonia.

In addition, the time of exposure to hyperbaric oxygen, when the induction of consumption coagulopathy and the symptoms of DIC can be observed, seems to precede the onset of pulmonary symptoms leading eventually to death. This may suggest that a primary event in the development of oxygen toxicity is involved which, in addition to activating the coagulation cascade, solicits a wide range of associated responses. These conclusions are supported by a recent report providing histological evidence that fibrin deposits in the lungs precede the formation of pulmonary hyaline membranes in rabbits that have been exposed to 100% oxygen at 1 ATA for 30-85 hours (1).

The fact that small amounts of lead acetate can dramatically accelerate the onset of oxygen-dependent consumption coagulopathy and DIC, as well as hasten the animal's death, is both confirmative and alarming. It is confirmative in that it suggests that the activation of the intrinsic coagulation pathway may be a primary site for the pathological action of hyperbaric oxygen. Lead enhances such activation, which leads to accelerated oxygen toxicity and reduced survival rates. It is alarming, however, to observe the enhancement effects of lead because appreciable body lead loads occur frequently. Lead poisoning, in conjunction with an exposure to increased oxygen partial pressures, might hasten some or all oxygen-toxicity-related effects. The fact that the skeletal system is the major site for the deposition of lead in chronic lead poisoning may link lead poisoning, via its enhancement effect on the coagulation system and its ability to hasten the onset of DIC, to the development of aseptic bone necrosis. This may be of particular interest since aseptic necrosis of the femoral head has been linked with hemorrhagic and thrombotic tendencies in such patients (7).

Finally, it is tempting to try to correlate susceptibility and resistance to oxygen toxicity with the presence of a complete intrinsic coagulation pathway. Fortunately, a limited store of data was available from the literature, permitting comparisons in the animal kingdom. In some instances, where it did not exist, the lacking data were determined and added (Table V). This table shows that short mean survival times in 1 atmosphere of oxygen are always

TABLE V

ANIMAL SURVIVAL TIMES IN 1 ATA O_2 AND FACTOR XII PRESENCE

Species	Mean Survival Time, hr	Factor XII
Rat	95	+
Mouse	92	+
Guinea pig	96	+
Rabbit	77	+
Dog	72	+
Cat	83	+
Frog	>1300	−
Turtle	> 552	−
Chicken	> 672	−
Duck	> 500	−
Whale	N.D.	−
Porpoise	N.D.	−

N.D. = not determined; + indicates presence of Factor XII; − indicates its absence.

associated with Factor XII activity, while long survival times, under similar conditions, coincide with the absence of this factor. Table V also indicates those animal species which are frequently exposed to hyperbaric oxygen, e.g., in breath-holding dives after air inhalation, as an inherent part of their normal and physiological activities. Such animals, for example whales, porpoises and frogs, are, however, Factor XII-deficient by nature. It may be the oxygen-dependent induction of consumption coagulopathy and subsequent development of pulmonary oxygen toxicity that makes it essential for such animals to be Factor XII-deficient, to avoid such effects. On the other hand, this relationship may also help to eliminate activation of those components of oxygen toxicity that lead eventually to pulmonary involvement and death. One such method may be through an appropriate inactivation of the intrinsic coagulation system. Chronic respiratory disease achieves this in the diseased rat, making the animal resistant to oxygen.

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PHYSICAL PERFORMANCE OF MOUSE COLONIES AS A MEASURE OF INERT GAS NARCOSIS, OXYGEN TOXICITY, AND THE CHOUTEAU EFFECT

M. A. Rokitka and H. Rahn

Identification of the limits which determine how far, how fast and for how long man can work underwater and the assessment of his performance underwater have been the objects of numerous investigations. Performance decrements have been attributed to a variety of factors such as inert gas narcosis, hydrostatic pressure, oxygen toxicity, breathing resistance, and neurological impairment. The degree of involvement of each of these factors has not always been clear, since under certain circumstances they interact with one another.

The aim of the present study was the identification of conditions which would maintain mouse performance at optimal levels at 100 ATA. The spontaneous running activity of colonies of deer mice provided a quantitative measure of physical performance; observations of various behavior patterns furnished qualitative information. Before studying performance at 100 ATA, it was decided to evaluate: (1) the narcotic effects of inert gases per se; (2) the toxic effects of elevated oxygen pressures; and (3) the possible therapeutic value of high oxygen pressures (Chouteau effect). The results of these preliminary studies were subsequently to be incorporated into a series of saturation dives to 100 ATA.

Methods

Experimental Animals

Colonies of 5 young adult deer mice, *Peromyscus maniculatus bairdi,* (16–20 grams) were housed and reared in specially adapted habitats (Fig. 1). Each colony consisted of weaned litter mates of both sexes. The nocturnal wheel activity of these laboratory-bred rodents was recorded on an hourly basis during both phases of an LD 12:12 light cycle which was reversed so that activity could be observed during laboratory work schedules. Control records (such as the one shown in Fig. 2) were obtained for several days prior to an exposure; the average distance run on the wheel ranged from 15 to 25 km/day/colony. This wheel activity represents a spontaneous, almost compulsive type of activity and is synchronous with the dark phase of a diurnal cycle (8, 9, 10).

Chamber Facilities

The spherical compartment of a 170 ATA chamber was used for all exposures to pressure.

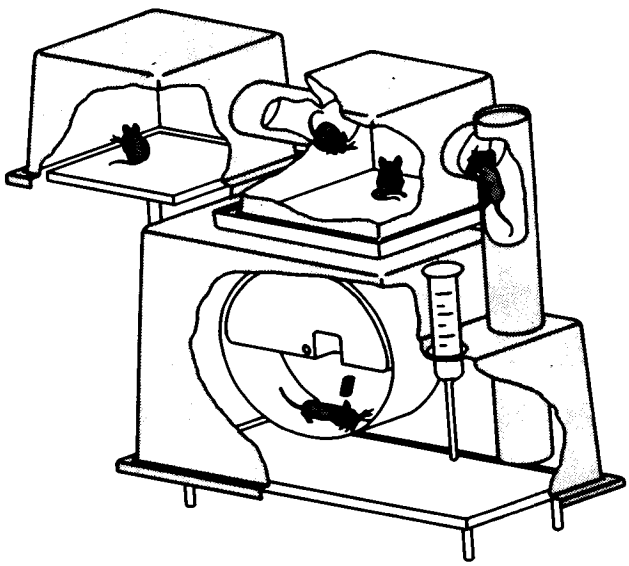

Fig. 1. Living quarters for a colony of 5 deer mice; 3-compartment habitat is equipped with an activity wheel, water reservoir, climbing tower and connecting tunnel; for details, see ref. (14).

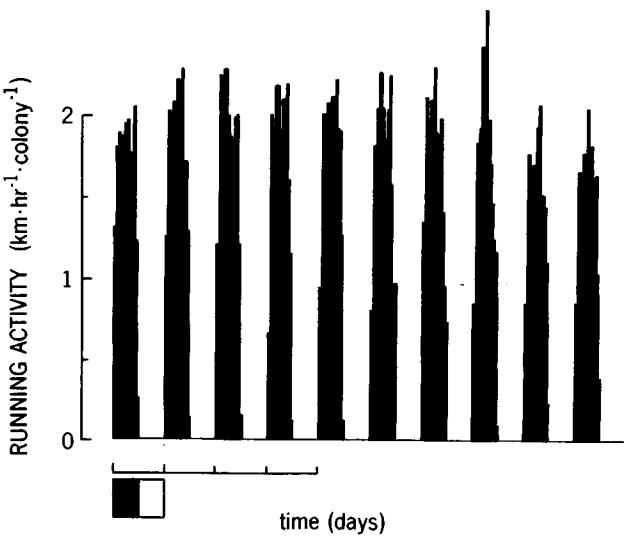

Fig. 2. Representative running activity profile for colony of 5 deer mice. Darkened areas correspond to activity observed during 12-hour dark periods which alternate with 12-hour light periods. Hourly totals for a colony are plotted against time of day.

To conserve valuable gases, the 5100-liter volume of the sphere was effectively reduced to 100 liters by enclosing the habitat which housed the mice in a lucite box (hereafter referred to as the environmental box). This box was continuously maintained at a slight overpressure with the desired gas mixture while the high-pressure chamber was pressurized with air to a predetermined depth equivalent. Carbon dioxide, ammonia and other trace contaminants were removed from the closed environmental box system by means of a biosupport package described by Morin and Laraway (14). Details of temperature and illumination systems are also given in this reference.

Experimental Design

The purpose of each series of preliminary experiments was to establish the effect of a given gas environment on the physical performance of a colony of deer mice. The approach to quantitative assessment of performance was based on spontaneous wheel-running activity which represents a more integrated response than the simple righting or rolling reflex. In addition, it allowed study of the deer mice as a social unit and observations of social interaction. By rearing the mice in a habitat which was transferred from the breeding quarters to the high-pressure chamber as an intact unit, the stresses of unfamiliar quarters and physical restraints were removed. Thus the use of colonies of animals which show well-defined cyclic activity was incorporated into each series of studies on inert gas narcosis and oxygen tolerance.

Inert Gas Narcosis

The classical method for determining the relative narcotic potency of inert gases involves relying on specific reflexes as biological end points. To evaluate the effect of inert gases using a new end point (i.e., spontaneous running activity), a series of exposures to selected inert gases was designed.

Protocol

The narcotic potencies of nitrogen, argon, and nitrous oxide were determined during 2–3 day saturation exposures of mouse colonies to various pressures, as indicated in Table I. Compression was carried out at 0.25 atm/min. Wheel-running activity was automatically recorded. Tower climbing, social interaction, physical appearance and postural balance were observed continuously. Water consumption was also measured.

The gas volume of the environmental box was exchanged twice each hour. The carbon dioxide tension was kept below 3 torr while an oxygen makeup system maintained an oxygen pressure of at least 0.21 ATA. Gas temperatures within the environmental box were 25-27 °C during exposures to argon and nitrogen and 24 °C during exposure to nitrous oxide.

Decompression was carried out at 0.25 atm/min for argon and nitrous oxide exposures and at 0.5 atm/min for nitrogen exposures. Postexposure running activity was recorded after return of the habitat to the breeding quarters. Table I lists the chamber pressure for each of the 9 exposures and summarizes the composition of the gas mixture within the environmental box for each exposure; 0.21 ATA oxygen accounts for the difference between chamber pressure and total inert gas partial pressure.

Results

Behavior records and activity summaries demonstrate that the three inert gases are distinctive in terms of narcotic potency. The following comments summarize the behavioral and physical changes that were observed.

Behavior with increased nitrogen pressure. No significant changes in physical appearance, postural balance, tower climbing or water consumption were detected at 13.8 and 20.9 ATA nitrogen. However, at 30.8 ATA nitrogen, a severe loss of coordination and balance resulted in labored running. Whole body tremors and heaving undoubtedly contributed to the observed difficulties in movement and to the marked reduction in water consumption.

TABLE I

INERT GAS PRESSURES AND SPONTANEOUS RUNNING ACTIVITY OF A MOUSE COLONY

Inert Gas Partial Pressure, ATA		Chamber Pressure, ATA	Control Activity, %			Distance Run, km/12 hr		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
	Nitrogen							
	13.8	14	58	91	—	7.7	11.9	—
	20.8	21	77	44	50	11.4	6.5	7.5
	30.8	31	48	15	—	8.9	2.7	—
Argon	Nitrogen							
6.3	0.5	7	62	62	74	14.9	15.1	17.8
12.8	1.0	14	35	10	11	7.5	2.2	2.3
20.2	0.6	21	1	1	—	0.3	0.3	—
Nitrous Oxide	Nitrogen							
0.7	1.1	2	84	74	72	18.0	16.0	15.6
0.8	1.0	2	12	86	20	1.2	9.6	2.2
0.9	0.9	2	8	62	—	0.5	7.3	—

$n = 5$.

Behavior with increased argon pressure. Physical appearance was unaffected by increased argon pressures. The ability to maintain postural balance and to negotiate the climbing tower deteriorated as the inert gas pressure increased from 6.3 to 20.2 ATA. An erect posture could not be maintained at the water supply; this may account for the reduction in water intake at 20.2 ATA argon.

Behavior with increased nitrous oxide pressure. A pronounced change in physical appearance was noted during each of the exposures to nitrous oxide. The mice looked extremely disheveled; their coats were scruffy despite almost continuous preening. In addition, the tails and extremities were gnawed upon. Progressive deterioration in ability to maintain postural balance was reflected in the difficulty with which the mice scaled the tower and ran the wheel. Running resembled a lobbing stiff-legged motion and became progressively slower with additional nitrous oxide. Water intake remained near control values.

Wheel-running with increased inert gas pressures. To evaluate the narcotic potency of the three inert gases quantitatively it was decided to compare wheel activity observed on the second day of an exposure with that observed under predive conditions at 1 ATA. The decision to disregard activity observed during the first day was based on the premise that pressure changes and increased noise levels during compression have a disturbing effect on the mice; the delay provided for the possible development of adaptations. Table I summarizes running activity in terms of relative performance (percent of control) and absolute distance run for the duration of each exposure.

The values obtained on the second day (the first undisturbed 12-hour dark period) were plotted on a probit scale against the log of the inert gas pressure (Fig. 3). Slopes for argon and nitrogen were drawn by eye while the line for nitrous oxide was located by assuming that it would ideally parallel the slopes drawn for the other gases. The unusual behavior and running activity of the mice exposed to nitrous oxide leave some doubt as to validity of this assumption.

Discussion

The wheel-running activity of colonies of deer mice yields an index of inert gas narcosis. By plotting observed running activity against inert gas pressure, the gas pressure necessary to reduce activity to a given level can be predicted. For comparison, one can choose the conventional ED_{50} level (i.e., that gas pressure which will depress a response to 50% of its control value). The respective ED_{50} values for nitrous oxide, argon and nitrogen are 1.1, 7.2 and 20.5 ATA, respectively (determined by using wheel-running as the response of choice). Similar ED_{50} values were obtained by investigators who depended on the loss of a specific reflex for an end point (5, 6, 12, 13). The relative narcotic potency of nitrogen and argon established in this study is 20.5/7.2 or 2.8; for nitrogen and nitrous oxide it is 20.5/1.1 or 18.6. These calculated ratios are comparable to ratios obtained by investigators who studied loss of righting reflex, analgesia, and increased electroshock convulsion threshold, and are discussed in detail elsewhere (15).

Oxygen Tolerance

There is some evidence to suggest that inert background gases may exert a protective effect against oxygen toxicity (1, 16). This effect was examined by maintaining mouse colonies at elevated oxygen tensions both in the presence and in the absence of 13.8 ATA nitrogen. As previously mentioned, this nitrogen tension produced no noticeable signs of narcosis, judged by spontaneous running activity, nor did it seem to interfere with the normal physical or social activity of deer mice.

Protocol

The preparation of mouse colonies, habitat, environmental box and high-pressure chamber was identical to that described in the previous section on inert gas narcosis. Colonies of mice were exposed for 1-4 days at 23-26°C to 0.8, 1.0 or 1.5 ATA oxygen, either alone or in the presence of 13.8 ATA nitrogen. The wheel-running activity of the mice was evaluated against

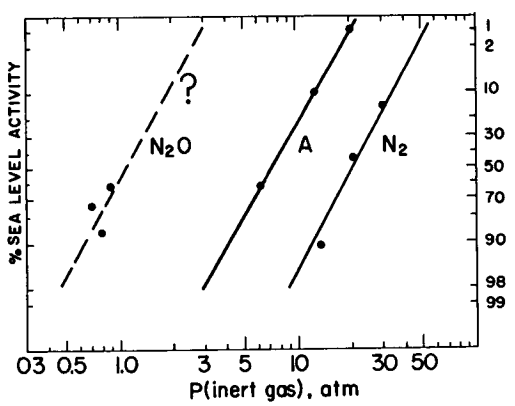

Fig. 3. Log-probability plot of running activity against inert gas partial pressure. Running activity was measured on 2nd day of exposure and was plotted as percentage of that observed in air at 1 ATA. Broken line was drawn parallel to other two. Scale represented on *ordinate* does not include 0 and 100% values.

control performance at 1 ATA. Behavioral changes associated with the onset of respiratory distress and survival time under different experimental conditions were used as indices of tolerance to oxygen toxicity. Records of postexposure activity were obtained for survivors.

Results

Figure 4 summarizes the wheel activity for *Series A* (0.8, 1.0 and 1.5 ATA oxygen) and for *Series B* (same oxygen pressures as in *Series A* with 13.8 ATA nitrogen).

Wheel activity: Series A. Performance on the activity wheel was interpreted as a function of both oxygen pressure and length of exposure. Figure 4 shows a severe depression in wheel use during the first day of exposure to 1.5 ATA oxygen. Neither the 0.8 ATA oxygen (P_{N_2} = 0.2 ATA) nor the 1.0 ATA oxygen exposures produced as drastic a depression in activity.

Activity observed on the succeeding 1 to 3 days of a given exposure was directly related to the oxygen pressure. At an oxygen pressure of 0.8 ATA, a 15-30% decrease below control values was recorded. Return to a normoxic environment was accompanied by a restoration to pre-exposure activity levels. Such was not the case with 1.0 and 1.5 ATA oxygen. Wheel use persisted for 3 days at 1.0 ATA oxygen and then came to an abrupt halt on *day 4.* In the presence of 1.5 ATA oxygen, the very limited activity of *day 1* was followed by death on *day 2.*

Wheel activity: Series B. The exposures to 0.8, 1.0 and 1.5 ATA oxygen with the addition of 13.8 ATA nitrogen produced results similar to those obtained in *Series A* in terms of the

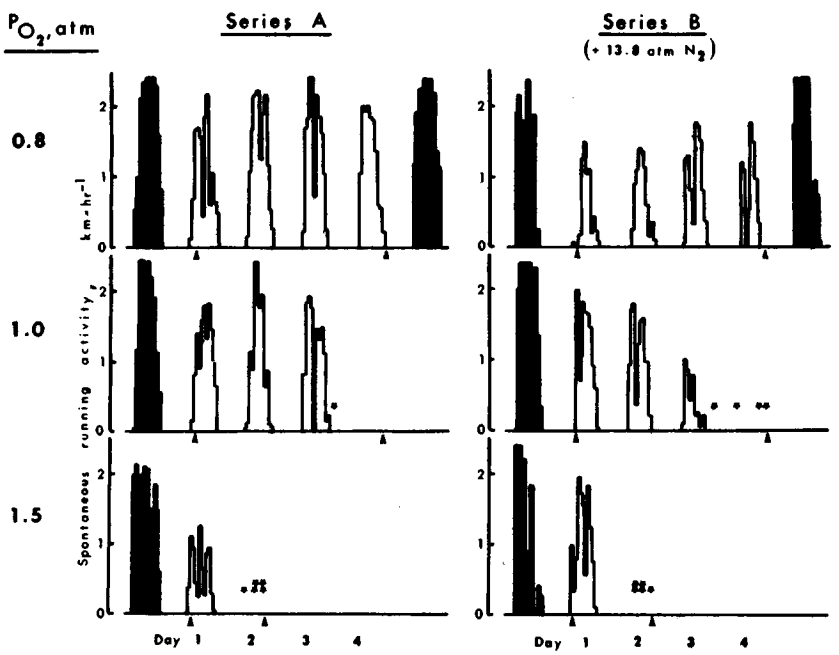

Fig. 4. Spontaneous running activity during exposure to various oxygen and nitrogen tensions. *Series A* (*left*) shows results obtained with 0.8, 1.0 and 1.5 ATA oxygen; *Series B* presents results obtained with 13.8 ATA nitrogen and oxygen tensions used in *Series A.* Control activity appears on left of each record. Pointers indicate beginning and end of each exposure; stars denote mouse deaths.

number of days of wheel activity. A noteworthy difference between *Series A* and *B* is in the level of observed activity. Except for the exposure to 1.5 ATA oxygen, there is less recorded activity in the presence of 13.8 ATA nitrogen.

Behavioral observations. The 4-day exposures to 0.8 ATA oxygen (with either 0.2 or 13.8 ATA nitrogen) showed no variations from control behavior. Pronounced changes in behavior noted at 1.0 and 1.5 ATA oxygen are reflected in the activity profiles of the colonies (Fig. 4).

Exposure to 1.0 ATA oxygen produced the first indications of respiratory distress at 53 hours (total exposure time). Several hours later the mouse that first showed dyspneic symptoms died. The 4 surviving mice became progressively lethargic and were totally inactive during the fourth day. A pattern of dyspnea, lethargy and death became apparent during the exposure to 1.0 ATA oxygen with 13.8 ATA nitrogen, with death occurring at 60 and 69 hours and two additional deaths at 82 hours.

The behavior of mice exposed to 1.5 ATA oxygen was not appreciably affected by the addition of nitrogen. No departure from normal behavior was observed during the first day in either series (with or without nitrogen). However, between 23 and 32 hours of exposure to 1.5 ATA oxygen, dyspnea was followed by death. The time course of the deterioration in the condition of the mice varied; the sequence was very well defined. Prior to death there was copious mucous production, active grooming of the naso-oral region, and convulsions which generally occurred about 5 minutes before death. These events were also characteristic of the exposure with added nitrogen. The only observable differences were a less intense dyspnea and a slight delay in the occurrence of the 5 deaths, which occurred 28-34 hours into the exposure.

Discussion

In the presence of high oxygen tensions, mouse performance on an activity wheel appears to be unaffected by the addition of nitrogen. When the oxygen tension is in the 0.8–1.5 ATA range, simultaneous exposure to 13.8 ATA nitrogen offers little or no protection. Both performance and survival appear to be dictated by the partial pressure of oxygen, indicated by the progressive physical and behavioral deterioration of the mice as oxygen tension increased.

Other investigators have examined the oxygen-inert gas interaction. Lanphier (11), Bennett (2), and Thompson et al. (17) attributed a synergistic effect to some inert gases. They demonstrated that elevated inert gas partial pressures can influence the rate of development of oxygen toxicity. An antagonistic effect was suggested by Almqvist et al. (1) and Smith et al. (16) who observed that the onset of convulsions and loss of consciousness are delayed. This was presumed to be in response to the addition of nitrogen to the inspired gas mixture.

The mechanism for oxygen-inert gas interactions is currently undefined. Until the extent of the influence of narcosis due to inert gases, the magnitude and effect of carbon dioxide retention, and the involvement of increased gas density can be determined, it will be impossible to attribute differences in performance solely to the oxygen-inert gas relationship.

Chouteau Effect

The incidence of hypoxic crises while breathing normoxic gas mixtures under pressure was first reported by Chouteau (7). His investigations demonstrated the reversibility of these crises by increasing the partial pressure of inspired oxygen; this recovery phenomenon has come to be known as the "Chouteau effect." In a pilot series of exposures of deer mice to

100 ATA ($He-O_2$), there was general lethargy while at maximum pressure. Considering the factors which may have contributed to this inactivity, it was decided to test the hypothesis that lethargy may be a manifestation of hypoxic crises.

Remarks and Discussion

Spontaneous running activity at 30, 50 and 100 ATA ($He-O_2$) was studied at oxygen partial pressures of 0.14-0.53 ATA. The lethargy observed during the first two days of exposure to 100 ATA was briefly reversed when the oxygen tension was increased from 0.17 to 0.40 ATA. The burst of activity which followed the addition of oxygen was immediate but transient; it lasted for 30-60 minutes.

At 30 and 50 ATA, the effects of elevated oxygen tensions were more sustained. Activity observed on the third and fourth days of exposure to 30 ATA was depressed by reducing the oxygen tension from 0.25 to 0.14 ATA. Restoration of activity followed return to 0.25 ATA oxygen. Further increasing the oxygen to 0.35 ATA did not affect the level of running activity at 30 ATA. The same held true for an increase to 0.53 ATA oxygen at 50 ATA; no further improvement in performance was noted. The impressive aspect of these maneuvers was the repeated reversibility of activity by adjustments in oxygen tension.

Thus it appears that activity in a helium environment at 30, 50, and 100 ATA can be induced or depressed by appropriate adjustments in oxygen tension. Behavioral changes associated with increases in oxygen tension appear to mimic the temporary remission of symptoms reported by Chouteau (7). His experiments with goats suggested that beyond a given pressure threshold, hypoxic crises develop during normoxic breathing under pressure. The experiments reported here suggest that the Chouteau phenomenon can be observed only after exposure to oxygen tensions below normoxic values.

Saturation Dives to 100 ATA

Inert gases have been shown to increase the threshold pressure for onset of the high pressure neurological syndrome (HPNS) in mice (4). They have also been reported to exert a protective effect against HPNS-related symptoms in human subjects compressed to 31 ATA (3). We chose to take advantage of the anesthetic effect of inert gases reported by Brauer et al. (4) and Bennett et al. (3) in planning a series of saturation dives to 100 ATA.

On the basis of the information obtained in our studies of inert gas narcosis and oxygen tolerance, it was possible to characterize the physical performance of deer mice in a number of gas environments under pressure. Therefore, a series of helium-oxygen dives was designed which provided for the addition of a second inert gas (either nitrogen or nitrous oxide) during compression to 100 ATA. It was also arranged to test the Chouteau effect; both high and low oxygen tensions were to be made available to the mice in an attempt to elicit changes in behavior and activity.

The following comments summarize the highlights of the 3-to-4 day saturation dives to 100 ATA.

Observations with Added Nitrogen

When 0-10 ATA nitrogen were added to the helium-oxygen environment during compression to 100 ATA, the onset of neurological disturbances was observed at 75-80 ATA. No

such disturbances were evident when up to 20 ATA nitrogen were gradually added during pressurization. That this level of nitrogen was well-tolerated is evidenced by the activity profile shown in Fig. 5. During the days which followed compression, activity remained at about half its control value, and was restored to control levels within 48 hours after returning to 1 ATA.

Not all exposures to 100 ATA (He-O_2, N_2) resulted in such a high level of running activity. In several instances, wheel activity was almost completely absent. However, during the periods of lethargy, the mice continued to explore their habitat and continued to eat and drink as they do at 1 ATA.

Observations with Added Nitrous Oxide

The saturation dives in which 0.4-0.8 ATA nitrous oxide was gradually added during compression resembled the dives with 10-20 ATA nitrogen, in that HPNS-related symptoms were not observed. There was some indication of slight narcosis as the nitrous oxide partial pressure approached 0.8 ATA. This condition disappeared as the chamber pressure approached 100 ATA. Even though no observable respiratory or neurological problems were reported during compression, wheel activity was negligible during the 3-to-4 day exposures with added nitrous oxide.

Observations with Elevated Oxygen Tensions

Adjustments in oxygen partial pressure were ineffective against the lethargy observed at 100 ATA. Despite increases in oxygen tension from 0.4 ATA to 0.6 or 0.8 ATA, restoration of activity was not observed. This applied to saturation dives with added nitrogen and to those with added nitrous oxide.

Summary

Observations of deer mouse performance at 100 ATA demonstrate the effectiveness of nitrogen and nitrous oxide in suppressing HPNS-related problems in a helium-oxygen environment. The ability to withstand pressures as high as 100 ATA for several days and the rapid restoration of activity following return to 1 ATA are further indications that the gas-

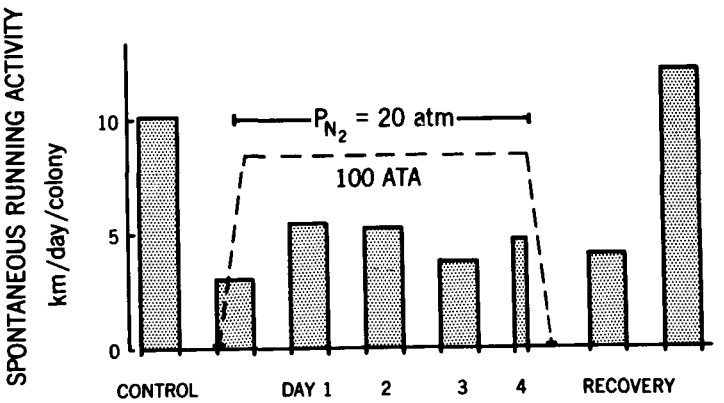

Fig. 5. Running activity observed before, during and after saturation dive to 100 ATA (He-O_2, 20 ATA N_2). Activity is plotted in km/day/colony; pressure profile is indicated by broken line.

eous environments of these saturation dives represent more favorable conditions than those in which helium is the only inert gas.

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PART IV. TOXICITY OF RESPIRATORY GASES

DISCUSSION

P. B. Bennett, Chairman

Dr. Weatherly: I would like to address my remarks to Dr. Kiesow. I am always a bit worried when people present information comparing different animals and in this case comparing different clotting systems. In man Factor XII is activated on being cut but so is Factor XI. You didn't say anything about that. You refer to the chicken as being Factor XII deficient. I am not sure how significant that is. Presumably when a chicken is cut it doesn't bleed to death because the clotting system is initiated. If Factor XII isn't present then presumably some other contact-activating agent must be. Perhaps this is the factor you should measure and not Factor XII.

Dr. Kiesow: I agree that the chicken is coagulation-factor-deficient in factors other than XII. We emphasized Factor XII because it is the beginning of the intrinsic clotting cascade, and furthermore we could demonstrate an activation of Factor XII. It also solicits a number of reactions that may be polarities of Factor XII activation, thereby amplifying the effects of the clotting activation system. Factor XI, Factor VII, and Factor V may also be activated, but consequent reactions apply only to Factor XII activations.

Dr. Weatherley: Yes, but they don't in the chicken, do they, because the chicken doesn't have Factor XII.

Dr. Kiesow: That's right. That is apparently why the chicken can accept hyperbaric oxygen quite well.

Dr. Weatherley: But what starts up the clotting system in the chicken if it doesn't have Factor XII? It can't be Factor XII, it must have some other factor, call it "x". Why didn't you measure Factor "x"?

Dr. Kiesow: We are dealing with the activation of the intrinsic clotting system. Certainly the chicken's blood clots if you mix it with tissue thromboplastins, it clots very well, in fact. But that has nothing to do with the activation of the intrinsic clotting system. Don't forget, when you cut your finger, you don't develop DIC.

Unidentified: Question to Dr. Kiesow. Some of the animals that you have shown in the last slide, which stayed a long time under oxygen, had metabolisms which lead to uric acid secretion instead of urea, for example the frog. Could you comment on this relationship with regard to the detoxification of lead acetate?

Dr. Kiesow: I would not like to tie lead acetate into this grouping of the animals. The detoxification of lead acetate, which is given iv, is a rather complex system. Let me add that it is the high insolubility of lead which confines iv-injected lead to the circulating bloodstream, at least for some period of time until redistribution occurs. That is what caused us to look into mechanisms that are associated either with the formed elements of the blood or with soluble components, including proteins.

Dr. Naquet: Dr. Rokitka, I was really impressed with your work. But I would like to ask, what do you call HPNS? We have heard many definitions of HPNS, and we are at the time where it is important to know what we are talking about. We know that HPNS in man is different with helium-oxygen and trimix. HPNS is probably not a single phenomenon, but many, depending on which gas is being used.

Dr. Rokitka: I will confine my definition to the symptoms as we see them in deer mice or as described for mammalian species other than man. We see the onset of tremors which are indistinguishable from shivering. They become so severe that seizures and finally paralysis result. We see this sequence develop when we compress to 75 or 80 atmospheres in a helium-oxygen environment where the oxygen is kept at normoxic levels. This is completely avoided by the use of enough inert gas. It is difficult to pinpoint the actual time of onset, but very careful observation does show a sequence that is fairly well defined. One can estimate the actual time to convulsion when the compression rate is maintained at levels such as the ones we use, namely 0.25 atmosphere per minute. So, HPNS in mice appears to be similar to that observed in man.

Dr. Naquet: I think it would be a good thing to know the exact mixture used, the definitions of the symptoms induced by this mixture. This precision is necessary if we want to know in a few years what we are talking about.

Dr. Sanders: To Dr. Banister. On the question of the glutamate-GABA-succinate shunt and the glutamate-glutamine protection theory that you are proposing at the time you show protection, you indicate that GABA remains

normal, glutamate is essentially normal and glutamine is elevated. It is interesting that at the same time in your electron microscopic print you show a filamentous type of mitochondria rather than the swollen type of mitochondria which you see at 24 hours, at which time you show that glutamate and glutamine are down and there is no protection from lithium chloride. You didn't show the GABA levels. Is this basically GABA maintenance and/or glutamic-to-glutamine synthesis? If this is correct, one would anticipate that blocking the GABA transaminase would shift the kinetics to the left, thus causing GABA, glutamate and glutamine to remain elevated. This theory would anticipate protection. But that is not the case, since Dr. Wood and other investigators have reported that blocking the GABA shunt does not provide protection. It is interesting that by blocking the GABA shunt, which would in theory cause GABA and glutamate to be elevated or normal, you lost OHP protection, but if you give succinate, you can still maintain protection. I do think that the electron microscopic data you have shown, combined with the fact that at the time you lose protection glutamate and glutamine are decreased, require that you look at the energy concept side of protection as well as the other possibility. I think that blocking of the shunt with loss of protection, at a point where there are normal or elevated glutamate and glutamine levels, tends to disagree with your theory.

Dr. Banister: I would agree with what you say. I'm not proposing that it is simply the inhibition of the glutamine-glutamate cycle that is important. I agree that energy levels are important. I think it is the levels of ATP that are available for maintaining an integrated cellular metabolism that are important. In fact, we are getting no protection at all when lithium is high in the brain. It potentiates the convulsive state and this is probably due to many other effects that lithium has in diagonal relationship with magnesium and calcium, and the way it may interfere with their biological actions at the cellular levels.

Dr. Sanders: I think we all agree that basically there are a lot of additional studies to be performed here. Even though we can get protection by certain of the basic agents, the primary or even secondary or tertiary mechanism is not specifically known at this time.

Dr. Banister: Yes, I'm saying that. Let us look precisely at these things with some kind of theory that you can shoot at rather than going along with other phenomenological investigations of several elements, searching for better protective agents with no comprehensive theory to explain why they are working or not working. Also, we should look at tissues other than heart and lung, with a view to giving some hierarchy to damaging events before effects even reach the brain or lung.

Dr. Sanders: If one examines the liver, kidney and brain grossly after severe oxygen toxicity, at 5 atmospheres of oxygen, for example, in Sprague-Dawley rats, one finds the kidney very severely damaged, similarly the liver and other organs. I definitely agree with you that there are other tissues involved, that the end point we may see as convulsions in CNS toxicity may be the result of an interplay between toxic responses taking place in different tissues.

Dr. Harvey: Dr. Rokitka, I wanted to add one more bit of information about the addition of oxygen to the high levels of nitrogen. We recently conducted an experiment at 198 feet with 3 subjects for 7 days. When we compressed, we took them down in a normoxic mixture, that is we started with air and added pure nitrogen. About 2½ hours after we reached bottom, two of the subjects developed nausea and over the next 7 or 8 hours had 3 or 4 hours of vomiting at the relatively normoxic level. We then added extra oxygen to go up to 0.3 atmosphere at that depth, intending to retreat to the 165-foot level to get out of the situation. The subject who had been on the headphones who had not had the nausea, then said that suddenly he could smell again and his speech became a bit more articulate. He hadn't been bad, but he had been a little slurred. The other two subjects were aroused and asked how they felt and they said they suddenly felt a good bit better and no longer had the nausea or any further symptoms. We continued for the remainder of the 7 days at the 0.3 atmosphere level. I can't say for sure that the oxygen was inadequate at the normoxic level, but we certainly saw some marked improvement, either due to accidental time sequence or to the addition of oxygen at that period.

Dr. Rokitka: It is difficult for me to actually point to a correlation between what you just noted and what we see in the mice. Unfortunately, our "communication" with the mice is somewhat limited.

Dr. Bennett: I have a question from the chair for Dr. Dougherty. In terms of your FVC depressions with oxygen, did you see any signs or symptoms associated with this, such as substernal pain?

Dr. Dougherty: Are you talking about the oxygen therapy for bends? In the bends cases, the two people in pre-SHAD I each had symptoms; they had been treated with Table 6, which is 240 minutes of oxygen, a pretty heavy dose on its own, and *subject P*, in the SHAD III dive, had some symptoms also. He mentioned dyspnea at rest, in addition.

Dr. Bennett: Were you able to ascertain whether the symptoms came before the fall in FVC or during it?

Dr. Dougherty: No. Unfortunately, the pre-SHAD I dive was basically a checkout dive. We did a few pulmonary function measurements, but none after the treatment. This dive was carried out to make sure that the equipment worked, and the subjects happened to get hit. During SHAD III, the initial drop in FVC, as I recall, was about an

hour and a half after the oxygen treatment. The subject was having some symptoms at the time, and then the FVC recovered at 14 hours. Then about 24 hours after the initial decrease in FVC, it went down to the lowest point, the 28% decrease, and then started coming back up.

Dr. Bennett: A question for Dr. Rokitka. I was very interested in your results, especially with the trimix. I quite agree with Professor Naquet about the difficulties of interpretation, but nevertheless there is a remarkable similarity between your results and human, whatever one calls it, HPNS or the effects of hydrostatic pressure. I wonder if you have tried a lower nitrogen pressure, since 20% nitrogen is a relatively high percentage. We did use that at Duke, as you saw perhaps in the trimix movie last night. With 18% we found that we had narcosis, as you have here. You may have a very interesting model to be able to work out for the human what is really effective, especially in regard to the lethargy which you reported. This is one of the characteristics of human HPNS and is a very serious problem if you are going to do trimix or deep helium dives with humans.

Dr. Rokitka: I couldn't help but see the similarities between the kinds of observations you were making with the divers during the film last evening and the observations we made with the mice. We did, in fact, try nitrogen partial pressures below 20%. We used 7, 14 and 18 percent nitrogen with no apparent success, that is, we had successful wheel activity during compression until 80–85 atmospheres at which time there was a very sudden onset of HPNS. It was almost as though there may have been an increase in the convulsion threshold with these lower nitrogen partial pressures, but they were not sufficient to completely abolish HPNS as we reported with the 20% nitrogen mix.

Dr. Bennett: Very interesting, and not dissimilar from the trials we recently completed in England, where we went to 1300 feet, the men were fit, and we were quite sure we were going to get to 1600 but we hadn't gone very far past 1300 feet before HPNS came on rather fast, primarily as fatigue and dizziness.

Dr. Rokitka: We suspect that the lethargy we see may well be a density-related problem and so are very cautious about using partial pressures in excess of those which we have already tried.

Dr. Banister: A question to the chairman and Dr. Rokitka. Did you observe the symptoms of tremor and convulsion to develop on one side of the body first, and then become general? Was there any such partial development?

Dr. Rokitka: We are not able to say which side of the body was involved at any one time. The only comment that I can make was that there seemed to be total involvement whenever a mouse was "hit". It was difficult to see whether one or another limb was more severely affected.

Dr. Banister: In most of our experiments we noticed that initiation occurred on one side.

Dr. Rokitka: Was this with mice or rats?

Dr. Banister: Sprague-Dawley rats.

Dr. Naquet: I agree with you, Professor Bennett. But species differences may exist. It is interesting to note in Dr. Rokitka's paper that mice run faster under trimix than under helium-oxygen. But in our experiments in men, when we use trimix and fast compression, the men are less able to work during the first part of the experiment than with helium-oxygen and slow compression. We found a diminution of activity with trimix, but you evidently did not find the same thing.

Dr. Rokitka: Are you referring to a type of adaptation that you see with human subjects?

Dr. Naquet: Yes, in humans there is adaption. The first hours after arriving at depth, men are not able to work, although they say they are able to work. But they don't work well and are fatigued. But after 24 hours they are almost normal. Did you find the same thing in your experiment?

Dr. Rokitka: It may well be that what we see reflects adaptation. The activity profiles from the 100-atmosphere dive showed that only half of normal activity was maintained. What is not immediately apparent from the profiles is that during the first morning after compression the mice were lethargic for several hours but resumed activity and performed on the wheel later in the day. We are now attempting to prolong our studies to see whether there could be a more complete recovery from pressure-related problems or any inert-gas-related problems. Adaptation is one of the situations we are looking for or at.

Dr. Naquet: I agree with you. We know that the men are not in good shape when they get to 300 meters after a fast compression with trimix, but they recuperate well. It would be interesting to know if they are in better shape a few hours after a slow compression with helium.

Dr. Bennett: Dr. Naquet, I think we are into the same problems we talked of early in the session. It is a question of rate of compression. With the correct rate of compression and trimix you don't get the fatigue, at least certainly we did not, and with one hour to a thousand feet, men with 10% nitrogen were very fit and able to work.

Dr. Naquet: I think that when the dives are deep and involve fast compression and trimix, the men need a period of adaptation, and if Dr. Rokitka has found the same thing, we agree perfectly.

Dr. Rokitka: I would like to mention another aspect of our study. We have observations other than running activity, namely behavioral and social observations, water drinking, and the like. They all reflect an increased burst

in activity following arrival at pressure. We even observed courtship and mating at these very high pressures. If we look at all of these other observations they may very well reflect what one could call adaptation.

Dr. Naquet: We all have to work on this problem of adaptation.

Dr. E. B. Smith: I want to comment on this difference between the concentrations of nitrogen that seem to be required to produce the optimum performances with men and mice. If we look at the reversal of nitrogen anesthesia by the application of high pressures of helium, the results indicate a rather higher optimum concentration of nitrogen in the mixture for mice than for men. I wonder if perhaps it is overgeneralizing to look for the same concentrations in men diving that we are looking for in mice.

Dr. Bennett: I certainly agree with you, Dr. Smith. I think it is very dangerous indeed to generalize from mouse to man. Only a very broad correlation is possible.

Part V. CELLULAR AND PHYSIOLOGICAL EFFECTS OF HYDROSTATIC PRESSURE

EFFECTS OF SHORT- AND LONG-TERM HYPERBARIC HELIUM-OXYGEN EXPOSURES ON GROWTH AND TISSUE WATER, LIPIDS, AND PROTEINS

T. K. Akers, B. K. Ross, D. Crittenden, M. J. Mason, G. Kosel and R. E. Thompson

The problem of inert gas narcosis in deep diving has been partially circumvented by the introduction of helium (He) as a diluent gas (3). However, both helium and nitrogen (N_2) have effects on the living organism. Very few studies delineating the effects of helium and nitrogen at pressure on body weight, body water distribution or standard blood indices exist. It was the purpose of the present study to determine the effect of hyperbaric helium and nitrogen on these parameters during prolonged exposure, with special emphasis on the first four days.

Methods

In the first set of experiments, Sprague-Dawley rats (165-190 g) were subdivided into the following experimental exposure groups, each consisting of 12 animals: 1 ATA room air for 4 weeks; 1 ATA normoxic helium for 1 week; 10 ATA He-O_2 (P_{O_2} = 200 mmHg) for 50 and 100 hours; and 10 ATA N_2-O_2 (P_{O_2} = 200 mmHg) for 50 and 100 hours. Chamber temperature measured between 22° and 25° C. For exposures at high pressure, compression was carried out at a rate of 20 ATA/hour. Decompression followed a modified exponential format (2). At the termination of decompression, body weight change from control value, O_2 consumption, and food and water consumption were determined, and a blood sample was taken for determination of Na^+, K^+, hematocrit, hemoglobin, and red blood cell count. The animals were killed, and tissue samples of the adrenals, kidneys, spleen, heart, lung, liver, and brain were removed for tissue water, lipid and protein determinations.

In the second set of experiments, guinea pigs (250-350 g) were exposed to 1 ATA normoxic helium for 24 days, 1 ATA room air for 24 days, 20 ATA He-O_2 (P_{O_2} = 200 mmHg) for 2, 4, 6, 12, and 24 days, and 40 ATA He-O_2 (P_{O_2} = 200 mmHg) for 2 and 4 days. Chamber temperature was held at 33° C. Compression and decompression procedures were carried out, as previously described. Body weight changes were determined immediately after decompression. The animals were killed and tissue water measured. Lung samples were obtained from some animals for scanning electron microscopic examination.

Blood indices were measured using the microcapillary technique for hematocrit and Hycel cyanmethemoglobin method for hemoglobin. Serum Na^+ and K^+ were measured with a Beckman 105 flame photometer.

Tissue samples were either freeze-dried or oven-dried at 80° C for 24 hours to determine the water content. Protein content was determined by the micro-Kjeldahl method. Lipid content was determined after extraction for 8 hours with commercial ether in a Soxhlet extraction apparatus.

Lungs prepared for scanning electron microscopy were perfused via the trachea with buffered aldehydes, dehydrated in a graded series of acetones, critical-point-dried using liquid carbon dioxide (CO_2) and coated with palladium-gold. Sections were viewed using a Cambridge Stereoscan S4 scanning electron microscope.

Results

Shortly after being pressurized to 10 ATA, the rats breathing nitrogen-oxygen appeared narcotic. The lethargic phase lasted through the first day of hyperbaric exposure. After that, the animals were alert and appeared to have adapted. During the 50- and 100-hour exposures, the rats lost an average of 24 g, in contrast to the air controls which showed a net gain of 6 g/day. These changes in body weight are expressed as percent of pre-exposure weights in Fig. 1. Over half of the animals exposed for 100 hours died before the end of the decompression phase of the experiment.

As shown in Fig. 2, food and water consumption were 2.41 g/hour and 3.79 ml/hour for the rats exposed to nitrogen-oxygen for 50 hours, and 2.40 g/hour and 3.58 ml/hour for those exposed to nitrogen-oxygen for 100 hours. These values did not differ from the control values, despite the weight loss seen.

The 32 air control animals had an oxygen consumption of 85.61 ± 9.57 cc O_2/min/kg$^{3/4}$. Of the rats exposed to nitrogen-oxygen at 10 ATA for 50 hours, 2 had an average oxygen consumption of 99.99 ± 0.10 cc/min/kg$^{3/4}$ on postexposure measurement, while 5 which had been exposed for 100 hours demonstrated an oxygen consumption of 80.67 ± 3.73 cc/min/

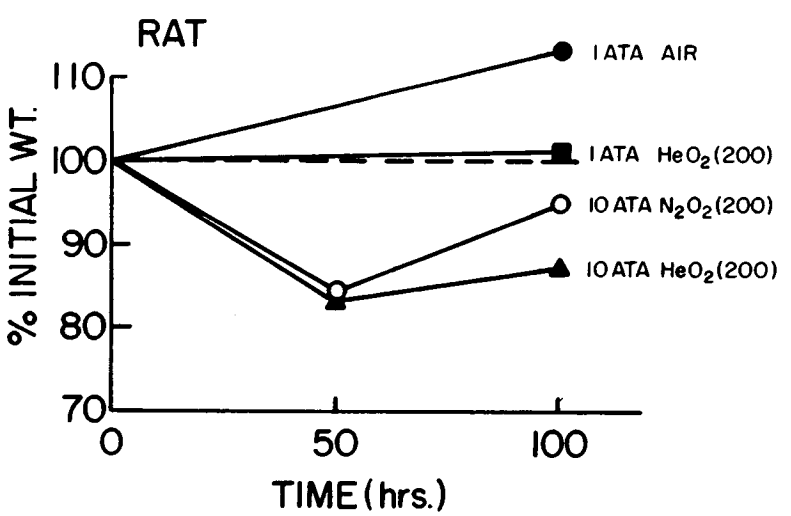

Fig. 1. Changes in body weight after exposure of rats to different gaseous environments.

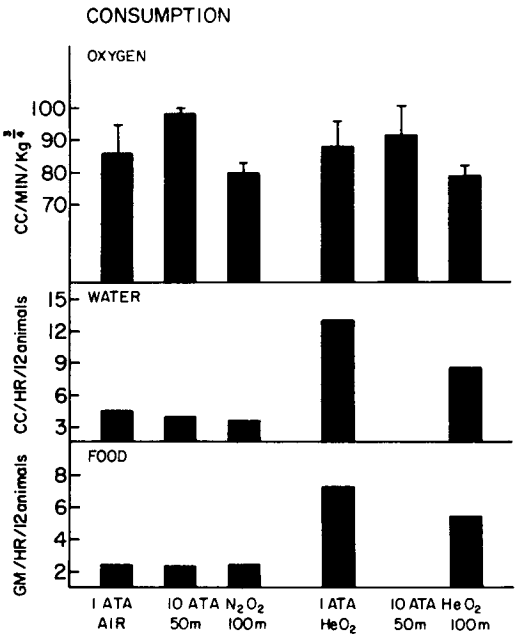

Fig. 2. Food, water and oxygen consumption of rats exposed to different gaseous environments. Food and water consumption values are for 12 animals; oxygen consumption values are means ± SD of individual post-exposure measurements.

$kg^{3/4}$. These data are also shown in Fig. 2. No significant differences were found between groups.

Results of blood analyses on nitrogen-exposed rats are shown in Table I. Serum Na^+ in rats exposed for 100 hours was slightly elevated over control values ($P < 0.05$), while K^+ concentration was depressed below control values in rats exposed for 50 hours ($P < 0.05$) and for 100 hours ($P < 0.001$). Hematocrit and hemoglobin values were lower in rats exposed for 50 hours, indicating hemodilution, but the RBC count was not significantly decreased in any of these animals.

The percentages of water in the tissues studied are summarized in Table II. The only tissues showing significant changes in water content after exposure to hyperbaric nitrogen and subsequent decompression were the adrenal glands and the lungs. Adrenals showed a decrease in lipid content from 59.05% ± 14.08% of dry weight in the control animals to 41.79% ± 4.22% after decompression from 100 hours of exposure to 10 ATA nitrogen-oxygen. There were slight but significant decreases in lipid content in the kidneys as well, while very small increases were seen in the liver and brain. Adrenal, kidney, and liver protein content increased slightly, but significantly, in the prolonged exposures.

In contrast to the nitrogen-exposed rats, pressurization to 10 ATA with an oxygen-equivalent helium mixture had no observable narcotic effect on the rats. The animals did huddle together and exhibit pilo-erection, because no attempt was made to increase the chamber temperature for these studies.

The rats exposed to helium at 1 ATA showed only slight increases in body weight com-

TABLE I

BLOOD ANALYSES OF RATS EXPOSED TO DIFFERENT GASEOUS ENVIRONMENTS

Exposure Conditions	Na^+, mEq/liter	K^+, mEq/liter	Hct, %	Hb, g/100 ml	RBC, 10^6/cmm
1 ATA air, 100 hours	142.6 ± 6.9 (27)	7.13 ± 1.4 (26)	44 ± 3 (51)	15.0 ± 1.1 (49)	7.63 ± 0.8 (44)
10 ATA N_2-O_2, 50 hours	142.8 ± 1.6 (6)	5.22 ± 0.7 (6)‡	41 ± 2 (12)†	13.9 ± 0.6 (12)‡	7.45 ± 0.5 (10)
100 hours	146.6 ± 2.1 (5)*	5.54 ± 1.4 (5)*	43 ± 5 (10)	14.4 ± 1.8 (10)	7.83 ± 0.8 (9)
1 ATA He-O_2, 100 hours	147.0 ± 3.0 (8)	5.09 ± 0.3 (8)	43 ± 2 (15)	13.8 ± 0.7 (15)	7.70 ± 0.7 (16)
10 ATA He-O_2, 50 hours	138.6 ± 5.2 (13)‡	7.28 ± 2.8 (13)*	49 ± 2 (11)‡	14.7 ± 0.7 (12)*	8.77 ± 1.0 (11)†
100 hours	143.8 ± 6.0 (8)	5.18 ± 0.5 (5)	48 ± 2 (20)‡	15.9 ± 0.5 (19)‡	9.17 ± 1.4 (18)‡

Values are means ± SD; number of animals studied appears in parentheses. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

TABLE II

PERCENTAGES OF WATER IN TISSUES OF RATS EXPOSED TO DIFFERENT GASEOUS ENVIRONMENTS

Exposure Conditions	Adrenal	Kidney	Spleen	Heart	Lung	Liver	Brain
1 ATA air	60.0 ± 10.49 (10)	70.64 ± 8.65 (10)	76.95 ± 5.95 (10)	77.89 ± 1.92 (10)	79.21 ± 0.02 (8)	72.53 ± 2.06 (10)	78.32 ± 1.69 (10)
10 ATA N_2-O_2, 50 hours	78.36 ± 3.52 (6)‡	62.79 ± 15.24 (6)	77.82 ± 0.56 (6)	77.76 ± 1.66 (6)	79.73 ± 0.67 (6)	74.05 ± 1.92 (6)	78.37 ± 1.27 (6)
100 hours	74.46 ± 2.19 (3)†	76.83 ± 0.40 (3)*	78.01 ± 0.37 (3)	81.84 ± 4.65 (3)	80.04 ± 0.11 (3)‡	71.96 ± 0.68 (3)	78.46 ± 0.46 (3)
1 ATA He-O_2	65.11 ± 1.37 (6)	74.83 ± 0.43 (6)	77.81 ± 0.44 (6)	78.02 ± 0.25 (6)	79.42 ± 0.32 (6)	71.10 ± 0.67 (6)	78.90 ± 0.20 (6)
10 ATA He-O_2 50 hours	71.38 ± 3.07 (6)†	72.34 ± 8.24 (6)	78.50 ± 0.63 (6)	78.55 ± 0.51 (6)	79.42 ± 0.40 (6)	72.07 ± 1.00 (6)	79.21 ± 0.27 (6)
100 hours	73.62 ± 2.70 (6)‡	75.09 ± 0.62 (6)	78.30 ± 1.74 (6)	76.91 ± 0.12 (6)‡	78.27 ± 1.03 (6)*	70.52 ± 0.89 (6)	78.50 ± 0.13 (6)

Values are means ± SD; number of animals studied appears in parentheses. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

pared to the weight of 1-ATA air controls, while the 10-ATA animals lost up to 18 g/animal (Fig. 1). No deaths occurred in rats exposed to helium-oxygen mixtures. Food and water consumption were significantly increased compared to that of 1-ATA air controls, with values of 7.34 g/hour and 13.11 cc/hour, respectively, in rats breathing helium at 1 ATA, and 5.65 g/hour and 8.74 cc/hour in rats exposed to helium at 10 ATA for 100 hours. No successful collections were made from the 50-hour group. The postexposure oxygen consumption mea-

surements were not significantly different for any helium group compared with air control values.

The animals which had been exposed to helium-oxygen at 1 ATA demonstrated significantly increased serum Na^+ and decreased serum K^+, compared to air control values. The hematocrit and hemoglobin were slightly decreased. Rats exposed to hyperbaric helium-oxygen showed a drop in Na^+ and a rise in K^+ after decompression. This was accompanied by a rise in hematocrit, hemoglobin and RBC count, indicative of hemoconcentration and sodium loss. These animals also exhibited diuresis. Adrenal gland size and water content were increased over helium-oxygen control values at 1 ATA, while heart and lung water decreased. The lipid content of the adrenals, liver and kidneys and the protein content of the kidney were greater than control values.

These changes indicate that hyperbaric exposure profiles can produce a large body weight loss, a shift in tissue water distribution which depends upon the diluent gas used, and a reduction in lipid content in specific tissues of the body. It should be noted that on autopsy all rats which had been exposed to hyperbaric nitrogen-oxygen, and all helium-oxygen-exposed rats exhibited very little adipose tissue compared with the 1-ATA air control animals. All of these animals showed diuresis, but only those which had been exposed to helium-oxygen demonstrated a compensatory increase in food and water consumption. The rats exposed to hyperbaric nitrogen may have been too narcotic to eat and drink normally, although their behavior after one day of exposure appeared relatively normal.

Studies on guinea pigs exposed to helium-oxygen at 20 and 40 ATA showed results similar to those described for rats. Weight loss after decompression from 2- and 4-day exposures to helium-oxygen at 20 and 40 ATA was greater than after 10-ATA exposures (Figs. 1 and 3). The most prominent shifts in water occurred in the adrenal, with significant increases occurring after decompression from prolonged exposures to pressure (Table III). The kidney water content of guinea pigs was significantly increased following pressurized helium exposures compared to 1-ATA air control values (Table IV).

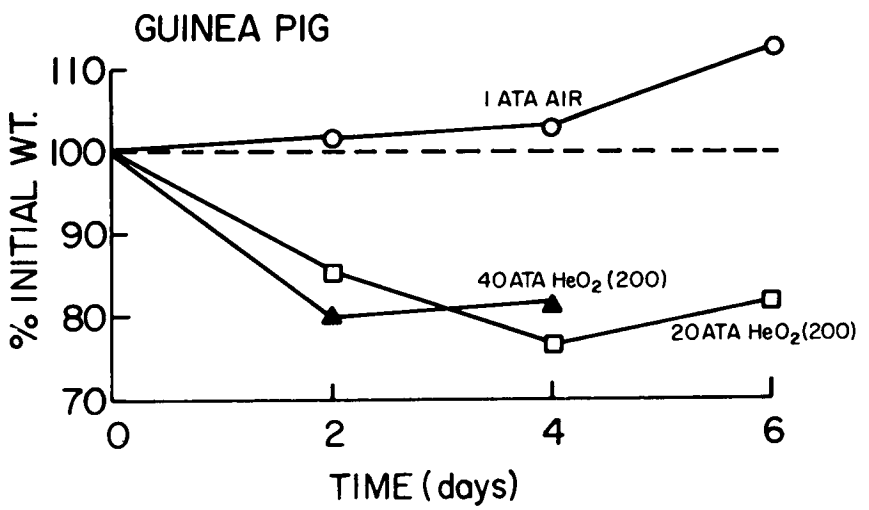

Fig. 3. Changes in body weight after exposure of guinea pigs to different gaseous environments.

TABLE III

ADRENAL WET-TO-DRY WEIGHT RATIOS IN GUINEA PIGS AFTER EXPOSURE TO DIFFERENT GASEOUS ENVIRONMENTS

Exposure Conditions	Exposure Time		
	2 days	4 days	6 days
Air, 1 ATA	1.09 ± 0.53	1.40 ± 0.25	1.62 ± 0.42
He-O_2, 20 ATA	2.11* ± 0.74	2.58† ± 0.74	2.65† ± 0.44
40 ATA	1.66* ± 0.39	2.15† ± 0.43	

Values are means ± SD; * $P < 0.05$; † $P < 0.01$; $n = 8$.

TABLE IV

KIDNEY WET-TO-DRY WEIGHT RATIOS IN GUINEA PIGS AFTER EXPOSURE TO DIFFERENT GASEOUS ENVIRONMENTS

Exposure Conditions	Exposure Time		
	2 days	4 days	6 days
Air, 1 ATA	3.39 ± 0.17	3.18 ± 0.32	3.20 ± 0.21
He-O_2, 20 ATA	3.72* ± 0.28	3.74† ± 0.26	3.62* ± 0.26
40 ATA	3.51 ± 0.20	3.73† ± 0.16	

Values are means ± SD; * $P < 0.05$; † $P < 0.01$; $n = 8$.

The lung structure of guinea pigs exposed to helium-oxygen at 1 ATA and 20 ATA showed very few differences when viewed with the scanning electron microscope. The alveolar epithelium appeared thin, so that red blood cells were apparent behind it. The Kohn's pores were patent in both cases, and the alveolar Type II cells appeared normal (compare Figs. 4 and 5).

Discussion

The low survival rate of rats exposed to nitrogen-oxygen at 10 ATA demonstrates the potency of nitrogen at that pressure. It is likely that emaciation and death were caused by interference with nutritional drive. Workman, Bond, and Mazzone (6) also found nitrogen at 200 fsw to be deleterious to rats.

The Na^+ retention shown by rats following hyperbaric nitrogen-oxygen exposures agrees with human electrolyte changes in response to hyperbaric air exposures reported by Baddeley and Fleming (1). The other blood indices which were altered after 50 hours of exposure but which tended toward normal after 100 hours at 10 ATA may indicate adaptation on long exposure.

Although the majority of the organs analyzed had normal water contents, adrenal water was considerably elevated upon completion of both the 50-hour and 100-hour exposures.

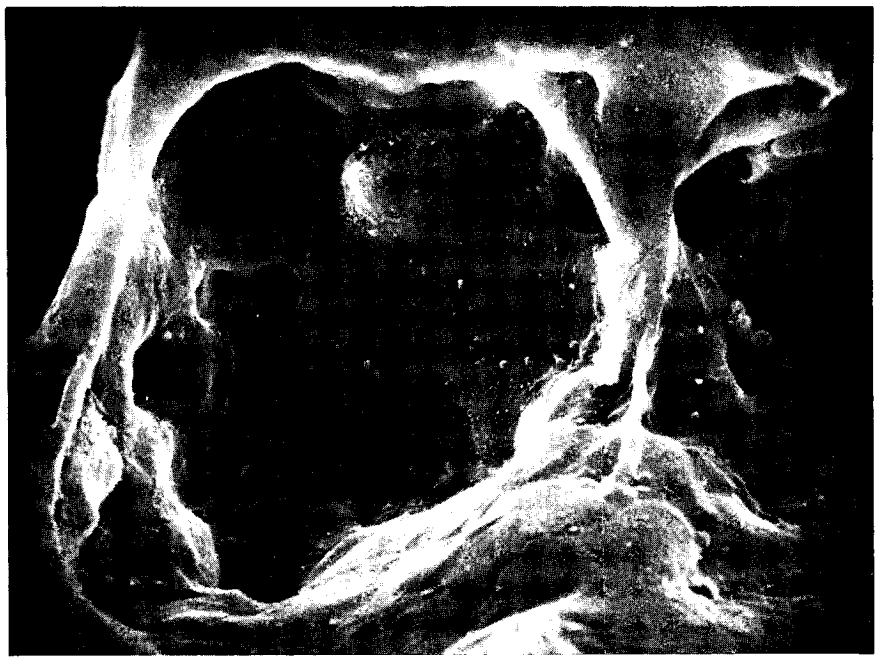

Fig. 4. Scanning electron micrograph of alveoli from normal guinea pig breathing air at 1 ATA. Note Kohn's pores and indication of red blood cells behind epithelium. × 2800.

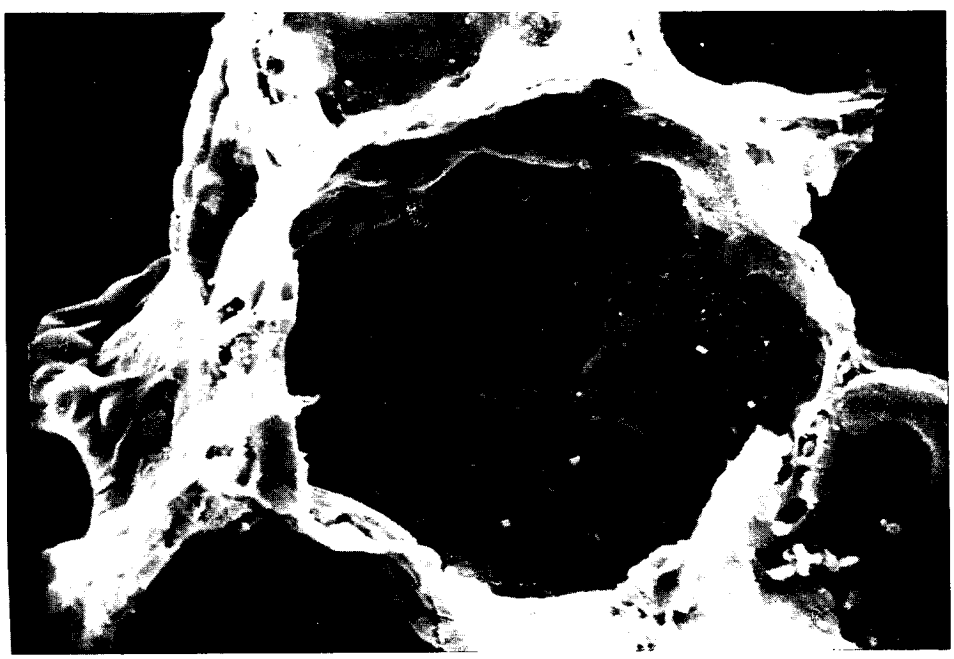

Fig. 5. Scanning electron micrograph of alveoli from guinea pig exposed to 20 ATA He-O_2 for 4 days. × 1050.

This may be an indication of the stress of pressure. Kidney and lung water were increased after 100 hours of nitrogen exposure. Increased kidney water may reflect increased blood flow to these organs, which results in diuresis. Recent reports by Boerboom and Boelkins (5) seem to support this possibility. Gas density injury to the lungs could account for pulmonary edema.

Lipid analyses indicate that the liver was a depot for sequestered substances from breakdown of adipose tissue and hormonal products. Many steroids which are metabolized in the liver could have originated in the adrenal tissue, and release of these steroids through environmental stress would deplete the adrenal gland of lipid content. The protein increases seen may represent only a relative increase concurrent with decreases in lipid content, rather than synthesis. Since liver protein increased along with lipid increases, protein anabolism is indicated.

Rats exposed to helium with a P_{O_2} of 200 mmHg at 10 ATA survived up to 100 hours at that pressure. Guinea pigs exposed to helium with the same P_{O_2} at 20 ATA and 40 ATA survived at least 24 days. However, in all cases, the animals lost weight, while the 1-ATA air control animals did not.

In the helium environments, electrolyte patterns were reversed from those found in the nitrogen environment, with Na^+ loss and K^+ retention. This effect, which needs to be explored further, could indicate a subtle difference in action between nitrogen and helium at pressure. All blood indices were elevated because of plasma water loss, which indicates hemoconcentration. Adrenal water elevation at every pressure studied reflects either increased blood flow or breakdown of water-containing tissue. Bitter and Nielsen (4) reported increased urinary corticosterone from rats hyperbarically exposed to helium. The decrease in lung and heart water indicates dehydration of the animals exposed to helium and agrees with the increased blood indices. The lipid and protein values were not altered as much in helium as in nitrogen exposure, yet the body weight loss was greater. The latter may be due to helium alteration of thermal stress in addition to the pressure stress. Though we consider helium to be an inert diluent gas, useful for prolonged hyperbaric exposure, it is not without its effect on the living organism. Which of the reported effects are caused by hydrostatic pressure stress, which are due to decompression, and which are caused by the specific gases breathed is impossible to ascertain at the present time. Further carefully controlled studies examining electrolyte shifts and hormonal responses during and after specific pressure profiles may elucidate the controlling factors.

Acknowledgment

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EFFECT OF HYPERBARIC HELIUM ON ANESTHETIC ACTION OF THIOPENTAL

R. E. Tobey, L. E. McCracken, A. Small and L. D. Homer

The possibility that an injury or an acute illness requiring surgery would occur in a deep sea diver under pressure has always been present. With the advance of underwater technology and the increasing number of man-hours spent at saturation depths, the likelihood increases that general anesthesia under hyperbaric conditions will be necessary. One such case has already occurred, requiring major abdominal surgery in a chamber at a simulated pressure equivalent to 190 feet of seawater (fsw). Local anesthesia, a narcotic and an antihistamine were employed in this case to obtain the anesthetic state required for surgery (2).

In future, surgical situations calling for more refined and profound anesthetic techniques may be indicated. There is, however, a paucity of information to serve as a guide to the practical use of anesthetic drugs and techniques under hyperbaric conditions. These studies were designed to devise a technique for general anesthesia which could be used in a hyperbaric environment. Since thiopental is one of the most commonly used drugs for induction of anesthesia at sea level, experiments were carried out to determine what influence a hyperbaric helium environment had on thiopental-induced sleep time.

Experimental Approach

A total of 180 female guinea pigs were randomly divided into six groups of 30 animals each. Two days prior to the experiment a No. 20 polyethylene catheter was placed in the jugular vein and threaded into the superior vena cava under intraperitoneal pentobarbital anesthesia. The catheter was filled with heparinized saline solution, brought out the back of the neck through a subcutaneous tunnel and folded into a protective plastic tube which was secured to the neck wound.

Approximately 48 hours after surgical preparation, each guinea pig was placed in a restraining stall, and arrangements were made for remote intravenous injection within the hyperbaric chamber (10).

The six groups were tested in the following environments: air at 1 ATA; helium-oxygen at 1 ATA; helium-oxygen at 20 ATA immediately upon reaching depth; helium-oxygen at 20 ATA two hours after reaching depth; helium-oxygen at 30 ATA immediately upon reaching depth; and helium-oxygen at 30 ATA two hours after reaching depth. A system of stratified randomization was used to determine the order of experiments.

For all except the group in air, the chamber was flushed with helium-oxygen (79/21 vol %) until no nitrogen could be detected by a Perkin-Elmer Fractometer. For hyperbaric exposures,

compression was carried out at approximately 2 atm/minute. The two groups of animals which remained at 1 ATA were exposed to continuous ventilation of the chamber for 10 minutes with either air or helium-oxygen, as appropriate. Oxygen partial pressure was monitored and maintained between 0.2-0.3 ATA, usually at 0.24–0.25 ATA when the animals were under pressure. Carbon dioxide concentration was monitored and was kept below a partial pressure of 0.002 ATA by a Baralyme scrubbing system. Rectal temperature was monitored continuously and maintained at 37 ± 0.5° C by controlling the chamber temperature.

After injection of thiopental (20 mg/kg, 1.25 % solution), the restraining stall was removed by a remotely operated motor, and the animals fell to their sides asleep. Sleep time was measured as the interval between the end of thiopental injection and recovery of the righting reflex. The data among groups were analyzed by the rank sum method.

In a second series of experiments, the effects of helium-oxygen environments and pressure on the 50% effective induction dose (ED_{50}) for thiopental were determined. In preliminary experiments, the intravenous ED_{50} was found to be 1.48 mg/kg in air at 1 ATA. The doses used in this experiment were chosen with a constant logarithmic interval, and were administered in a randomized sequence.

Four groups of 25 guinea pigs each were tested in the following environments: air at 1 ATA; helium-oxygen at 1 ATA; helium-oxygen at 20 ATA and helium-oxygen at 30 ATA. A system of stratified randomization was used for the order of experiments.

The guinea pigs were prepared as described previously, and given intravenous thiopental (1.5 mg/kg) 25 minutes after placement in the chamber. No time was allowed for saturation of the animals in these studies. The presence or absence of righting reflex was taken as the end point of drug effect. All animals which failed to lose the righting reflex were autopsied to confirm intravenous placement of the catheter. The data were analyzed by the chi square test for statistical significance.

Results

Thiopental sleep time in the helium-oxygen environments was significantly shorter than that in air at 1 ATA (Tables I, II). This effect appeared to be correlated with the partial pressure of helium, with the exception of the group saturated in helium-oxygen at 20 ATA, which was not statistically different from the group in helium-oxygen at 1 ATA. No differences in sleep time were found between the nonsaturated and saturated groups in either the 20- or 30-ATA environments.

The results of studies which compared the effects on righting reflex of an ED_{50} dose of thiopental in air at 1 ATA with helium-oxygen at 1 ATA and increased ambient pressures are shown in Table III. There was a statistically significant difference between the effects of 20 ATA helium-oxygen and 1 ATA air, and 30 ATA helium-oxygen and 1 ATA air. No significant statistical difference was found between the results using air and helium-oxygen at 1 ATA, or between helium-oxygen mixtures at 20 and 30 ATA.

Discussion

The results of these sleep time studies demonstrate that, with one exception, helium produces a pressure-dependent antagonism of thiopental anesthesia in guinea pigs that was apparent even at 1 ATA. Moreover, the effect seemed to be virtually immediate, since allowing

TABLE I

EXPERIMENT I, MEDIAN SLEEP TIMES OF GUINEA PIGS AFTER INTRAVENOUS THIOPENTAL UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Gas Environment	*n*	Median Sleep Time, min
Air, 1 ATA	30	44.1
He/O_2, 1 ATA	30	25.5
He/O_2, 20 ATA (I)	30	21.0
He/O_2, 20 ATA (S)	30	24.6
He/O_2, 30 ATA (I)	30	16.4
He/O_2, 30 ATA (S)	30	16.3

I = tested immediately after reaching depth; S = tested in a saturated condition, 2 hours after reaching depth. Dosage was 20 mg/kg.

TABLE II

STATISTICAL COMPARISONS OF SLEEP TIME UNDER DIFFERENT ENVIRONMENTAL CONDITIONS BY RANK SUM TEST

Comparison, ATA	*P* Value
Air, 1 ATA to He/O_2, 1 ATA	< 0.003
Air, 1 ATA to He/O_2, 20 ATA (I)	< 0.001
Air, 1 ATA to He/O_2, 20 ATA (S)	< 0.027
Air, 1 ATA to He/O_2, 30 ATA (I)	< 0.001
Air, 1 ATA to He/O_2, 30 ATA (S)	< 0.001
He/O_2, 1 ATA to He/O_2, 20 ATA (I)	< 0.0025
He/O_2, 1 ATA to He/O_2, 30 ATA (I)	< 0.0027
He/O_2, 1 ATA to He/O_2, 20 ATA (S)	NS
He/O_2, 1 ATA to He/O_2, 30 ATA (S)	< 0.009
He/O_2, 20 ATA (I) to He/O_2, 20 ATA (S)	NS
He/O_2, 30 ATA (I) to He/O_2, 30 ATA (S)	NS
He/O_2, 20 ATA (I) to He/O_2, 30 ATA (I)	< 0.013
He/O_2, 20 ATA (S) to He/O_2, 30 ATA (S)	< 0.027

Data from Experiment I; I = tested immediately after reaching depth; S = tested in a saturated condition, 2 hours after reaching depth.

a longer period of time for tissue saturation with ambient helium did not enhance the effect of the gas. The finding that the antagonism of sleep time in the 20-ATA saturated group was not significantly greater than the effect of helium-oxygen at 1 ATA is a puzzling discrepancy in the otherwise orderly data.

Although other effects of helium at relatively low pressures have been reported (3, 9, 11), the authors are unaware of any previous reports of an effect at such low partial pressures of helium on the action of drugs. In other studies, possible effects of hyperbaric helium on drug action were sought but not observed. Greenbaum and Evans (5) found no difference in the analgesic effects of morphine in mice in helium-oxygen at 20 ATA compared to air at 1

TABLE III

COMPARISON OF SLEEP RESPONSES (LOSS OF RIGHTING REFLEX) AFTER ED_{50} DOSE OF THIOPENTAL UNDER VARIOUS HYPERBARIC CONDITIONS

Condition	*n*	No. Asleep	% of Total
Air, 1 ATA	25	14	56
He/O_2, 1 ATA	25	16	64
He/O_2, 20 ATA	25	3*	12
He/O_2, 30 ATA	25	1†	4

Dosage = 1.5 mg/kg, intravenous. * $P < 0.005$ compared to air at 1 ATA; † $P < 0.0005$ compared to air at 1 ATA. Data from Experiment II.

ATA. Similarly, Small (10) determined the acute lethal toxicity of ethanol, pentobarbital, morphine and lidocaine in rats and found no effect of helium at 20 ATA on the acute toxicity of these drugs. The effect of hyperbaric helium on anesthesia as manifested by the righting reflex was not studied, and those results do not, therefore, necessarily contradict the positive findings in the present study. They do, however, suggest that the antagonism of helium observed here may be due to a particular pharmacologic property of thiopental. This hypothesis is strengthened by preliminary observations which have shown that ketamine anesthesia is not antagonized by hyperbaric helium (6).

It is desirable to discuss the possible mechanisms by which helium could produce such antagonism of anesthesia. One explanation is that this effect may simply have been the result of physical factors that were not adequately controlled. Thus, increased sensory input to the central nervous system may have been caused by the animal's perception of increasing pressure, e.g., via the tympanic membrane, or by enhanced auditory stimulation from noises accompanying pressurization as a result of increased sound pitch and transmission in the denser hyperbaric atmosphere. However, the fact that antagonism of anesthesia was observed when animals were allowed to remain undisturbed for two hours at elevated pressure before being tested makes it unlikely that the effect was due to such physical factors.

The absence of nitrogen in the atmosphere has been shown to have measurable effects on certain biological systems (11), but such a simple explanation is not adequate to explain the graded response observed with increasing helium partial pressure.

There is little to suggest that helium acts as a physiological antagonist to thiopental. First, certain inert gases of the helium series are themselves depressant; second, even though hyperbaric helium has been associated with the phenomenon of central nervous system stimulation known as the High Pressure Nervous Syndrome (HPNS), this effect is most pronounced at pressures in excess of 21 ATA and is thought to be caused by a hydrostatic pressure effect rather than a pharmacologic helium effect (1, 5).

The observed antagonism of narcosis elicited in newts and mice with several different volatile anesthetics when helium at 150 ATA is applied (8) may be related to HPNS. Such effects have been attributed to compression by high hydrostatic pressure of neural membranes thickened by anesthetic molecules; this mechanism forms the basis for the "Critical Volume Hypothesis" of anesthetic mechanisms (7). Since even helium at 1 ATA was found to have some effect in these sleep time experiments, it seems unlikely that such a mechanism contributed to the present results.

Other mechanisms that could be responsible for the observed effect of helium are: (1) a specific antagonistic effect of helium which decreases the effectiveness of the interaction between thiopental and its receptor site, or site of action, e.g., through competitive antagonism, or by inducing a conformational change at the site of action; or (2) an effect of helium on initial distribution or later redistribution of thiopental. The experiment described above, in which the presence or absence of the righting reflex was observed after an ED_{50} dose of thiopental, was designed to provide information that would aid in differentiating between these two possibilities.

The efficacy of an ED_{50} dose of thiopental was decreased under increased ambient pressures in a manner similar to that which characterized the reduction in sleep time observed in the first series of experiments. Whereas there was ample time available for helium to affect drug redistribution when sleep time was measured, the efficacy of an ED_{50} dose of thiopental was determined immediately after injection, and could not, therefore, have been influenced by altered redistribution. This observation implies that the effect of helium at 20 or 30 ATA was due either to an alteration of the initial distribution of the drug, or to a specific antagonistic effect.

It should be noted, however, that helium at 1 ATA did not significantly antagonize the response to an ED_{50} dose of thiopental. This result suggests that drug redistribution may have played a role in the effect of helium at 1 ATA on thiopental sleep time. Alternatively, failure to detect an effect of helium at 1 ATA on response to the ED_{50} dose could have been due to the low statistical sensitivity of the experimental design. It is also possible that other factors, such as temperature in the chamber or the P_{O_2} of the breathing mixture, played a role.

The reduced effectiveness of thiopental under increased partial pressure of helium is important clinically, because these results indicate that a twofold increase in the dosage may be required to induce anesthesia in a patient being treated under hyperbaric conditions. Further investigations are necessary to establish an ED_{100} for thiopental under increased ambient pressures, since that is the clinical requirement. It will also be important to determine if the peripheral effects of thiopental are antagonized to the same extent by helium as cerebral effects. For example, the increased dose required to induce unconsciousness under hyperbaric conditions may produce such profound cardiovascular depression that use of the drug in these conditions will be impractical.

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HYDROSTATIC PRESSURE EFFECTS ON TADPOLE TAIL MUSCLE

Yoshihiro Mano

It is well known that paralysis occurs in organisms exposed to very high hydrostatic pressures. This phenomenon was first described by Regnard et al. (1). However, very little research has been devoted to correlating macroscopic paralysis of tissue with histological analysis. In the studies reported here, histological examinations were carried out on muscle tissue from tadpoles exposed to hydrostatic pressures ranging from 1 to 500 ATA in a wet chamber.

Experimental Approach

Tadpoles were chosen as experimental subjects because they are aquatic animals which can be readily obtained at any time of the year (2, 3). Since tadpoles develop into frogs, they offer a convenient means for comparing bronchial respiration with pulmonary respiration in the same animal.

In the center of the normal tadpole tail there is a notochord which is surrounded by connective tissue. Muscle is present between the notochord and skin (Fig. 1) (10, 11). This muscle is called a myotome, but it is striated and is functionally a voluntary muscle. Tadpole tail muscle begins to be reduced or absorbed when the metamorphosis from tadpole to frog begins. In these experiments, all tadpoles were selected in accordance with the classification of Taylor and Kollros (9), and were exposed to high pressures before their tail muscles were reduced.

Total number of subjects in the high pressure exposure group was 204, while 51 subjects made up the control group. In each experiment, the tadpoles in the control and high pressure groups were at the same ontogenetic stage of development. All experiments were carried out in a specially constructed wet high pressure chamber (Fig. 2). Water temperature in the chamber was set at 20° C in the summer and 15° C in the winter. Experiments were conducted at pressures ranging from 7 to 500 ATA, and exposure times varied from 30 seconds to 120 minutes. Decompression took several seconds. Most of the tadpoles were either fixed in formalin, Bouin's, or Susa's solution or frozen within 3 minutes of return to 1 ATA (Fig. 3). Others were kept at normal laboratory temperature for an additional period of from 2 hours to 10 days to investigate the effects of decompression. Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin or Mallory's stain (8).

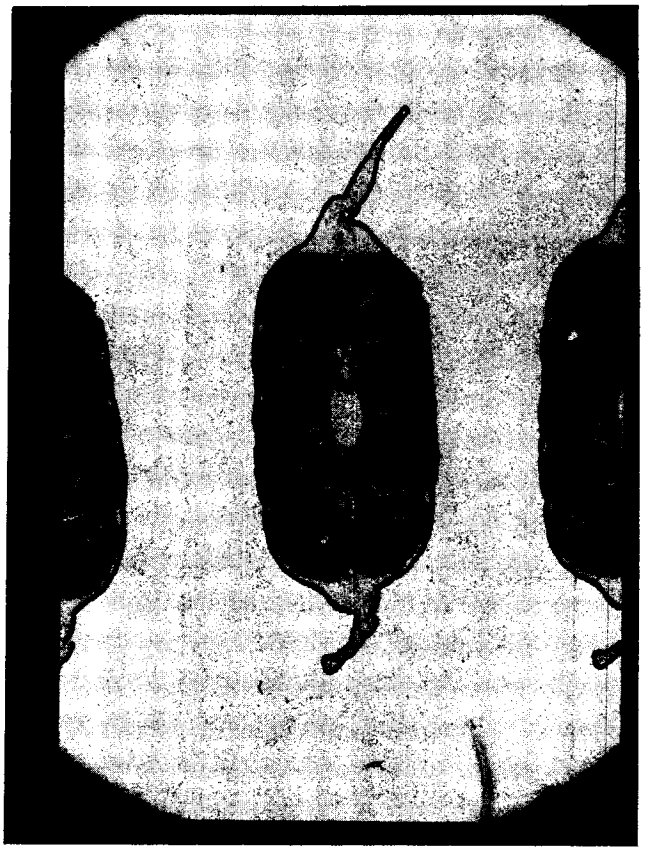

Fig. 1. Normal tadpole tail in cross section, from 8th stage. × 11.

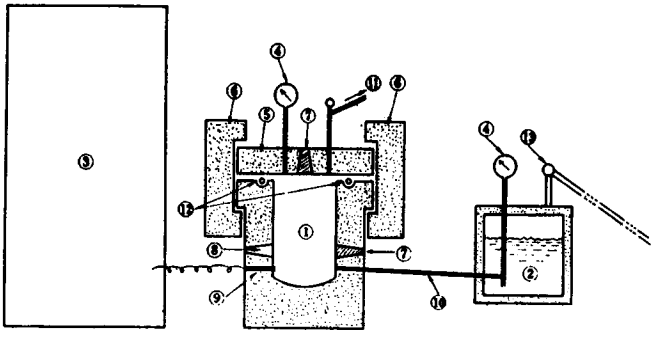

Fig. 2. Diagram of wet high pressure chamber. (1) Inside of animal chamber; (2) liquid tank; (3) multipurpose polygraph; (4) pressure gauge; (5) upper lid; (6) fixation annex; (7) observation window; (8) window for light; (9) polygraph wires; (10) liquid supply pipe; (11) drainage pipe; (12) O-ring; (13) manual compression pump.

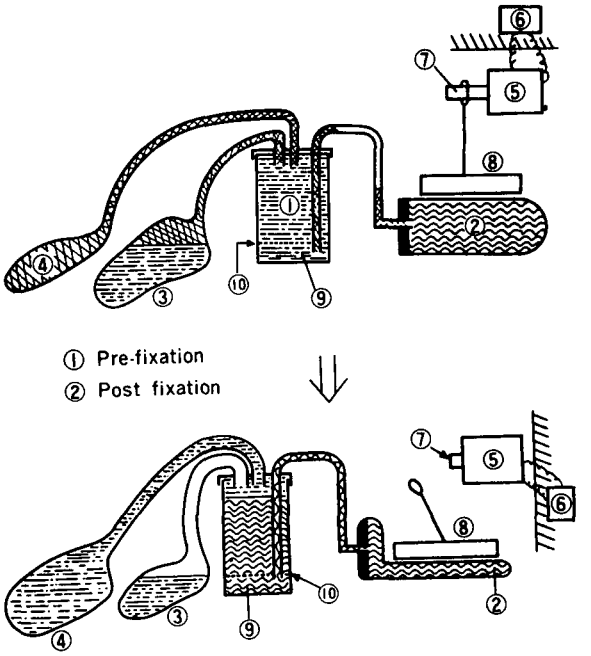

Fig. 3. Diagram of equipment developed to fix tadpoles under increased hydrostatic pressures. (1) Main cistern; (2) bag for fixation liquid; (3) bag for pressure control; (4) bag for drainage at decompression; (5) solenoid; (6) 24-V battery (outside chamber); (7) prop; (8) weight (1 kg); (9) subjects (tadpoles); (10) net to prevent tadpoles from surfacing.

Results

Results of these studies are shown in Table I. No macroscopic or histological abnormalities were found in any tadpoles exposed to hydrostatic pressures less than 20 ATA, whether the tadpoles were killed immediately or 10 days after decompression. This finding confirms earlier work at these pressures in which the exposure times were less than 10 minutes (7). Tadpoles exposed to 50 ATA for 10 minutes and killed 180 minutes after decompression demonstrated interstices between muscle fibers although these tadpoles had not displayed overt paralysis. These interstices were considered to be caused by decompression, since they were different from the ones made by histological fixation in the control group. Similar interstices, also assumed to be caused by decompression, were found in tadpoles exposed to 500 ATA for only 30 seconds and killed 120 to 180 minutes after decompression. These tadpoles were alive after decompression from 500 ATA, lying still for the first 5 minutes and beginning to swim 12 minutes after decompression was completed. Though macroscopic paralysis was not seen, muscle damage was evident on histological examination (7). Muscle fiber, nucleus and mitochondria were thinner and histochemical activity was lower than in sections from control animals (7).

Since interstices have rarely been found in tadpoles exposed to 500 ATA for 10 minutes if they were fixed or frozen within 3 minutes of completion of decompression, this provides additional evidence that such interstices were probably caused by decompression.

Paralysis was observed in tadpoles exposed to 500 ATA for at least 60 seconds. Degree of

TABLE I

MUSCLE DAMAGE IN TADPOLES EXPOSED TO HIGH HYDROSTATIC PRESSURES AND DECOMPRESSED BEFORE KILLING

Pressure, ATA	Exposure Time, min	Time between Decompression and Killing	*n*	No. of Subjects with Paralysis	No. of Subjects with Compression Effects	No. of Subjects with Decompression Effects
7	10	10 days	9	0	0	0
10	10	10 days	9	0	0	0
20	10	10 days	9	0	0	0
50	10	130–180 min	12	0	0	11
	10	Immediately	6	0	0	0
100	10	100–150 min	12	0	1	12
	10	Immediately	9	0	3	0
150	10	Immediately	6	0	2	0
200	10	75–135 min	12	0	0*	12
	10	Immediately	6	0	2	0
300	10	60–120	12	0	3*	12
	10	Immediately	6	0	5	0
310	10	Immediately	3	0	3	0
400	10	120–180 min	12	1	4*	12
	10	Immediately	6	0	6	0
500	0.5	120–180 min	12	0	1*	12
	0.5	Immediately	6	0	3	0
	1	120–180 min	6	3	0*	6
	1	Immediately	6	2	4	0
	3	1880 min	6	5	1*	6
	5	150 min	6	6	1*	6
	10	90–120 min	12	9	3*	12
	10	Immediately	9	8	9	2
	30	120 min	6	6	4*	6
	120	120 min	6	6	2*	6
Total			204	46	57	115

Compression effects are defined as rounded fibers; decompression effects are defined as muscle interstices and damage without rounded fibers. * It is difficult to observe rounded fibers in animals which are not killed immediately after decompression, because the muscle fibers have been seriously damaged.

paralysis was proportional to developmental stage, and hypodermal bleeding was sometimes seen in the abdominal region or the tail fin. The extent of paralysis was increased by a 3-minute exposure time, and apnea was noted. As the exposure time increased, paralysis became worse. However, tadpoles are more resistant to exposure than surface fish (7).

Cross sections of tail muscle examined histologically demonstrated that muscle fibers were compressed, degenerated, and rounded by these pressure profiles (Fig. 4). The frequency of this occurrence correlated with the macroscopic degree of paralysis. There was a higher incidence of muscle fiber degeneration when tadpoles were exposed to higher pressures for longer times. Such degeneration was therefore presumed to be a hydrostatic pressure effect (7).

In longitudinal sections taken from paralyzed tadpoles, disordered arrangement of muscle fiber was observed to a much greater extent than in the control group. This variation was also considered a hydrostatic pressure effect (7).

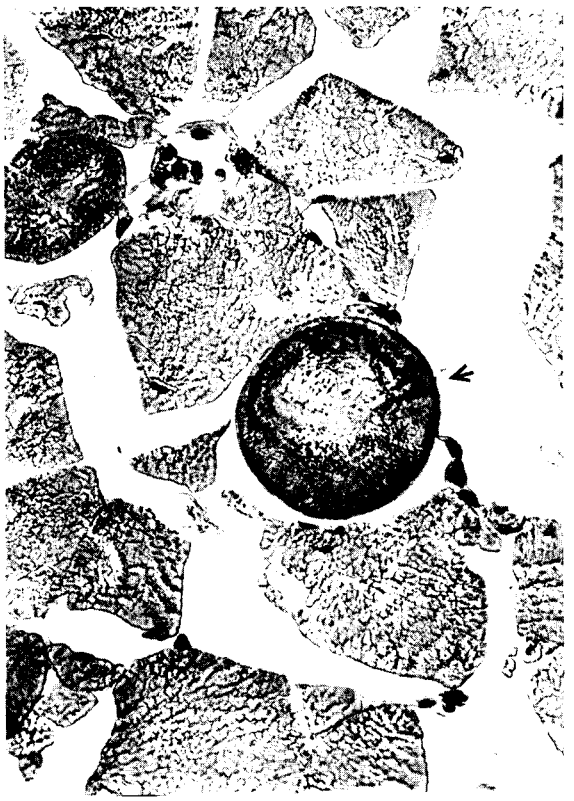

Fig. 4. Rounding of muscle fiber in tadpole tail after exposure to 100 ATA for 10 minutes (arrows). Cross section, from 8th stage. × 500.

These changes in muscle fiber were occasionally seen in tadpoles which did not display paralysis, but the frequency of occurrence was very small (Table I). Muscle fiber degeneration, defined as a change from the normal to a round shape and hereafter referred to as "rounding," was also found in low incidence in tadpoles exposed to 100 ATA for 10 minutes, a result which is considered to be caused by individual differences or variation. The incidence of rounding in muscle fibers increased with pressure exposures over 300 ATA, as discussed previously (7). Whether this rounding of fibers seen in cross section can be correlated with the disordered arrangement of muscle fiber observed in longitudinal sections is not clear. The size of rounded fibers increased when exposure conditions were severe.

Destruction of muscle was distinct in tadpoles exposed to 500 ATA for 10 minutes and killed 180 minutes after decompression; destruction was not so clearly seen in tadpoles exposed to 500 ATA for 10 minutes and killed immediately after decompression. It was concluded, therefore, that this muscle damage was caused not by compression but by decompression.

To gain more information about the rounding effect, tadpoles in the internal gill stage were selected, exposed to high pressure and decompressed, and their tissues sectioned and embedded. Muscle fibers from tails of these tadpoles were more severely damaged than muscle

fibers seen in the earlier experimental animals. The myotome appeared to have been torn, constricted and layered, forming a scar (Fig. 5). This finding supported the expectation that younger subjects would suffer greater damage (12). This result also suggested that rounded fibers were probably cross-sectional views of the degenerated muscle fiber seen in longitudinal sections.

Some unknown physical and biochemical factors changed normal muscle fibers into degenerated fibers under very high hydrostatic pressure conditions. A special process was needed that would permit the time course of development to be followed. Changes in blood vessels under high pressure can easily be observed; the author has observed blood vessel spasms in the cheek pouches of hamsters under high pressure and in the external gills of tadpoles during and after hyperbaric exposure. These effects are probably caused by spasms of the smooth muscles in the vessels.

The muscle fiber of the tadpole tail is striated rather than smooth. Though spasms have not been observed in tadpole tail, it was predicted that such spasms would occur in striated muscle under extreme hyperbaric conditions. Paralysis is considered the likely end result, since the tadpole tail has no bones and only a notochord in the center, and the tail muscle is not able to protect itself against contraction. The muscle fibers of the tail will therefore be torn, constricted, layered and built up into scars.

To prove whether or not decompression had an effect on muscle fibers, special equipment was developed which would permit tadpoles to be killed and fixed while exposed to high hydrostatic pressures. After tadpoles were exposed to 500 ATA for 10 minutes, they were placed in fixative for 5 minutes and then decompressed to sea level. Tadpoles of the same ontogenetic stage were exposed to 500 ATA for 10 minutes, decompressed, and then fixed with the same (Bouin's) solution. Both groups were compared with sea level controls.

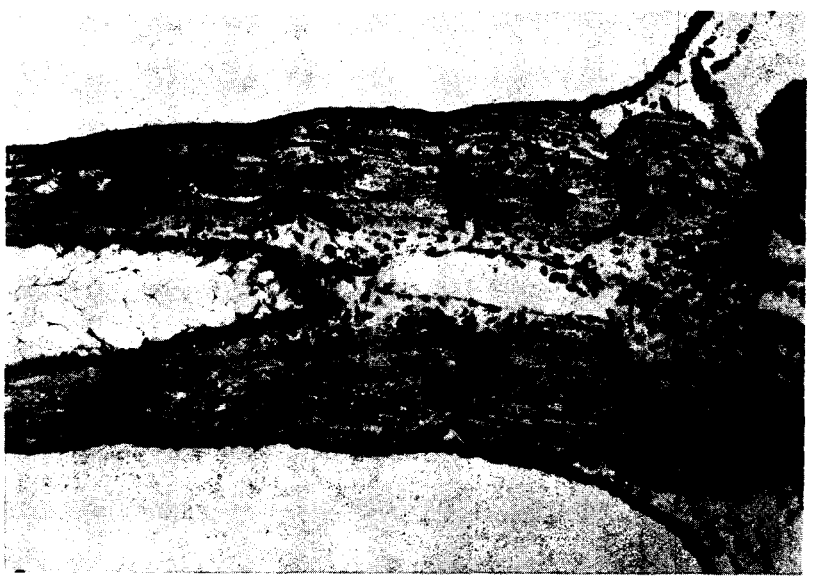

Fig. 5. Degenerated, layered fibers forming scars in muscle from tadpole tail after exposure to 500 ATA for 10 minutes. Longitudinal section, from internal gill stage. × 120.

Results indicated that tadpole tail muscle was damaged, constricted, and layered due to high hydrostatic pressure, but that some parts of the fibers retained their normal striated structure. Tadpoles killed immediately after decompression from 500 ATA exhibited even greater damage, although distinguishing between effects was difficult because the analyses were necessarily qualitative. However, all effects were more severe in younger tadpoles.

Decompression sickness has been considered to be caused by bubbles which are formed from surplus gas released from solution by inadequate decompression after diving. However, a tadpole is not a pulmonate animal, and the amount of gas in solution in its body is small. The tadpole is therefore not as likely to develop decompression sickness. Individual variation in the sensitivity of men to nitrogen narcosis is well established, and it is well known that some individuals are more prone to decompression sickness than others, even if the conditions of the exposure are the same (4, 5, 6). Tadpoles also exhibit individual variation in sensitivity to decompression effects, and in sensitivity to pressure effects. Future research should concentrate on the physical or physiological effects of hydrostatic pressure.

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INERT GAS REVERSAL OF HYDROSTATIC PRESSURE EFFECTS ON DIVIDING CELLS

A. G. Macdonald

Hydrostatic Pressure

High hydrostatic pressure imposes a special type of physiological stress. It is a broadly acting agent, but is only capable of perturbing a steady-state process if there is a reaction involving a significant molar volume change. Reactions involve activation volume changes analogous to activation energy and, if these are positive, pressure will retard the reaction rate; if they are negative, pressure will accelerate the rate. Often it is possible to regard the rate of a physiological or biochemical process as a function of a rate-determining equilibrium, in which case the visible effect of pressure may be treated as the result of a shift in the controlling equilibrium. This may be an enzyme-substrate or enzyme-ligand equilibrium, a change in ionic or nonpolar solutes, or a change in the structural components of cells.

Because pressure effects immediately attract molecular interpretations, experimenters in this area may find themselves seeking explanations of effects at a level of analysis which is likely to be more advanced than development of the subject being investigated. Such a situation is not unique to pressure physiology, but striking historical examples still dominate the literature. For example, in the 1930's, several highly competent workers in the field of high pressure neuromuscular physiology revealed a number of interesting hydrostatic pressure effects in muscle, e.g., pressure contracture, and in nerves (reversible modification of action potentials). Their line of investigation almost ceased, however, probably because the effects which they observed depended on other developments in molecular biology in general and in muscle and membrane chemistry in particular for their interpretation (3, 4). Early observations of how pressure affects muscles and nerves have yet to be interpreted, but it is now much more feasible to attempt to do so. Conversely, advances in high pressure physical chemistry make it possible that pressure will be a useful agent for the study of these systems.

During the 1930's, cell biologists were also investigating the effects of high pressure on another type of contractility, that involved in cell division, cytoplasmic streaming and in the function of motile organelles. This work produced a substantial body of knowledge which is now more a part of orthodox cell biology than neuromuscular effects of pressure are a part of neuromuscular physiology (15). High pressure cell biology continued to be investigated because a simple and general physical-chemical interpretation existed for the effects observed, and because the molecular sites of action of pressure were accessible, albeit indirectly, with the techniques available at the time. Light microscopy and, later, electron microscopy kept the cellular effects of pressure in focus over the years, and they continue to be valuable

techniques. In a recent study, birefringence measurements on pressurized cells have yielded thermodynamic data on macromolecular assembly processes of widespread significance (6).

The point of departure for this paper is the mechanism and effect of the action of high pressure on cell division. Pressure reversibly liquefies cytoplasm and reversibly inhibits the division of all eukaryotes which have so far been investigated (15). It also blocks similar forms of cytoplasmic motility. Although invertebrate eggs have been the main type of cell studied, vertebrate eggs, Protozoa and various other cells also conform to this rule. The decrease in "gel strength" (consistency) of cytoplasm is generally about 25% per 100 atm (15). The division block caused by pressure may be accounted for by the liquefaction of the cytoplasm, but other observations show that pressure also affects, to varying degrees, the metabolism of adenosine triphosphate (ATP), the permeability of the cell membrane, and numerous other cellular compartments.

Liquefaction of bulk cytoplasm by hydrostatic pressure, without any massive osmotic flux, implies a dissociation of the constituents of the cytoplasm, that is, a shift to the left in the sol $\rightleftharpoons$ gel equilibrium which is normally under physiological control of the cell. Specific components of the cytoplasm, certain microtubules and polysomes, are reversibly disaggregated by pressure. The microtubules forming the mitotic apparatus of several cells, and of invertebrate eggs in particular, exhibit a marked sensitivity to pressure both in vivo and in vitro (6, 9, 23). Microtubules and their component parts coexist in the cell in an equilibrium which is normally controlled to achieve chromosome separation during division.

Birefringence measurements provide a measure of the equilibrium constant K = (multimer/subunits), and by carrying out birefringence measurements on living cells subjected to high pressure, it has been shown that the molar volume change for the aggregation process is approximately + 400 ml/mol of polymerized subunits (6). Comparing this value with data from pure chemical reactions leads to certain conclusions regarding the mechanism by which microtubules are assembled, which are discussed below. For the present, the mitotic apparatus may be regarded as a rather pure type of colloidal structure which bulk cytoplasm resembles in its response to pressure. It is therefore reasonable to view the dividing cell, whose division is blocked by hydrostatic pressure acting on bulk cytoplasm as well as on the mitotic apparatus, as one whose motile machinery is mechanically uncoupled. Experiments with sea urchin eggs (a mitotic divider) and the ciliated protozoan *Tetrahymena* (which is amitotic) show that cells may be kept in such an inhibited state for a generation or so without undergoing irreparable damage. Thus, despite the metabolic dislocations which are doubtless slowly poisoning the cell, pressure acts as a surprisingly selective agent in blocking division.

This brief summary can be concluded with the observation that the pressure-induced liquefaction of bulk cytoplasm, the inhibition of cell division, and the disaggregation of pressure-labile ultrastructural components can be modified by other agents, of which deuterium oxide and temperature are particularly significant. It is generally the case that raising the temperature or substituting deuterium oxide for water increases the resistance of a cellular target to high pressure, and this paper will argue that inert gases do the same.

Inert Gases

Inert gases (helium, hydrogen, and nitrogen in this paper) are unable to react chemically in a physiological preparation; they do not form covalent bonds and can generally be treated as ultra-weak general anesthetics. An effective dose of an inert gas comprises a partial pressure term, implying a subsequent solubility of the molecules in a given solvent, and a purely

hydrostatic pressure term. It is well known that nitrogen, as an anesthetic, is only 5% as potent as nitrous oxide, and its effective dose involves a relatively small pressure term. Helium, which is also seen as a potential anesthetic, is so physiologically inert that its hypothetical, i.e., predicted, narcotic potency is only 1/130 that of nitrous oxide, or about 190 atm (19). When helium is applied to animals at such pressures it primarily exerts a hydrostatic pressure effect, and evidence that it actually acts like an anesthetic is both indirect and meager. Experiments which aim at separating the hydrostatic pressure and partial pressure (solubility) terms in an effective dose of inert gas are difficult to carry out with mammals (2), but in dividing cells the situation is much simpler and, as has already been discussed, the results of pressure experiments are amenable to physical-chemical interpretation. In the experiments described here, hydrostatic pressure and inert gases have been separately applied to dividing cells in such a way as to distinguish between the specific effect of the gas molecules and the action of hydrostatic pressure.

Tetrahymena pyriformis

The cells used in the experiments were *Tetrahymena pyriformis* W, a ciliated protozoan about 70 μ in length (5). It grows on a nutrient broth in sterile cultures and during the logarithmic phase of growth at 25° C (the temperature used in the experiments), it has a mean generation time of about 3½ hours. The cell contains a compound meganucleus which is unique to ciliates, and it also normally possesses a micronucleus whose role is to undergo fusion during cell conjugation. The strain of *Tetrahymena* used here lacks a micronucleus; it never undergoes conjugation but simply grows and divides into two. Division is a transverse constriction of the cell with the meganucleus simultaneously dividing in similar fashion. There is no mitosis because the genetic material is not condensed into chromosomes. The site of cleavage is morphologically well defined and appears at a specific stage in the morphogenesis of the two prospective daughter cells. The chief morphological structure which is duplicated in one of the daughter cells is the oral apparatus, which is a compound structure of fused cilia. *Tetrahymena* is a fascinating and instructive cell which is widely used by cell physiologists and is particularly useful in studies of cell division.

The effects of pressure on *Tetrahymena* have been investigated by Zimmerman using synchronous cultures and by Macdonald using asynchronous cultures in the logarithmic stage of growth (12, 13, 23). In log phase cells division is inhibited by 250 atm of pressure, which is far from being a lethal dose. Although gel strength measurements have not been carried out on *Tetrahymena* at high pressure, there is evidence to suggest that its cytoplasm is liquefied by 250 atm, and other evidence demonstrates that microtubules in the cell cortex are pressure-labile. There is also evidence that while pressure apparently inhibits cell division by direct liquefaction, metabolic disturbances are also taking place in the cell cycle.

Tetrahymena's division is also sensitive to anesthetics; it can be reversibly blocked by clinical doses of halothane and chloroform, and also by an appropriate dose of n-propane (7) (Table I).

EXPERIMENTS WITH INERT GASES

Cells in the logarithmic phase of growth were mounted in hanging drop cultures on the surface of a plastic window fitted to a high pressure vessel. Division was thus observed at high pressure by direct medium-power light microscopy (see reference 14 for details). Inert

TABLE I

HYDROSTATIC PRESSURE AND PARTIAL PRESSURES OF INERT GASES AND ANESTHETICS WHICH INFLUENCE THE DIVISION OF *Tetrahymena pyriformis* AT 25° C

	Subeffective Dose, atm		Effective Dose, atm		Reference
Halothane	0.001	No effect alone but combines with 100 atm hydrostatic pressure to inhibit division, i.e., additive interaction	0.01	Inhibitory alone; counteracted by 100 atm hydrostatic pressure, i.e., antagonistic interaction	(7)
Chloroform	0.001		0.005		(7)
n-Propane	0.25		0.75		(7)
Nitrogen	30		60		(8)
Hydrostatic Pressure	100	Indistinguishable from 1 atm	140–325	Inhibitory	(12, 14)
			400	Lysis	(this paper)
Nitrogen	100	Indistinguishable from hydrostatic pressure	175–250	Counteracts inhibition and lysis caused by hydrostatic pressure	(this paper)
Hydrogen	<130		130–400		(14)
Helium	<175		175–250		(14)

gas compression or hydraulic compression introduces minor differences in culture conditions, and these were carefully investigated. A full discussion of the validity of the comparison between growth rates under gas and under hydraulic conditions is found in reference 14, and this paper proceeds from the conclusions reached in that discussion.

Figure 1 shows that a pressure of 100 atm, whether applied hydraulically or with helium or hydrogen, exerts no effect on the rate of cell division, while at 250 atm the inert gases inhibit division to a much lesser extent than hydrostatic pressure does. Division in hydrogen is more rapid than in helium. At pressures in excess of 325 atm, hydrostatic pressure causes the cells to lyse (Fig. 2), while hydrogen protects the cells.

Nitrogen at 250 atm caused an initial inhibition of division which was followed by a marked recovery (Fig. 3*B*). Nitrogen (175 atm) counteracted hydrostatic pressure to an extent similar to helium (compare Fig. 3*A* in this paper with Fig. 4*A* in reference 14). Nitrogen at 100 atm yielded a rate of division similar to that found with a hydrostatic pressure of 100 atm.

The recovery of cell division rates in helium at 325 atm and in nitrogen at 250 atm is interesting; it is also apparent in experiments in which hydrostatically compressed cells are exposed to nitrogen pressure by a method which involves no significant change in total pressure (Fig. 4*A*). Previously published studies of this type report negligible lag after similar injection of hydrogen gas (14). Figure 4*B* shows the reverse experiment: cells beginning to divide in nitrogen at 250 atm are inhibited when the gas at constant high pressure is removed by switching to purely hydrostatic pressure. Figure 4*C* shows the same phenomenon in cells dividing in hydrogen. There is negligible delay before the onset of inhibition (14).

Nitrogen experiments were carried out at the same time as other experiments with helium and hydrogen, previously designated series B(14). Series B experiments showed that nitro-

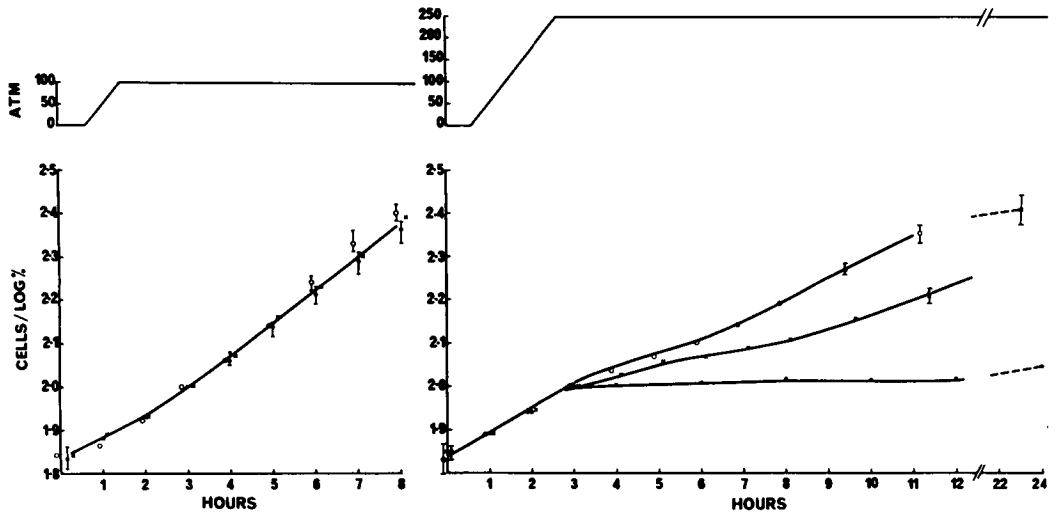

Fig. 1. Rate of cell division of *Tetrahymena* in selected pressures of inert gases. *A* (*left panel*), 1 or 100 atm; *B* (*right panel*), 250 atm; open circles = H_2; × = He; filled circles = hydrostatic pressure. Cell numbers (means of 4 experiments) are normalized at 3 h and logarithm of relative cell number is plotted on *ordinate*. Time course of compression is indicated in the upper graphs.

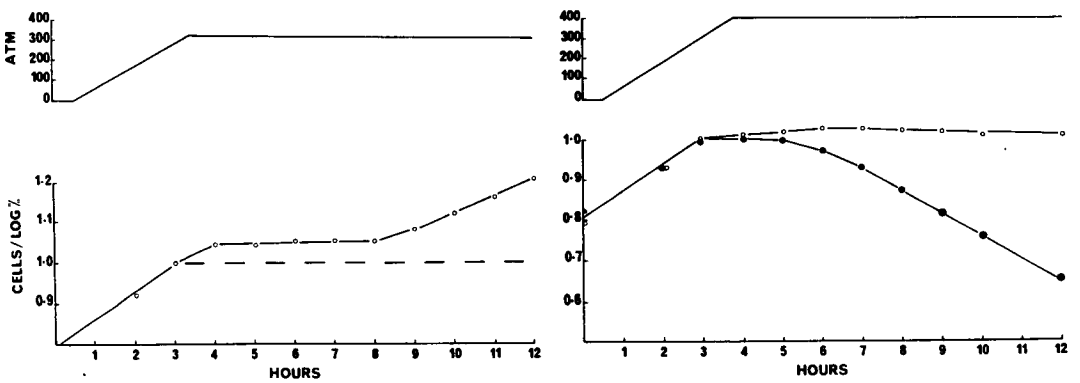

Fig. 2. Rate of cell division of *Tetrahymena* in hydrogen. *A* (*left panel*), 325 atm; *B* (*right panel*), 400 atm; open circles = H_2; closed circles = hydrostatic pressure. Cell numbers (means of 4 experiments) are normalized and logarithm of relative cell number is plotted on *ordinate*. In *A*, dashed line indicates absence of division in 325 atm hydrostatic pressure.

gen at 100 atm exerted no effect, which appears to contradict an earlier finding that it caused a marked delay in *Tetrahymena's* division (8). All likely explanations for this inconsistency have been investigated, leading to the conclusion that *Tetrahymena* is able to change its sensitivity to inert gases in large part independently of any change in cell division rate under normal control conditions. In the present type of experiment it appears that this base line is not completely satisfactory.

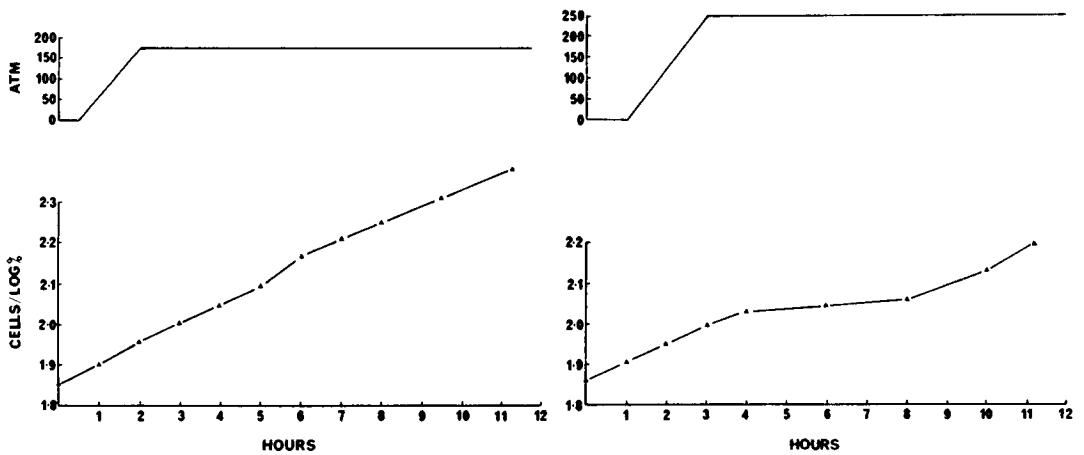

Fig. 3. Rate of cell division of *Tetrahymena* in nitrogen. *A* (*left panel*), 175 atm; *B* (*right panel*), 250 atm. Details as in Fig. 1.

Two additional pieces of evidence support this interpretation of the change in sensitivity to nitrogen. It has been demonstrated that *Tetrahymena's* sensitivity to helium shifted slightly between series A and series B experiments, which were carried out a year apart. In the series B experiments, *Tetrahymena* divided slightly more rapidly in helium at 250 atm than it did in series A. Furthermore, series B cells appeared to grow at a marginally faster rate under control conditions, and also showed a little more resistance to hydrostatic pressure at 250 atm than the series A cells did (compare Figs. 4*B* and 4*C* in reference 14). The change in the cells was probably caused by some change in growth conditions, even though media preparation and all other procedures were carefully standardized in this work. There are perhaps differences between cells in the early logarithmic phase of growth and those in a later stage of the same phase. Growth in the early part of the logarithmic growth phase may yield cells with somewhat variable substrate levels or with differences in the specific precursors required for division, which do not exhibit marked changes in division rate under control conditions. Whatever the causes of the variation in results obtained with *Tetrahymena*, these results have considerable interest. The prospect of manipulating *Tetrahymena's* susceptibility to inert gases and pressure, perhaps by specific nutritional means, exists.

Other Criteria

Cell division has been the criterion used quantitatively in these experiments, but two other features of the cells were visibly affected. First, the rate of ciliary swimming was reduced by hydrostatic pressure and, to a far lesser extent, by similar pressures of inert gases, particularly hydrogen. Second, cells exposed to high pressures of hydrogen became misshapen, almost ameboid, and appeared to possess an excessively large surface area for the volume of contained cytoplasm. In general, cells which exhibited a normal rate of division had a relatively normal swimming speed and appearance.

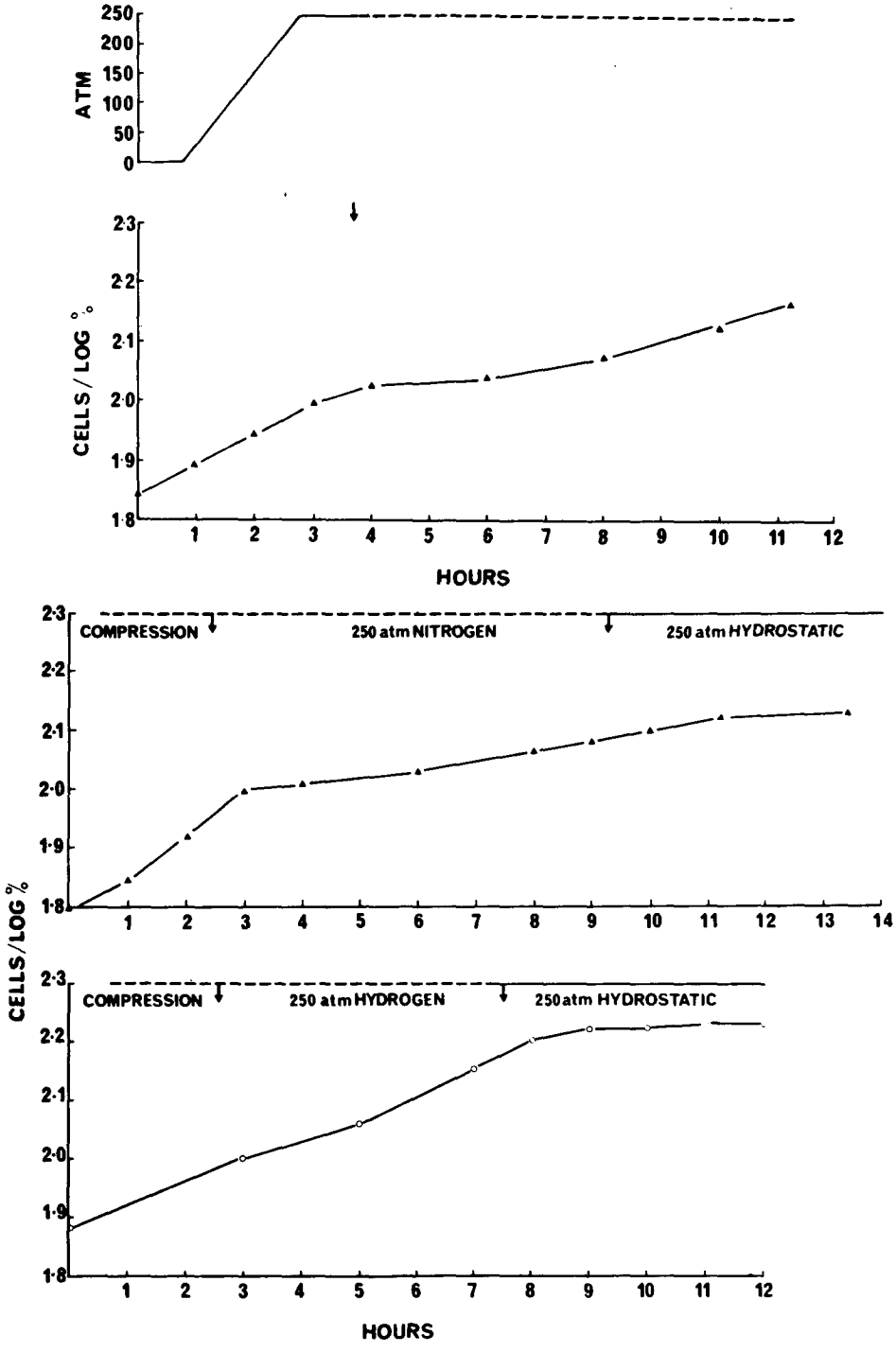

Fig. 4. Reversible effects of hydrostatic pressure and inert gases on rate of cell division in *Tetrahymena*. *A* (*upper panel*), high hydrostatic pressure is changed to nitrogen pressure at arrow. *B* (*center panel*), 250 atm N_2 pressure is changed to the same hydrostatic pressure (arrow). *C* (*lower panel*), 250 atm H_2 pressure is changed to the same hydrostatic pressure (arrow). Details as in Fig. 1.

Conclusions

Helium, hydrogen, and nitrogen counteract the deleterious effects of hydrostatic pressure in a readily reversible manner. More detailed data show that helium pressure is distinguishable from hydrostatic pressure at 175 atm, while the specific effect of hydrogen is detectable at 130 atm. These data correspond to the predicted narcotic dose for helium and that directly determined for hydrogen in mice in Brauer's work (2, 19). The results for nitrogen, hitherto unpublished, are more complicated. In view of the delayed effect of nitrogen at 250 atm, and the drift in *Tetrahymena's* sensitivity to it, no threshold pressure for a specific nitrogen effect can be selected.

It was noted that in cells in which division proceeded at an intermediate rate in the presence of an inert gas, the cleavage process took a nearly normal length of time and constricted the cell in a normal fashion. Thus the preparation for division, and not the contractile process itself, is affected. The sensitive component in the preparation may be the organization of the contractile machinery, or it may be more remote from the division process and closer to the cell's intermediate metabolism. The lag in division during exposure to nitrogen at 250 atm suggests additional metabolic or preparatory complications.

The results strongly suggest that multiple pressure-labile targets are in some way stabilized by the gases, although the possibility of a single, pressure-labile target being stabilized cannot be ruled out. The stabilizing effect of inert gas molecules acting on T, a pressure-labile molecular target whose normal functioning is necessary for cell division or any other activity, may be formally described in two equilibria.

$$\mathrm{Tp} \underset{-\Delta V}{\overset{+\Delta V}{\rightleftharpoons}} \mathrm{T} \qquad \frac{\mathrm{T}}{\mathrm{Tp}} = \mathrm{K}_1 \tag{1}$$

$$\mathrm{G} + \mathrm{Tp} \underset{-\Delta V}{\overset{+\Delta V}{\rightleftharpoons}} \mathrm{TpG} \qquad \frac{\mathrm{TpG}}{\mathrm{GTp}} = \mathrm{K}_2 \tag{2}$$

where Tp is the pressurized target whose function is impaired, G stands for inert gas, and TpG an inert gas-pressurized target molecular complex. TpG and T are functionally indistinguishable, and K_1 and K_2 determine the rate of division in hydrostatic pressure and gas pressure, respectively. ΔV indicates the molar volume change involved in the processes.

The nature of the interaction between an inert gas and pressure-labile target molecules is of wide significance. In view of the physical properties of the inert gases, we may assume they do not act by forming covalent bonds nor by forming clathrates. Their most probable mode of action is by way of hydrophobic interaction. There is, in fact, good evidence that clinical anesthetics and the physiologically potent inert gases interact with specific target molecules in a hydrophobic manner (1, 18, 21, 22). Nitrogen is merely a weak anesthetic gas, and hydrogen behaves physiologically as an ultra-weak anesthetic, so the only novel postulate here is that helium also acts as an ultra-weak anesthetic gas. This argument is strengthened by the good correlation between the partial pressures at which specific effects of helium and hydrogen appear in *Tetrahymena*, and their hypothetical (for helium) and demonstrated (for hydrogen) partial pressures which elicit narcotic effects in mice.

How might hydrophobically interacting molecules oppose a pressure-induced change in a target molecule or molecular aggregate? Two plausible possibilities are considered here. If the

pressure-labile target is a large protein with a hydrophobic interior or phase, such as the lipid part of a cell membrane, pressure may condense the system, increasing its orderliness, viscosity or rigidity, and thereby altering its function in some way. The dissolution of inert gases into the hydrophobic core of a protein or membrane would tend to dilate it, render it more fluid, and thus restore its normal function (Fig. 5*A*). Several workers have considered this as the basis for the reversal of anesthetic effects by hydrostatic pressure (16, 20). It is conceivable that by titrating inert gas partial pressure against increased hydrostatic pressure a functional state of the hypothetical target could be maintained, as manifest in the present experiments by a nearly normal rate of cell division. It is worth mentioning that while there is evidence to indicate that hydrostatic pressure renders artificial membranes more dense

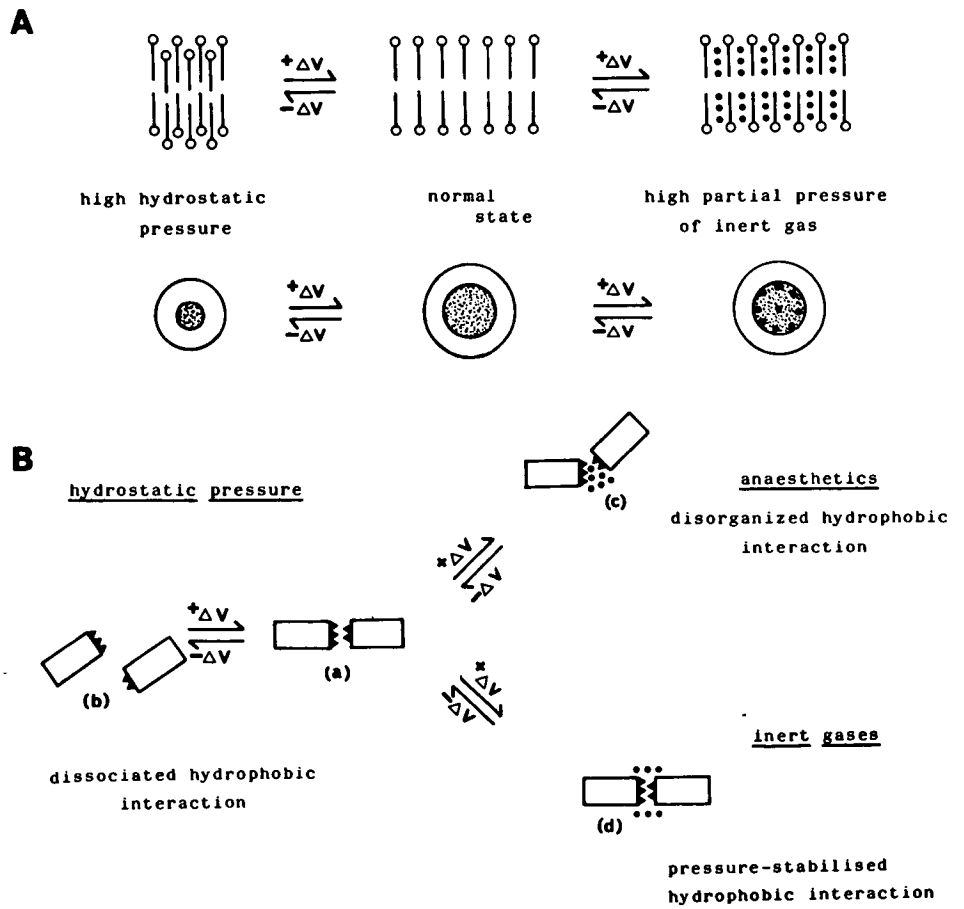

Fig. 5. Speculative interactions between inert gases and pressure-labile targets. *A* (*upper row of upper panel*), idealized lipid bilayer; *lower row,* protein with hydrophobic core. Normal functional state is shown in center column; left column shows nonfunctional, pressurized states; right column shows functional states achieved by presence of inert gas molecules shown as dots (16, 20). *B* (*lower panel*), functional state of some critical hydrophobic interaction is shown at (a); dissociated, pressurized state shown at (b). Anesthetics disrupt hydrophobic interaction (21, 22) at (c), but high partial pressures of inert gases stabilize it against hydrostatic pressure at (d). In all equilibria depicted, molar volume changes are indicated, ± ΔV, but ambient water molecules, whose differential ordering around the apolar groups primarily determines volume changes, are not shown.

and rigid, there is not yet any evidence to show that it exerts a physiological effect in this way. There is evidence that clinical anesthetics dissolve in red cell membranes, causing some lateral expansion, and that *Tetrahymena's* appearance in high pressures of hydrogen could be accounted for by the same phenomenon.

There is a second possibility. If the pressure-labile molecular target is a structural cytoplasmic polymer or multimeric enzyme in which pressure acts to dissociate hydrophobic interactions, then inert gases will have to stabilize the interaction to antagonize the effect of pressure. Hydrophobic interactions contribute to the normal conformation of many proteins, and a number of cases have been reported in which hydrostatic pressure acts fairly specifically to dissociate or hydrate such hydrophobic interactions (6, 17). It seems reasonable to postulate that hydrophobic interactions might be reinforced by the adsorption, or some other effect, of apolar, inert gas molecules.

Consider the simple hypothetical case of the polymerization of subunits, as in poly-L-valyl ribonuclease, or perhaps even in pressure-labile microtubules. The positive entropy, enthalpy, and molar volume changes which occur when the pressure-sensitive equilibrium shifts to the associated state in this type of hydrophobic interaction is due primarily to the release of ordered water around previously exposed apolar sites (Fig. 5*B*). Apolar molecules, such as inert gases, will tend to adsorb at hydrophobic sites, and might conceivably enhance their hydrophobicity and stability at high pressure either directly or by solvent perturbation (11) (Fig. 5*B*). Hydrogen should be more effective than helium since it is more soluble in hydrophobic solvents, and this is borne out in the results reported here. Nitrogen should be more effective than hydrogen, but the results show that this is not always the case. There seem to be complications with nitrogen which the simple treatment adopted here cannot cope with.

Although no chemical precedents for the stabilization of hydrophobic interactions by apolar solutes are known to the author, this would be the simplest mechanism which would account for division proceeding at pressures which liquefy cytoplasm. There are two antagonists of pressure whose mode of action is relevant here. The dividing sea urchin egg and the pressure-labile microtubules of the mitotic apparatus within it are rendered more resistant to the liquefying (depolymerizing) action of pressure by replacing water with deuterium oxide (10, 15). Although it is not entirely clear how deuterium oxide acts, one possibility is that it strengthens hydrophobic interactions. In dividing cells (15), raising the temperature opposes the liquefying effect of pressure on both bulk cytoplasm and pressure-labile microtubules. While an increase in temperature will exert numerous effects in cells, it is generally agreed that hydrophobic interactions in specific molecules are strengthened by a temperature increase and may well stabilize pressure-labile targets.

In summary, inert gases counteract the effects of hydrostatic pressure in dividing cells, probably by mechanisms involving the fluidizing or expansion of a bulk, compressed, hydrophobic phase and by stabilizing pressure-labile hydrophobic interactions by adsorption or a longer range phenomenon. An understanding of the role of hydrophobic interactions in cellular colloids has been arrived at largely by thermodynamic reasoning. Anesthetics are potent hydrophobic probes, and it is significant that they are also capable of disrupting pressure-labile microtubules, as well as reversibly inhibiting the division of the whole cell (1, 7). Figure 5*B* shows how an effective dose of anesthetic molecules might disrupt a pressure-labile, hydrophobically bonded polymer at atmospheric pressure, and this effect itself should, on general grounds, be antagonized by pressure.

With the easily studied but obviously complicated process of cell division in *Tetrahymena*,

it has been shown that hydrostatic pressure at 100 atm does indeed offset the inhibitory effects of anesthetics (7). Table I summarizes the effects which both anesthetics and inert gases have on *Tetrahymena's* division. Inhibitory clinical doses of the potent agents are antagonized by 100 atm but, mysteriously, subeffective doses are enhanced by pressure. The intermediate case of nitrogen has produced, in apparently sensitive cells, inhibitory effects which were antagonized by pressure at 100 atm. In less sensitive cells, nitrogen only counteracted the effects of hydrostatic pressure. The very weak agents, helium and hydrogen, only exert their physiological effect by counteracting hydrostatic pressure. The data in Table I may be broadly accounted for by the equilibria and hypotheses proposed in this paper. It is also of interest that these views (Fig. 5) may also apply to the way in which anesthetics and inert gases counteract some of the disturbances seen in the central nervous system of mammals subjected to high hydrostatic pressure.

ACKNOWLEDGMENT

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PART V. CELLULAR AND PHYSIOLOGICAL EFFECTS OF HYDROSTATIC PRESSURE

DISCUSSION

C. E. G. Lundgren, Chairman

Dr. Bennett: Dr. Tobey, could you comment on the temperature control in your studies? Knowing of the thermal problems with helium and of the data in the paper by Dr. Cromer, was body temperature measured and was there any attempt to keep chamber temperature constant in your experiments?

Dr. Tobey: We monitored the rectal temperature in the first experiment continuously in all of the animals and maintained their body temperature between 36.5 and 37.5° C by controlling the environmental temperature in the chamber. We found that by keeping the environmental chamber at about 37 to 38° C, we were able to keep a very precise level of body temperature in the animals, and so we did not monitor them in the second series of experiments.

Dr. Winter: I would like to ask Dr. Tobey a question, but first I'd like to say that Dr. Ray Smith and I have done work which very much confirms what you have presented. We used a longer-acting barbiturate in a different species and at higher pressures, but we got very similar results. We got an increase of approximately 40% in ED_{50} for the phenobarbitol requirement in mice at 100 atmospheres. However, one of your results surprised me greatly. You stated that the substitution of helium for nitrogen at 1 atmosphere cut your sleep time in half. Now I presume that your hypothesis for the general effect you described is based on an assumption of an alteration in critical volume. How does that fit in with the change in sleep time at 1 atmosphere achieved simply by switching gases?

Dr. Tobey: I don't have an explanation for that. Do you?

Dr. Winter: No. I'm very surprised by it, frankly. I would not have expected it.

Dr. Tobey: We did not confirm the results of Experiment I in our second experiment testing the effectiveness of ED_{50} doses of thiopental at 1 atmosphere in helium-oxygen. However, we used the chi square test for the data of our second experiment, and our statistical sensitivity was not as good as in the first experiment. I don't know whether the first experimental result is just happenstance and not true, or whether the second result is not true.

Dr. Kiesow: I wanted to ask Dr. Macdonald if he could fill another gap in my limited knowledge of protozoology. Is *Tetrahymena* an anaerobic organism?

Dr. Macdonald: No, biochemists tell me it is just like mammalian liver cells.

Dr. Kiesow: Then it does require oxygen for growth?

Dr. Macdonald: Yes.

Dr. Kiesow: What did you do then about incorporating oxygen in your experimental environments?

Dr. Macdonald: The liquid paraffin was equilibrated with air. The gas experiments started with air filling the pressure vessel, so essentially, the P_{O_2} was normal.

Dr. Kiesow: During exposure to all pressures?

Dr. Macdonald: Yes.

Dr. Hong: Dr. Akers, did you measure urine flow?

Dr. Akers: No, we didn't because we had a combined urine sample from 12 rats, but we did assess the volume and it was considerably increased, two- to three-fold in a 50-hour period.

Dr. Hong: You used combined urine samples, and analysis of these samples indicated an increase in excretion of ketone bodies.

Dr. Akers: Yes; it was so strong that the minute you pulled the sample out you knew there were ketones present.

Dr. Hong: In your case, the reduction in body weight amounted to 10% of the initial body weight, due to elimination of body fats and water secondary to the elimination of the ketone bodies.

Dr. Akers: The most notable finding was the absence of aggregates of adipose tissue in the bodies of animals on autopsy, and we strongly suspect that what accounts for this 10 to 20% weight loss within the first 24 to 48 hours was a mobilization of all this adipose tissue plus water loss.

Dr. Hong: Was diuresis maintained throughout the entire experiment, or was it confined to the first three days, in which you observed a marked reduction in body weight? After the first three days, body weight seemed to increase again, didn't it?

Dr. Akers: In the experiments that Dr. Boelkins ran for 84 days, diuresis continued throughout, as did lag in body weight and increase in water consumption. Our rat experiments were only 100 hours, as were the ones on the guinea pigs; I didn't get adequate data on the urine to follow that.

Dr. Walder: I have a comment to make on Dr. Macdonald's paper. It seems to me that there is a difference between your hydraulic compression experiments and your gas compression experiments in that in the one you have a hanging drop in a liquid phase, and in the other you have a hanging drop in a gas phase. Why didn't you saturate your liquid phase, which I think you said was medicinal paraffin, with helium? There is the possible objection that metabolites might not be able to escape so readily into a liquid phase as they can into a gas phase.

Dr. Macdonald: But I did precisely that.

Dr. Walder: But you made no mention of the fact that the liquid phase was saturated with the helium before you introduced it.

Dr. Macdonald: No; I'm referring to the situation where the cells are pressurized in liquid paraffin and then gas is injected.

Dr. Walder: But the liquid paraffin is not saturated with the gas.

Dr. Macdonald: It is within a few milliseconds.

Dr. Walder: I doubt that saturation is possible in that time span.

Dr. Macdonald: You are dealing here with a hanging drop which is 1 mm in diameter and about a fraction of a millimeter deep. It is covered with a film of paraffin with a gas bubble beneath it. I think it is self-evident that it will equilibrate very rapidly. Furthermore, in the first series of experiments, I used 25-ml volumes of cell suspensions containing the growing cells. These were equilibrated with various gases in a sort of "monster setup," and I compared these to the same volume of suspensions hydraulically compressed in glass syringes. The results fit the general interpretation I'm offering.

Dr. Brauer: I have two comments, one of which is directed to Dr. Tobey. I am not quite so surprised about the 1-atmosphere helium effect; if you look at metabolic rates in mice, replacing air with helium even at temperatures on the order of 28 or so degrees ambient, you do get a fairly sizable increase in metabolic rate, and then as you go up to higher pressures this continues to increase rapidly. I think your figures sound quite compatible with the metabolic rate figures we have on the smaller animals. Perhaps oxygen uptake data might help to clarify your observations. If you have any data, they would be interesting.

My second comment is directed to Dr. Mano, and it is really more an expression of delight to see a terribly important type of experimentation begun. I am particularly interested in the beginning you are making, of collecting data concerning morphologic changes associated with decompression in hydraulic media. I hope that series will continue, because I think it is a very important line of inquiry.

Dr. Miller: I have a question for Dr. Macdonald. Sears and Gittelson reported narcosis studies of paramecium in 1961, similar to those you performed in *Tetrahymena*. They studied the effects of high hydrostatic pressures of inert gases on activity and morphology, and found that at very high pressures of xenon, the paramecia stopped swimming, rounded up and, as I recall, contractile vacuole activity stopped. They postulated that this effect was caused by a change in the volume of the membrane which inhibited activity of the animals. Do you think such sol gel changes, rather than a change in volume, may account for the changes you have observed?

Dr. Macdonald: I don't know where to begin with that one. The sol gel equilibrium shift I think you are referring to will necessarily involve a molar volume change. Otherwise, it would not happen with change in pressure. So, I don't think I can distinguish between the two parts of your question; they are one and the same.

Dr. Miller: So you are saying there could be a sol gel transformation as a reflection of change in volume?

Dr. Macdonald: Yes. More conventionally, we say there is a sol gel change due to the effect of pressure and the molar volume change.

Dr. Miller: Did you see any indications of rounding up of the cells, loss of motion, or anything that would indicate sol gel change?

Dr. Macdonald: Yes. It is fairly well documented even with these *Tetrahymena* which have a complex cortical structure to their cell. You do get liquefaction, liquefying change in the cytoplasm; the changes you see in *Tetrahymena* are quite consistent with this. The most striking morphological change I saw was in the hydrogen experiments in which the cells began to look rather ameboid, as if their cell membranes were excessively large for the amount of cytoplasm they contained. This is very suggestive of membrane expansion. I don't think it has anything directly to do with their ability to divide, which is essentially a contractile process.

Dr. E. B. Smith: I was very struck with Dr. Macdonald's growth rate results, which show a remarkable parallel with studies on amphibians at comparable pressures. There is one experiment that I think would tend to confirm the parallel. Thus, if you took a 20% nitrogen, 80% helium mixture you should on the whole do rather well (perhaps as well as with hydrogen) in maintaining the growth rate. I wondered if you have the facilities and had thought of doing mixture experiments?

Dr. Macdonald: I did start some experiments with mixtures, but using the pure nitrogen gas gave unexpected results.

Dr. E. B. Smith: Not at all; I think because of the narcotic effect, you wouldn't do as well, because you would have tremendous inhibition. What I think you have to show is that if you dilute the nitrogen to the appropriate value you could do as well as with pure hydrogen.

Dr. Macdonald: Yes. You're suggesting making a synthetic hydrogen.

Dr. E. B. Smith: Clearly, with pure nitrogen the results are worse than with pure hydrogen. Our argument would be that there is a balance between a hydrostatic effect and the dissolution of the gas. With helium the balance is way over toward hydrostatic effects, while with hydrogen it is fairly neutral. With nitrogen you are dealing with narcotic effects. If our hypothesis is right, or if the parallel is to be maintained with amphibians, a 20% nitrogen, 80% helium mixture should do nearly as well as hydrogen.

Dr. Van Liew: I would like to make a reckless attempt at synthesizing between two papers. Perhaps Dr. Akers and Dr. Macdonald would like to comment when I'm finished. Disturbances of cell division in a whole animal hit on the cells that are most rapidly dividing, the gastrointestinal cells and the blood cells. Perhaps in our exposures of whole animals to high pressure we should be looking for the kinds of changes which are seen in radiation sickness and also in vitamin deficiencies.

Dr. Akers: Some of the changes in the blood compartments are fluid shifts, and not production of additional red cells to account for the hemoconcentration. But I do agree, on the basis of earlier studies in our lab, that the effect of increasing pressure on transmitter receptor phenomena indicates that the membrane is changing and we should expect all sorts of transport problems.

Dr. Balldin: It has been suggested, Dr. Tobey, that barbiturate potentiates oxygen toxicity during hyperbaric oxygenation. Have you any comment on this as it relates to the breathing of high oxygen concentrations in helium-oxygen mixtures and thiopental anesthesia?

Dr. Tobey: No, I was not aware of that suggestion. Our oxygen concentrations were not that high, since they were just 0.24 to 0.26 atmospheres, which is a surface equivalent of about 25%. It is difficult for me to associate the rather striking changes that we found in the thiopental effect with this minimal increase in oxygen partial pressure.

Dr. Ross: Dr. Akers, did you separate your animals or were they allowed to huddle?

Dr. Akers: The guinea pigs were each in a separate cage, so they were isolated animals. The rats in the long experiment were caged in pairs, and the rats in the short term were aggregated. We have repeated part of this with single, isolated rats, and we get the same kind of results.

Dr. Ross: We have done some work in our lab at 2° C in normal air at 1 ATA and we got almost exactly the same diuresis, increase in food consumption, and weight loss, and some of the changes in the lung. We then took a similar group of animals and placed them in normoxic helium at 7 ATA and we got a slight increase over the effect of cold alone. My comment is, could it not be that much of the effect you're seeing is from the cold temperature, or the cold temperature effect of the helium?

Dr. Akers: We adjusted our temperature up to 35° C which, based on previous experiments and the literature, is supposed to be a thermoneutral level for these animals at 20 atmospheres of helium. I suspect what we have here is thermal stress, pressure stress and an aggregation stress all acting on these animals.

Dr. Lundgren: In this connection, Dr. Akers, were these thermoneutral levels of 34 or 35° C that have been determined in other experiments made on adult animals?

Dr. Akers: Yes, we used adult rats.

Dr. Lundgren: In principle, we have reason to suspect that thermoneutral conditions for small growing animals would be higher than for adult animals, because of the disadvantage of the younger animal in terms of the relationship between body surface and body mass.

Dr. Akers: That is true, but when we observed our animals' behavior in these atmospheres, they appeared perfectly comfortable. When we boost them to 40 atmospheres, however, they curl up and pilo-erect. I suspect we don't have the right temperature, but we may also have other problems at 40 atmospheres. At 20 atmospheres, however, the animals appear to be perfectly at home.

Dr. Lundgren: Dr. Mano, have you had the opportunity to observe any signs of reversibility in the effects of

high pressure that you saw on your tadpoles? I'm asking because of some observations I once made in an adult frog. It was in connection with testing a pressure chamber, and I was amazed to see this frog, which was compressed hydraulically in water, go into very severe contracture, pressure contracture, at something like 350 atmospheres. We were of course convinced that it was dead, but after taking it out (after very rapid decompression, incidentally) and bending its legs a little, to our surprise we saw it behave quite as a normal frog. I would like to have your view on this return of function, in view of the damage to your tadpole tails.

Dr. Mano: Paralysis is caused by the degree of pressure; at first the muscle fibers stiffen under high pressure, and then they tear. After decompression, the fibers return to a flexible state, but the bent tail is a sign that the animal is dead. Some of the more mature tadpoles do show reversibility of pressure effects, but there are large individual differences in response to pressure among tadpoles.

Part VI. DETECTION AND PREVENTION OF BONE NECROSIS

ASEPTIC BONE NECROSIS IN JAPANESE NAVY DIVERS

H. Ohiwa and A. Itoh

Many authors have reported that bone lesions are associated with inadequate decompression (2, 12, 17) and that dysbaric osteonecrosis should be classified as an occupational disease.

In Japan, studies on bone necrosis were done in connection with research in orthopedic and occupational medicine (6, 11, 13). The studies of Kinoshita (7, 8, 9, 10) reported on a group of about 400 divers, who spent ten out of every 15 days from April to November diving for shell fish in the southern Pacific Ocean, for a period of nine years. These divers worked continuously below 35 meters, and evidenced an incidence of decompression sickness as high as 70%.

The incidence of decompression sickness was directly related to the effects of the piecework system under which these divers worked. This system made them reluctant to spend time on the surface or to report symptoms of minor bends. Of the 400 divers followed in Kinoshita's work, 138 suffered disabling compensable injuries and received Workmen's Compensation. Sixty percent of the 400 divers had radiographic evidence of osteonecrosis, including 16 with osteoarthritis.

In 1968, Ohta (14, 15) performed radiographic surveys on 301 shellfish divers, and found bone lesions in 152 (50.5%) of this group. Those with type A lesions (juxta-articular) constituted 11.3% of this group, while 35.9% had type B lesions (head, neck, or shaft). Asahi, Ohiwa, and Nashimoto (1) also reported that 16 of 79 divers (19%) from Ko-zu Island had radiographic evidence of bone lesions, but were otherwise asymptomatic.

These early studies made the correlation between working conditions and incidence of bone necrosis in healthy, asymptomatic divers clear. For example, Elliott (3) reports a bone lesion incidence of less than 6% among Royal Navy divers. The following points have been well established in the literature:

(1) The majority of divers with bone lesions had more than one such lesion;
(2) Type B (head, neck, or shaft) lesions, and occasionally, type A (juxta-articular) lesions are found among divers who have never suffered clinical bends;
(3) Pain or neurological symptoms are not necessarily associated with the site of the lesion;

(4) Type A lesions may have been incorrectly diagnosed as type B lesions because of faulty radiographic interpretation; and
(5) The incidence of the disease may be higher than originally believed, if questionable cases are included in the incidence figures.

This study will discuss the results of examination conducted over the last six years, involving a total of 95 divers who are associated with the Yokosuka Naval District and the MSDF Diving School at Edajima.

Radiographic Examination

Over a six-year period (1969–1975), antero-posterior radiograms of the shoulder, elbow, hip, and knee joints were taken every six months. Bone lesions were classified in accordance with Ohta's classification scheme (Table I).

At the first examination, 50 out of the total of 95 divers had radiographic evidence of bone damage. Whether this damage was diving-related or not could not be established with certainty, so follow-up examinations were made.

Results

After six years of radiographic observation, 13 of the divers were classified as positive (Table II). No significant correlation could be made with type of diving, age, years of diving, number of diving hours, or experience with overt decompression sickness (Tables III, IV, V). However, divers who had spent the most time diving did have a high incidence of positive cases (Table VI).

TABLE I

CLASSIFICATION OF BONE LESIONS IN DIVERS

Type A, juxta-articular lesions
Intact articular cortex
1) Segmental opacities
2) Linear opacity
3) Mass opacities
Structual failure
4) Translucent subcortical band
5) Collapse of cortex
6) Sequestration of part of cortex
Osteoarthritis
7) Osteoarthritis
Type B, head, neck and shaft lesions
1) Dense areas (not bone islands)
2) Irregular calcified areas
3) Translucent areas and cysts

Classification according to Ohta and Matsunaga (15).

TABLE II

CORRELATION BETWEEN POSITIVE EVIDENCE OF BONE LESIONS AND OCCURRENCE OF OVERT DECOMPRESSION SICKNESS

Case No.	Overt Symptoms of Decompression Sickness
1	none
2	none
3	none
4	none
5	pain-only bends
6	none
7	none
8	pain-only bends
9	pain-only bends
10	none
11	none
12	none
13	none

TABLE III

CORRELATION BETWEEN BONE LESION INCIDENCE AND TYPE OF DIVING

Type of Diving	Number of Subjects in Class	Number with Lesions	% of Total
Air, SCUBA	1	0	0
Air, helmet	13	1	7.6
EOD	50	8	16.6
Deep Sea	31	4	12.9

TABLE IV

CORRELATION BETWEEN YEARS IN DIVING AND INCIDENCE OF BONE LESIONS

Number of Years	Number of Men	Number with Lesions	%
< 1	3	0	0
< 3	39	3	7.6
< 5	16	1	6.2
< 7	14	3	21.4
< 9	8	1	12.5
10 or more	15	5	33.3

TABLE V

CORRELATION OF BONE LESION INCIDENCE WITH AGE

Age Range	Number of Subjects in Class	Number with Lesions	%
< 20	8	1	12.5
< 25	27	2	7.4
< 30	33	3	9.1
< 35	14	2	14.2
< 40	12	4	33.3
40 or more	1	1	100.0

TABLE VI

CORRELATION OF INCIDENCE OF BONE LESIONS WITH HOURS OF DIVING EXPERIENCE

Hours	Number of Subjects in Class	Number with Lesions	%
< 500	20	0	0
< 1500	27	3	11.1
< 3000	24	2	8.3
< 5000	19	4	21.0
< 6000	3	2	66.6
6000 or more	2	2	100.0

Figure 1*A* shows a dense area on the left femoral head; Figure 1*B* shows the same subject six years later; the trabecula is blurred and dirty-looking. The same subject showed an opaque shadow on a radiogram of his left humeral head, but this did not progress over the 6-year examination period. Two other subjects, classified as type B, had similar dense areas on radiograms of the left humeral head. Neither of these areas showed radiographic evidence of change over the course of the study.

Figures 2*A* and *B* show markedly increased density of the left humeral head in two divers who participated in the study. Figures 3*A* and *B* demonstrate irregularities of calcification of the left humeral head which did not deteriorate over the course of the study. Of the 13 divers designated positive cases, 3 were classified as type B, and 10 were classified as type C (doubtful cases). Classification and site of lesion are shown in Table VII.

Discussion

Studies by several authors (12, 13, 15) and the radiographic evidence obtained in this study have led to the following conclusions:

(1) With type A lesions, early signs predict later ones;
(2) Questionable cases should be followed serially.

Questionable radiographic signs are: small, scattered opacities surrounded by rings, which may or may not be calcified and which may be honey-comb-like in appearance; local calcifi-

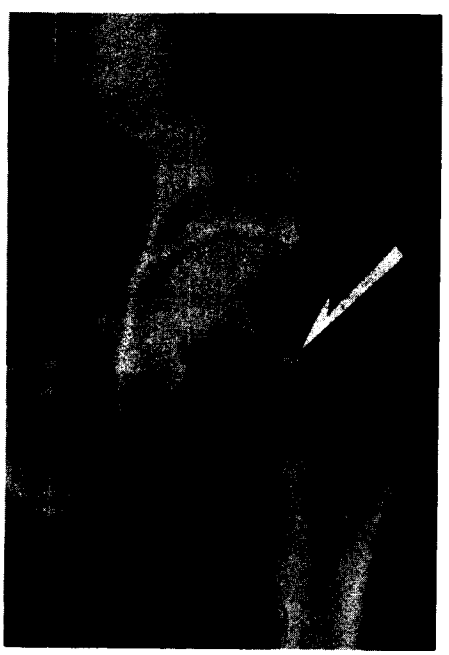

Fig. 1*A*. Dense area of left femoral head.

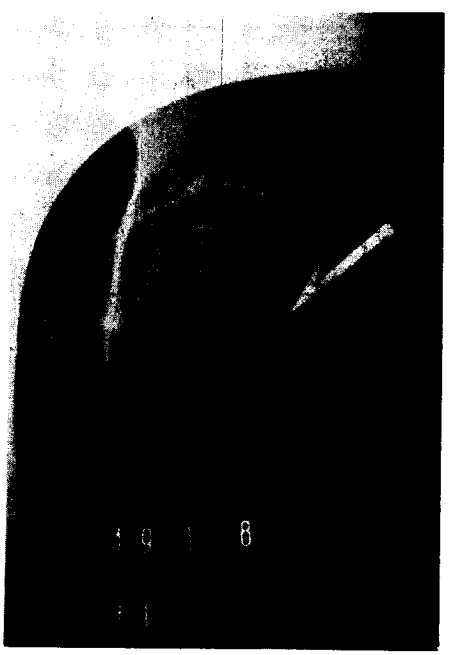

Fig. 1*B*. Same area, six years later.

Fig. 2*A*. Area of left humeral head, showing increased density.

Fig. 2*B*. Another example of area of increased density of left humeral head.

Fig. 3*A, B*. Irregularities of calcification in left humeral heads of two divers.

TABLE VII

CLASSIFICATION AND SITE OF LESION

Type	Humerus, Head and Shaft, Right	Humerus, Head and Shaft, Left	Femur, Head and Shaft Right	Femur, Head and Shaft Left
B, 1	1	1		1
B, 2				
B, 3				
C, 1	3	2	3	2
C, 2	1		1	

Classification according to Ohta and Matsunaga (15); Type C are questionable cases.

cations with drop-like or scattered, dense areas; or irregular patterns with ripple-like or network-like trabeculae which look "dirty" on the radiogram.

It was impossible in this study to establish with certainty that infarction of the bone resulted from diving-related damage. Classifying radiolucent areas was often difficult (4, 5, 7, 8, 9, 14, 16); these studies will have to be continued for a longer period before incidence can be established. Tentatively, however, this study showed a comparatively low incidence (3%) of confirmed bone lesions in Japanese Navy divers; if the questionable cases are included, this figure is 13%.

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EXPERIMENTAL DYSBARIC OSTEONECROSIS: FACTORS INFLUENCING INCIDENCE AND LATENCY

Chryssanthos P. Chryssanthou

Dysbaric osteonecrosis has long been recognized as a lesion induced by exposure to large changes in ambient pressure. Recently, the disease drew added attention primarily because it became evident that this potentially disabling disorder is widespread in divers and compressed-air workers. The incidence of the disease, determined in relatively extensive surveys, ranges from 4% in Royal Navy divers (3) to 50–60% in Japanese diving fishermen (6,7). This wide variation in incidence is due primarily to differences in the conditions of exposure to pressure. Certain conditions, such as degree of pressure, duration of exposure, rate of decompression and frequency of exposure, are known to influence the incidence of the lesion. Other factors, including rate of compression and obesity, may also play a role. In addition, it has not yet been established whether the development of osteonecrosis can be correlated with the acute manifestations of decompression sickness. Such uncertainties in our understanding of dysbaric osteonecrosis are primarily due to our ignorance of the etiology and pathogenesis of the disorder.

Finding an animal model for dysbaric osteonecrosis would contribute significantly to the solution of these problems, by allowing evaluation of the influence of various factors on the incidence, severity and latency of the lesion under various controlled experimental conditions. Furthermore, an animal model could be used to study the etiology and pathogenesis, as well as the prevention and treatment, of the disease. Dysbaric osteonecrosis has been experimentally produced in several animal species, including mice (1), rabbits (5) and miniature swine (9). Using the mouse as a model for such studies allows large scale experiments, which are required for statistical purposes, to be undertaken. Further, if mice are used, large numbers of animals can be simultaneously subjected to compression-decompression in the same chamber, which ensures that exposure to environmental conditions will be identical.

This paper reports on experiments designed to study the influence of obesity, number of exposures and rate of compression on the incidence and latency of dysbaric osteonecrosis in mice.

Material and Methods

The animals employed were male, hereditarily obese, hyperglycemic mice and their thin siblings (Jackson Memorial Laboratories, Bar Harbor, Me.). Thin animals weighed 18–35 g (average 24 g). There were two weight ranges of obese mice: 38–60 g (average, 54 g) and 60–90 g (average, 78 g). The animals were subjected to 75 psig air pressure in a pressure

chamber for 2–6 hours (usually for 3 hours). The compression was either rapid (to 40 psig in 30 seconds or to 75 psig in 60 seconds) or staged. The staged compression for both obese and thin animals involved stops at 15, 30, 45 and 60 psig for 10, 20, 10 and 20 minutes, respectively. The decompression phase was always staged with stops at 50, 40, 30, 20 and 10 psig for 5, 25, 35, 75 and 120 minutes, respectively, for obese mice, and at 50, 30, 20 and 10 psig for 2, 5, 15 and 30 minutes, respectively, for thin mice. The animals were subjected to these conditions once (single exposure) or 3–8 times (multiple exposure). Corresponding obese and thin mice which were not subjected to compression-decompression were used as controls. The groups to be compared were composed of mice of approximately the same age and weight. The animals died or were killed at intervals ranging from 24 hours to 17 months after initial dysbaric exposure. The bones of the hind limbs and at least one of the front limbs and the sternum were processed for histologic examination. A total of 2,219 bones from 390 animals was examined.

Results

During an observation period of at least one hour after decompression, the animals exhibited no (apparent) clinical signs of decompression sickness, except in a few instances in which the animals died within 24 hours after decompression. Histologic examination of the bones of these animals revealed marked hyperemia of the bone marrow, with occasional hemorrhagic foci. Gas bubbles were present in both the diaphysis and epiphysis, particularly in obese mice. These appeared as round or oval clear spaces of different sizes, which sometimes equalled the diameter of the medullary canal. Often, they were irregularly shaped, with smooth outlines distorted by bony trabeculae which protruded into the bubble.

In animals which died or were killed at intervals of 2 to 17 months after the initial hyperbaric exposure, histologic examination revealed definite osteonecrotic changes in 33% (49/147) of the obese animals and in 6.2% (10/162) of their thin siblings. In most cases, the lesion involved the epiphysis of the proximal end of the tibia bilaterally or unilaterally. In some cases, necrosis was seen in the epiphysis of the distal end of the femur. Occasionally, a lesion was observed in the head of the femur and in the bones of the upper extremities. The necrotic lesion always involved the spongy tissue of part or all of the epiphysis. In early stages of the lesion, pyknosis and karyorrhexis of the osteocytes in epiphyseal trabeculae were apparent. The hematopoietic cells exhibited loss of nuclear staining and indistinct cellular boundaries. In more advanced lesions, the intertrabecular marrow spaces contained amorphous masses of granular debris and the lacunae in the necrotic trabeculae were devoid of osteocytes (Fig. 1). Several microcracks (fissures) which sometimes extended to the surface of the trabeculae were seen between lamellae. A few microscopic trabecular fissures, however, were also observed in control (nonexposed) animals, and the significance of such microcracks is therefore questionable. The Haversian canals in the necrotic areas were either empty or contained granular debris, which apparently resulted from degenerated tissue. At later stages, fibrovascular tissue was seen to invade and replace necrotic marrow. Resorption of bone was evident in some cases, but appositional new bone formation on pre-existing necrotic trabeculae was only seen occasionally. In a few cases, necrotic epiphyseal trabeculae appeared fractured, with collapse of the articular surface. In other instances, there was erosion of the epiphysis of the proximal tibia with concave defects in the articular surface. These osteonecrotic changes were seen in 4 of 36 control obese mice, and in none of the 45 control thin mice.

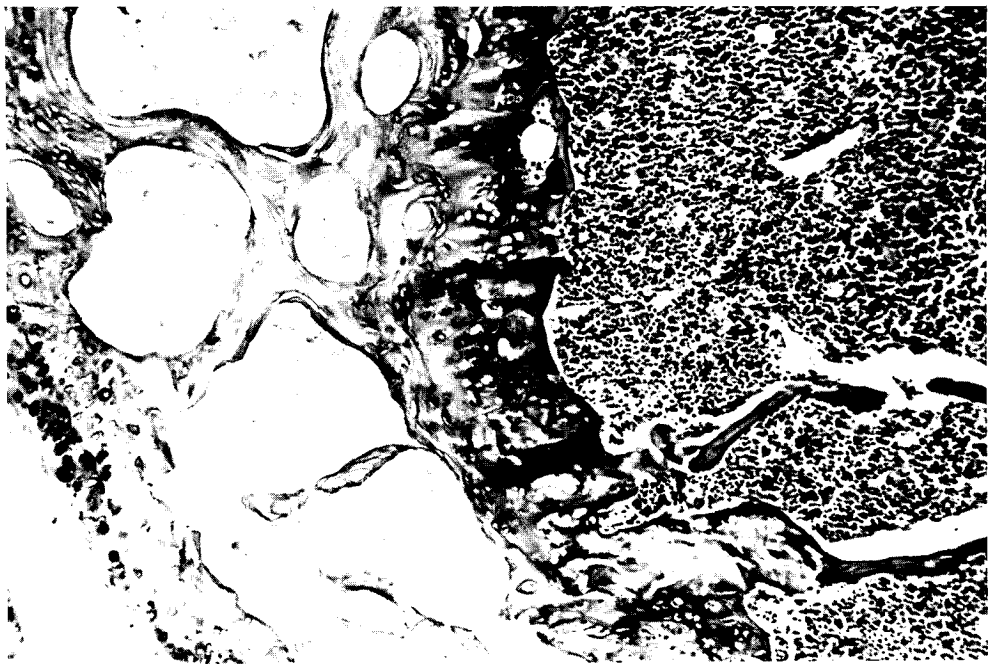

Fig. 1. Proximal end of tibia in a mouse killed 5 months after exposure to pressure, showing necrosis in the epiphysis. Note marrow spaces containing granular debris and trabeculae with lacunae devoid of osteocytes. Microcrack extending to trabecular surface can be seen near upper left corner. (Hematoxylin and eosin stain, × 32)

Osteonecrosis first became histologically evident 2 months after the initial exposure to compression-decompression. Table I shows that within the first 3 months after exposure, the incidence was 0% in thin mice and 11.7% in obese mice. Four months or more after exposure, the incidence rose to 8.2% in thin and 48.3% in obese animals. These results also suggest that the latent period is shorter in obese mice.

Table II shows a striking correlation between animals' weight and incidence of dysbaric osteonecrosis. The incidence was also influenced by the number of exposures to pressure. Table III shows that in obese mice the incidence was 31.1% after a single exposure, and 64.7% after multiple exposures. Rate of compression was another factor which appeared to play a role in the development of osteonecrotic changes. Table IV shows that in thin mice there were no lesions in animals which underwent staged compression, while 7.6% of those animals which were compressed rapidly developed necrosis. In obese mice, an incidence of

TABLE I

INCIDENCE OF DYSBARIC OSTEONECROSIS IN MICE AFTER HYPERBARIC EXPOSURE

Type	0–4 Months	4 Months or More	*P*
Obese	11.7% (7/60)	48.3% (42/87)	< 0.001
Thin	0% (0/40)	8.2% (10/122)	N.S.

N.S. = not significant.

TABLE II

INFLUENCE OF OBESITY ON INCIDENCE OF DYSBARIC OSTEONECROSIS IN MICE

Type	Average Weight, g	Incidence	*P*
Thin	24	6.2% (10/162)	
Obese	54	29.1% (16/55)	< 0.001
	78	35.9% (33/92)	< 0.001

TABLE III

INFLUENCE OF NUMBER OF HYPERBARIC EXPOSURES ON INCIDENCE OF DYSBARIC OSTEONECROSIS IN OBESE MICE

Type	Single Exposures	Multiple Exposures	*P*
Obese	31.1% (14/45)	64.7% (11/17)	$0.02 < P < 0.05$
Thin	4.3% (2/46)	5.1% (3/59)	N.S.

N.S. = not significant.

TABLE IV

INFLUENCE OF RATE OF COMPRESSION ON INCIDENCE OF DYSBARIC OSTEONECROSIS IN MICE

Type	Rapid Compression	Staged Compression	*P*
Obese	64.7% (11/17)	26.7% (8/30)	$0.02 < P < 0.05$
Thin	7.6% (10/132)	0% (0/30)	N.S.

N.S. = not significant.

64.7% with rapid compression was reduced to 26.7% with staged compression, a statistically significant difference.

The number of exposures and the rate of compression also influenced the time of onset of the lesion. There is an appreciable shortening of the latent period when rapid compression is used, compared to staged compression, or when multiple exposures are compared to single exposures.

Discussion

It is apparent from these results that dysbaric osteonecrosis can be experimentally produced in mice. The occurrence of lesions in four of the control obese mice could be associated with the obesity and/or hyperglycemia of these animals. Both conditions have been impli-

cated in the etiology of nondysbaric aseptic bone necrosis. Obese mice also have fatty livers, and it has been suggested that a fatty liver is capable of spontaneously releasing embolic fat globules into the circulation (8). None of the thin controls developed bone necrosis.

The incidence of osteonecrosis in mice subjected to compression-decompression was far greater in obese than in thin animals. In fact, there was a correlation between the weight of the animal and the incidence of bone lesions. It has been reported previously that the degree of obesity of these animals correlates with their susceptibility to decompression sickness (1). These correlations, however, do not imply that the delayed dysbaric osteonecrosis is associated with the acute manifestations of decompression sickness. On the contrary, with the diving profiles used, except for a very few obese mice, the animals which developed bone lesions did not exhibit clinical signs of decompression sickness. Although dysbaric osteonecrosis appears to be independent of decompression sickness, it is still possible that the pathogenetic mechanisms of the two conditions share some initiating or contributing factors associated with obesity. These factors could be related to the high solubility of nitrogen in adipose tissue and in the fatty bone marrow of the obese mice. Fatty bone marrow exchanges nitrogen slowly, and decompression could thus cause great supersaturation of dissolved gas. It is therefore conceivable that even "safe" decompression rates may permit release of gas bubbles from fatty marrow tissue over long periods of time. Intravascular and/or extravascular gas bubbles could cause circulatory impairment and ischemia, which could in turn precipitate necrotic bone changes.

These speculations do not intend to limit pathogenetic considerations of dysbaric osteonecrosis to the direct effect of local gas bubbles. Circulating nitrogen bubbles could trigger a chain of secondary events, including aggregation of platelets and erythrocytes, coalescence of unstable lipids or disruption of fatty tissues by expanding bubbles, and changes in the coagulation process. Any of these alterations might result in embolization and circulatory stasis in the bones. Still it is apparent that, in all of these mechanisms, the protagonist is the gas bubble which directly or indirectly causes circulatory impairment. Alternatively, however, there is the possibility that factors other than gas-bubble-related events may play a role in the pathogenesis of dysbaric osteonecrosis. For example, it has been suggested that gas-concentration gradients resulting from rapid pressure changes can produce osmotic changes and fluid shifts that could contribute to the production of bone lesions (4). It is evident in this case that the rate of compression may influence the development of bone changes. Our finding that the incidence of dysbaric osteonecrosis is higher with rapid than with staged compression supports this hypothesis. In this connection, it has also been reported that the severity and frequency of hyperbaric arthralgia are reduced in divers subjected to slow compression rates, and gas-induced osmosis has been implicated in this phenomenon (2).

It therefore seems reasonable to propose that dysbaric osteonecrosis may develop as a result of several initiating and contributing factors acting in concert or in sequence. The findings of the present study regarding the latency in the development of bone necrosis and the influence of multiple dysbaric exposures are consistent with well-known observations on human subjects. The influence of obesity and the rate of compression on the incidence of the disease has not yet been established. Although the results of this study indicating an increased incidence of dysbaric osteonecrosis in obese and in rapidly compressed animals must be extrapolated with caution, they should draw attention to the possible role of these factors in the development of the lesion in humans.

Conclusions

1) Dysbaric osteonecrosis can be produced experimentally in mice, particularly in obese strains.
2) Latency of the lesion ranges from 2 to 4 months after the initial dysbaric exposure.
3) With multiple exposures the incidence is higher and the latent period shorter than with a single exposure.
4) With staged compression the incidence is lower than with rapid compression.
5) In obese mice, the incidence is greater and the latent period shorter than in thin siblings.
6) Dysbaric osteonecrosis appears to be independent of decompression sickness.

ACKNOWLEDGMENTS

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INFLUENCE OF INADEQUATE DECOMPRESSION ON PATHOGENESIS OF OSTEONECROSIS

P. J. Stegall, T. W. Huang and K. H. Smith

In 1971, we induced aseptic bone necrosis experimentally in miniature swine as the result of multiple compression/decompression exposures (2). The following year, initial hematologic studies noted that platelet adhesiveness increased by as much as 50% immediately postdive, while platelet survival rates dropped from 5 days (the normal interval) to 2 days, and fibrinogen survival rates dropped from 4 days to 3 days at the end of the first week after a single hyperbaric exposure (5, 6). In 1973, kinetic measurements demonstrated that neither platelet function inhibitors nor anticoagulants could alter the consumption of these hematologic factors after diving, but that a combination of these two medications could normalize the survival rates (1, 3, 4, 7). Platelet counts were not relied upon as an index to hemostatic change because their numbers may reflect increased rates of production as well as release from storage pools as compensatory mechanisms.

Since more than one mechanism could be at work to produce the increased utilization of platelets and fibrinogen, these data led to histopathology studies on animals subjected to single and multiple exposures. Findings from these studies included hyaline thrombi in the blood vessels of Haversian canals, changes in the medium-size arteries of the bone marrow (specifically, myointimal cell proliferation with resultant lumen narrowing), hemorrhage in the trabecular area of the metaphysis, and bone necrosis (8, 9).

In our continued search for the initiating event of bone necrosis, we exposed four animals to the same profile (60 fsw for 6 hours, with decompression at a rate of 30 feet per minute), which produced a 100% incidence of osteonecrosis in previous experiments involving over 50 animals. The animals so exposed were killed at 1 hour, 2 hours, 4 hours, and 26 hours after decompression to observe temporal histologic and cellular changes. None of the pigs exhibited any signs of decompression sickness by the time of killing. Lung and kidney tissues were obtained, as were the humeri and femora of all animals. The tissues were fixed and prepared for both light and electron microscopy, using standard techniques.

Histopathology

In kidney specimens examined from the pig killed one hour after decompression, a bubble was rarely seen (Fig. 1). In the animal killed at 4 hours, numerous bubbles were seen in the

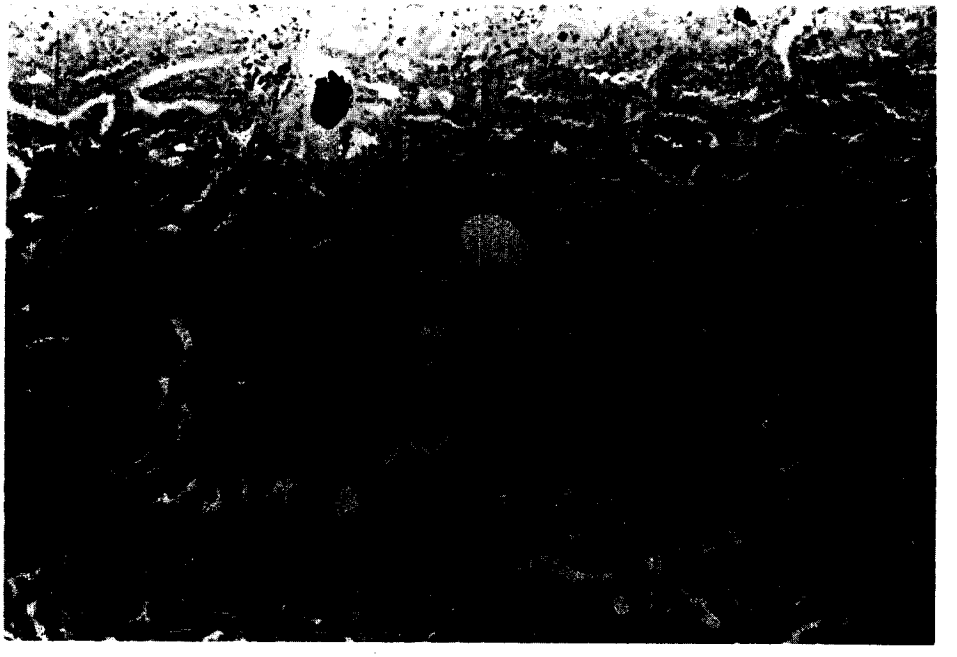

Fig. 1. A rare air bubble (arrow) found in kidney 1 hour after decompression. Glomeruli (G) appear normal.

peritubular capillaries of the cortex (Fig. 2); by 26 hours, however, the bubbles were no longer present. In the 2-, 4-, and 26-hour animals, thrombi were present in the glomeruli (Fig. 3) and peritubular capillaries, and glomerular necrosis was seen as early as 4 hours after decompression (Fig. 4). Tubular necrosis was evident at 26 hours (Fig. 5).

In comparison with normal bone taken from control pigs showing well-defined blood-filled vessels within the Haversian canals (Fig. 6), the specimens of cortical bone taken from the 2-hour pig showed a complete absence of blood in any vessels within these systems, presumably because of air filling (Fig. 7). Four hours and 26 hours after decompression, hyaline thrombi had occluded most of the vessels (Fig. 8).

In this series of animals, no thrombi were found in lung tissue. Air-distended pulmonary arteries were found occasionally in the 2-hour specimen, but more prominently and extensively in the 4-hour animal (Fig. 9).

Electron Microscopy

Electron microscopic examination of the tissues provided more detailed information on the damage which resulted from this single dive. The bone specimen area examined in Fig. 10 does not include the injured vessel which preceded the hemorrhage noted; however, the presence of erythrocytes as well as plasma proteins indicates the severity of the damage.

Hemorrhagic areas such as these (seen extensively by light microscopy) were observable in 4- and 26-hour bone marrow specimens. The configuration of a normal capillary (Fig. 11) surrounded by normal marrow fat cells contrasts sharply with a capillary removed from a pig 4 hours after decompression, which shows vessel walls distended by a large air bubble whose

Fig. 2. Numerous air bubbles (arrows) seen in kidney 4 hours after decompression.

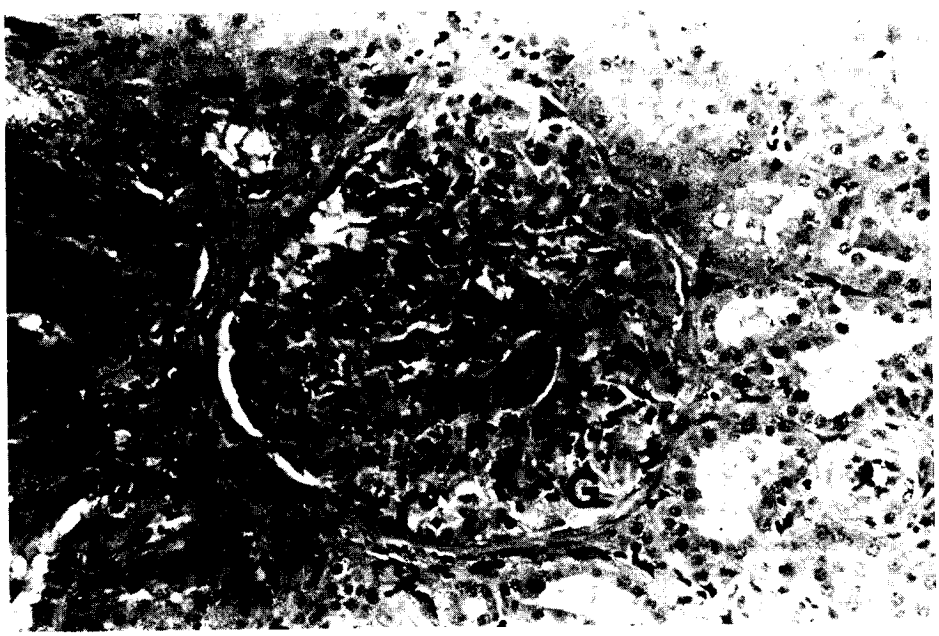

Fig. 3. Thrombus (arrow) seen in a glomerulus (G) 4 hours after decompression.

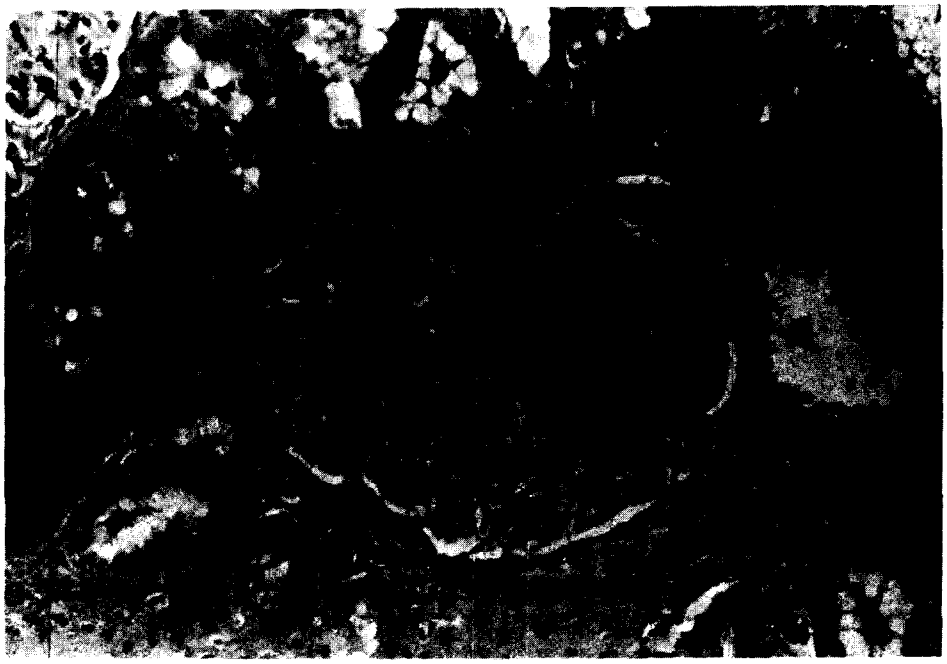

Fig. 4. Necrosis (arrows) seen in a glomerulus (G) 4 hours after decompression.

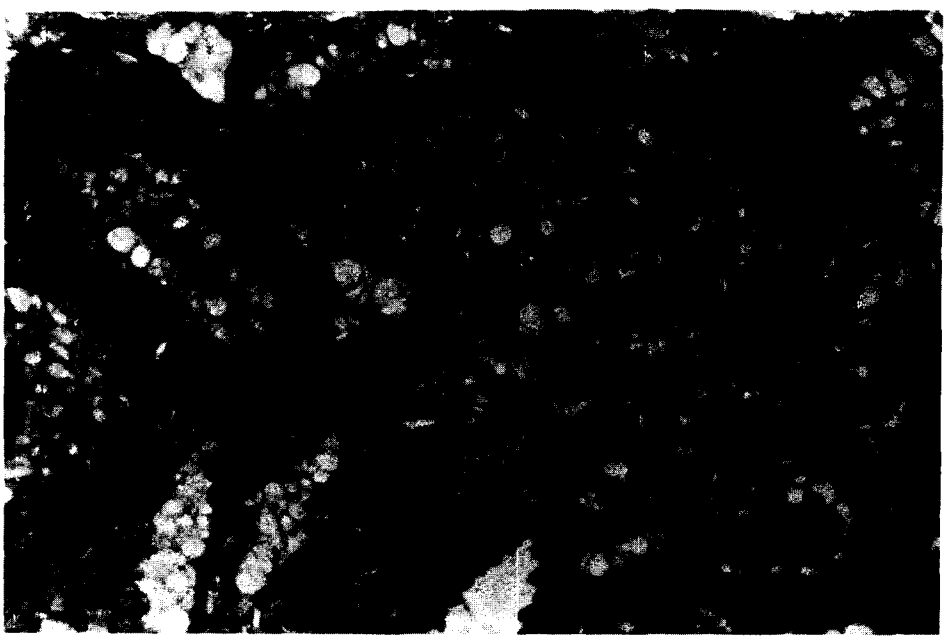

Fig. 5. Tubular necrosis (N) found in animal killed 26 hours after decompression.

Fig. 6. Cortical bone from normal control animal showing Haversian canals with blood-filled vessels (arrows).

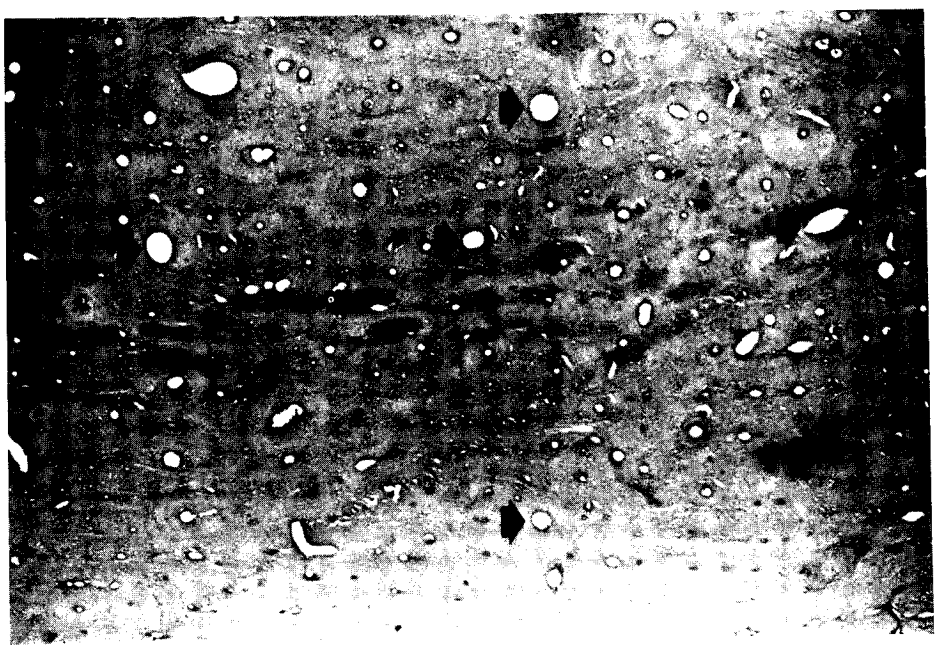

Fig. 7. Cortical bone taken from a pig 2 hours after decompression, showing empty Haversian canals (arrows), presumably because of air filling.

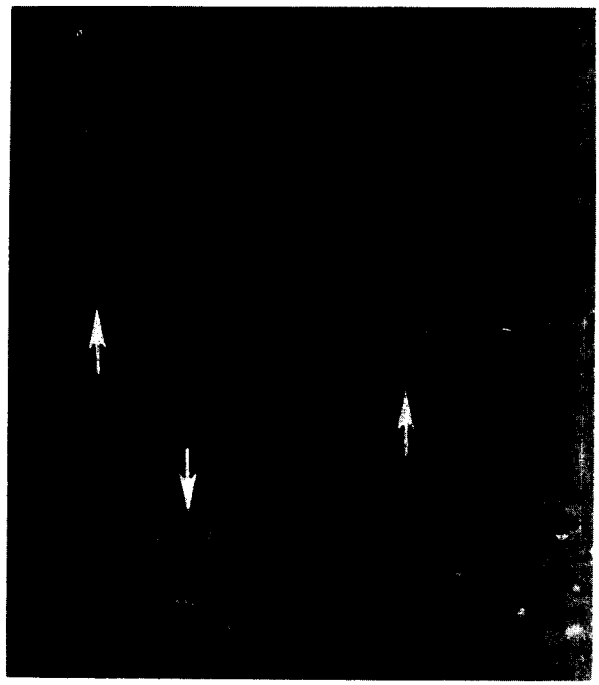

Fig. 8. Hyaline thrombi (arrows) occluding vessels in cortical bone 26 hours after decompression.

Fig. 9. Pulmonary arteries (A) distended by air in an animal killed 4 hours after decompression.

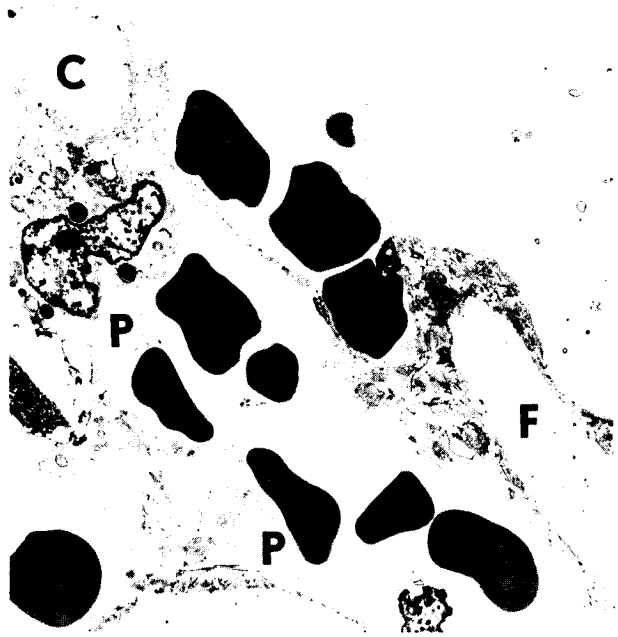

Fig. 10. Evidence of vessel damage seen by extravasation of erythrocytes (E) and proteinaceous material (P) into interstitial spaces. A capillary (C) and fat cell (F) are seen in this bone specimen from a pig killed 4 hours after decompression.

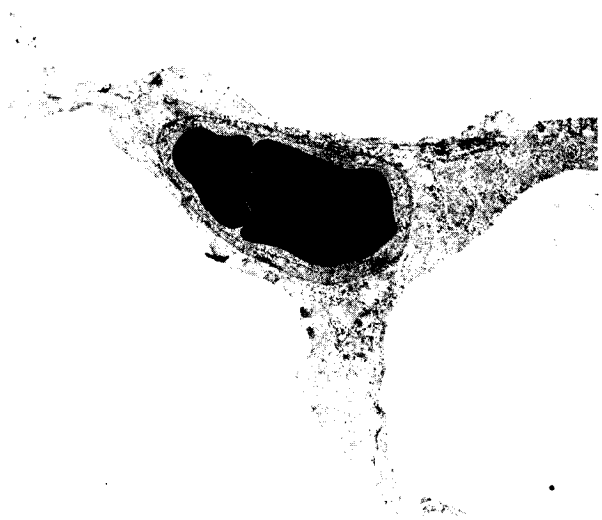

Fig. 11. Configuration of a normal capillary.

interface with blood is characterized by a distinct lipoprotein skin (Fig. 12). A helmet-shaped red cell which has undergone this transformation in response to stress can be seen in the plasma pushed aside by the bubble.

Vessels which were not distended by air were often contracted, as seen in Fig. 13. Degenerating endothelial cells projecting into the lumen of the vessel were also noted. Ultramicroscopic studies revealed several other ways in which the endothelium responded to blood-borne bubbles. In some vessels (Fig. 14), the endothelial lining was completely scraped or stripped away, and there were even more occurrences of a simpler interruption of the endothelial lining (Fig. 15). In either case, the basement membrane was unprotected.

Figure 16 shows a single platelet adhering to the membrane of a degenerating endothelial cell. Although the capillary in Fig. 17 looks relatively normal (no vessel injury can be seen at this power), the adherence of a platelet to the vessel wall is an unusual occurrence and a strong indication of vessel trauma.

We conclude from this study that a single inadequate decompression in miniature pigs, who exhibited no signs of decompression sickness before the time of killing, introduces tissue-damaging bubbles which initiate marked microvascular alterations. These changes are endothelial damage, platelet consumption, extravasation of vascular contents, edema, glomerular and tubular necrosis, peritubular capillary thrombi in the kidney, and vascular thrombi in cortical bone.

We believe that these very early changes, combined with our earlier histopathic studies of decompression-related osteonecrosis, define the initial course or pathogenesis of osteonecrosis.

Fig. 12. Lipoprotein skin (L) marking interface between an air bubble (A) and blood. Helmet-shaped red cell (E) is present in this distended capillary.

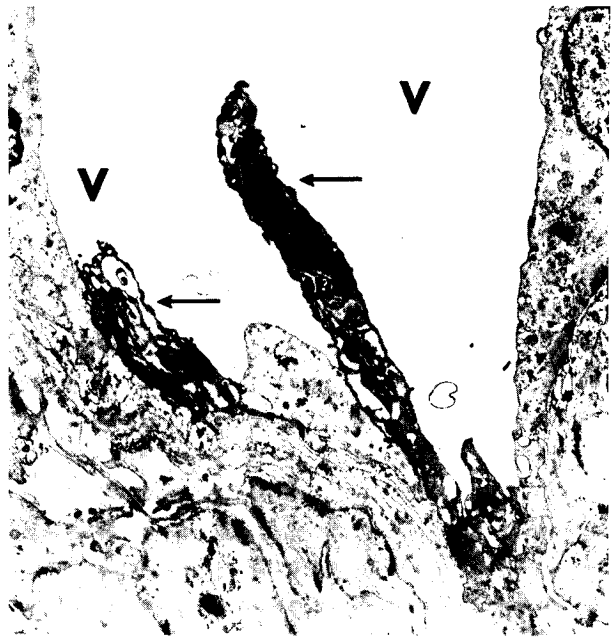

Fig. 13. Degenerating endothelial cells (arrows) projecting into a vessel lumen (V).

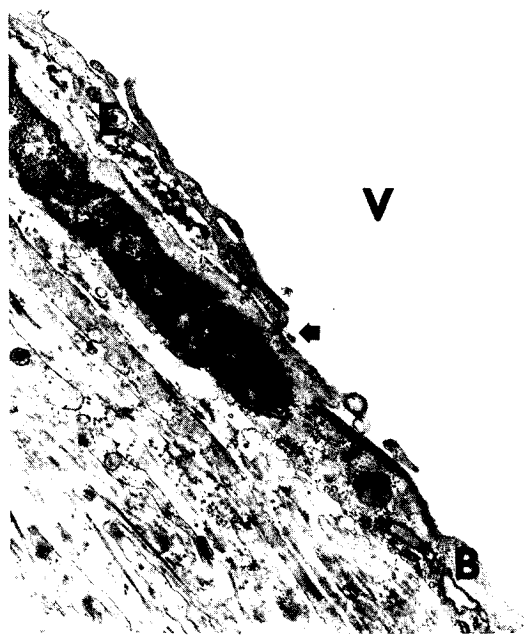

Fig. 14. Endothelial lining (E) of lumen (V) stripped or scraped away, exposing basal lamina (B).

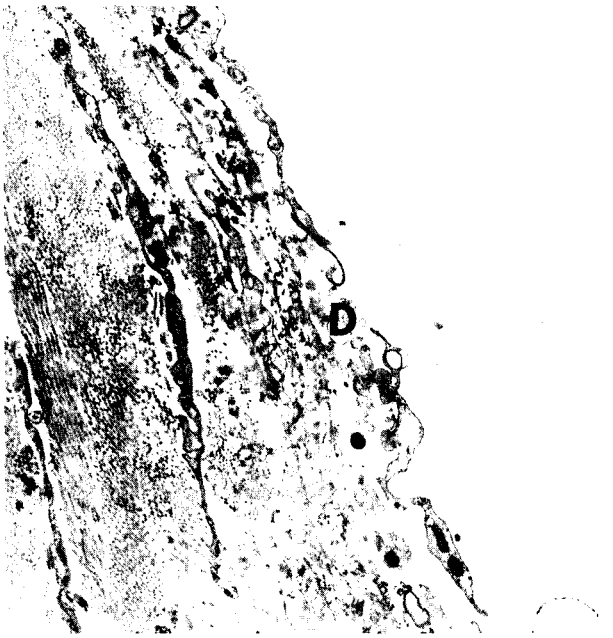

Fig. 15. Cellular debris (D) exposed when endothelial lining was interrupted.

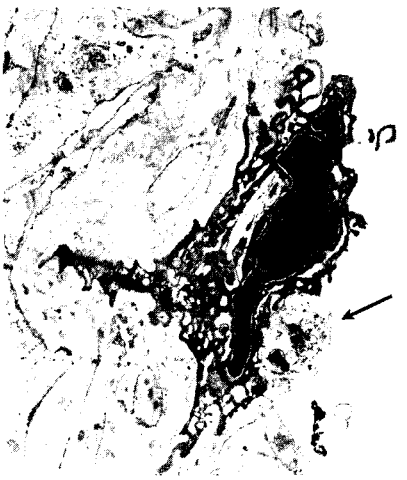

Fig. 16. Single platelet (P) seen adhering to membrane of degenerating endothelial cell (arrow).

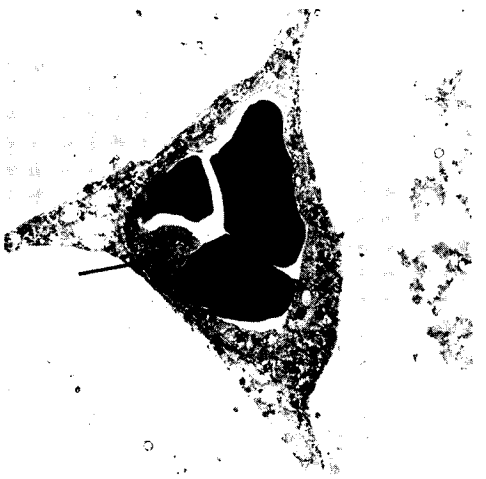

Fig. 17. Platelet (arrow) attached to capillary wall.

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PART VI. DETECTION AND PREVENTION OF BONE NECROSIS

DISCUSSION

D. N. Walder, Chairman

Dr. Kawashima: In 1964, Kim described 42 bone islands (0.8% in a series of 5,000 patients who had some type of bone radiological examination). No new bone islands were reported that had not been seen previously. On the other hand, we found a very low percentage of bone islands in nondivers, but we found bone islands in 197 of 450 divers, 21.6% of whom had bone lesions. We performed an examination series from 1965 to 1972; the number of divers was 123; in 1965 we found bone islands in 15 divers, and in 1972 we found them in 28. In nondivers, I found only 15 with bone islands (0.54%). Dr. Spencer, I would like to ask, do you think we should add bone islands to the classification of bone lesions in divers or vocational workers?

Dr. Spencer: I am not an expert in this field; may I defer to the chairman for an opinion?

Dr. Walder: We have compared two groups, each of about 100 men, compressed air workers and heavy manual laborers. I can't give you the exact figures from memory, but there was no statistical difference between the prevalence of bone islands in the two groups. This is also true for commercial divers. I think I'm also right in saying that the British Navy has looked at the question of the incidence of bone islands in divers and nondivers, and has decided that there is no significant difference between the two groups.

Dr. Hills: As regards the bone islands, Captain Harrison of the Royal Navy did let me have two bone islands and cortical bone taken from the same bone on autopsy. We found that the calcium-phosphorus ratio was the same in both. We also found that when we did a very slow cooling curve which indicates the degreé of crystallinity, there was no real difference between the two.

Dr. Jones: First, Drs. Sealey and Oppenheim are to be congratulated on an excellent roentgenographic follow-up study in compressed air workers, probably the most extensive study performed in this country.[1] We had originally thought that if we could keep the compressed air exposure to less than 11 psig, we could avoid decompression sickness altogether, and if we kept the exposure under 17 psig, we could avoid dysbaric osteonecrosis. Dr. Ribeiro has had experience with compressed air tunneling operations in Brazil, with pressures to 14 psig, and he has seen decompression sickness with possible dysbaric necrosis in these workers. Our initial findings may not be absolutely certain. I noticed in Dr. Chryssanthou's interesting study that there were microcracks in trabecular fractures in some of the animals in a later part of the series. We have noticed the same thing, and we don't believe this is architectural or artifactual distortion caused by the processing of these slides; we believe these are actual microscopic cracks that extend out to the surface of these trabecular cleavage planes, which subsequently result in gross architectural distortion. Also, as an orthopedic surgeon, I have often seen a much higher incidence of post-traumatic fat embolism in obese individuals, and this has now been proved to be statistically significant clinically. The fat embolism after injuries is significantly greater in obese individuals; have you done fat stains on any of these mice?

Dr. Chryssanthou: In response to your first question, we have seen those microcracks and some of them extended to the surface, resulting in cleavage planes. However, we have also seen these microcracks in control nonexposed animals. I believe at least some of them could be due to artifacts. However, it is true that a greater number of micro-

[1] J. L. Sealey and E. B. Oppenheim presented a paper, "Aseptic bone necrosis survey in compressed air workers" at the Symposium in San Diego in July, 1975. The paper is not included in these *Proceedings.*

fissures was observed in the exposed animals and often they extended to the surface of the trabecula. This was rarely observed in control animals. Therefore, it is a little bit difficult to evaluate the possible significance of microfissures. As for your second point, we did not do fat stains on those bones. However, in the obese mice, as you pointed out before, one can see emboli, and we have found them in the lungs of those animals. If you noticed, in control obese mice the incidence of bone lesions was about 11%. This could have been caused by fat emboli from the fatty livers of those animals; it has been reported that fatty livers are capable of releasing embolic-size fatty particles that could cause ischemic lesions in the bone.

Dr. Walder: Dr. Chryssanthou, when you spoke about the delay in the appearance of the lesions, were you speaking about delay in histological change?

Dr. Chryssanthou: The only way we could establish the lesion was by histological examination; we didn't do any neurological studies because the animals are very small and you can't do that. So when I said there was a latent period of about three months, I was referring to the period until the occurrence of the first definite histologic change indicating dysbaric osteonecrosis. I would like to point out in this respect, because this is very pertinent, that our criterion for calling a case a definite lesion was based on several factors, not just on the absence of osteocytes in the lacunae or on the presence of necrotic marrow. Therefore, we may have missed some earlier occurrences, because the lesion was at its very early stage. For instance, I showed a slide representative of several cases in which we noticed change in the osteocytes such as karyorrhexis, necrosis of the nuclei, and other degenerative changes. Those we did not count as dysbaric osteonecrosis. Therefore, the onset of the lesion may have been a little earlier than this 3-month period.

Dr. Stegall: I would like to say that we did do a fat stain in our pigs and it was negative.

Dr. Bennett: I want to raise a point of discussion deriving from the papers of Dr. Chryssanthou and Dr. Stegall, and if you refer to the abstracts I think it becomes quite clear why. The abstract of Dr. Chryssanthou in fact says, "that development of dysbaric osteonecrosis appears to be independent of decompression sickness." The abstract of Dr. Stegall says, "the conclusion that inadequate decompression initiates a series of events which begins with marked microvascular involvement and ends with osteonecrosis is unavoidable." Obviously, the two are completely incompatible. I would like the panel to discuss this point further and try to resolve it for us.

Dr. Chryssanthou: I don't believe that these two statements are incompatible. Inadequate decompression doesn't necessarily mean inadequate enough to produce decompression sickness. My conclusion did not imply that decompression rates did not influence the development of osteonecrosis. What we observed and what I said was that we used profiles which did not result in the development of clinically or histologically detectable decompression sickness, and in those animals which did not develop decompression sickness, dysbaric osteonecrosis was produced. Even in those animals, the decompression may have been inadequate, but not inadequate enough to produce signs of decompression sickness.

Part VII. INERT GAS TRANSPORT: REDUCTION OF DECOMPRESSION TIME

EFFECT OF IMMERSION AND AMBIENT TEMPERATURE ON ELIMINATION OF ^{133}XENON FROM HUMAN ADIPOSE TISSUE

Ulf I. Balldin

The rate of elimination of inert gas from the tissues may influence development of decompression sickness in divers. The elimination rate of whole body tissue nitrogen in subjects immersed with the head above water is greater than that of sitting subjects in dry conditions (5). This increased gas elimination has been attributed to hemodynamic changes. For example, cardiac output increased during immersion (2). Also, the elimination of ^{133}Xe from the anterior tibial muscle increased during immersion, which probably reflected corresponding increases in muscle blood flow (6). Because fat has a large nitrogen-dissolving capacity, this study investigated inert gas elimination and blood flow from subcutaneous fat in human subjects under dry conditions and during immersion, using the ^{133}Xe-elimination technique (12).

Whole body nitrogen elimination is also enhanced in a warm environment, especially during immersion in warm water (3, 5). Because cutaneous blood flow increases greatly in a warm environment (9), there is reason to believe that blood flow and, therefore, inert gas elimination, will also increase in the deeper subcutaneous adipose tissue. The effect of a warm environment on the elimination of an inert gas from subcutaneous adipose tissue is the subject of this paper. Because environmental temperature is more easily controlled when subjects are immersed in water than when they are not, these experiments were made with immersed subjects whose heads were above neutral or warm water.

Methods

The physical characteristics of the subjects are shown in Tables I and II. Subjects had a light breakfast and were instructed to avoid physical exercise and smoking before the experiments. Clearance of ^{133}Xe from a local deposit in the subcutaneous adipose tissue was studied. A sterile saline solution (0.10–0.20 ml, corresponding to about 200 μC:) of ^{133}Xe (Studsvik, AB Atomenergi, Sweden) was slowly injected deep into the subcutaneous adipose tissue outside the anterior tibial muscle via a hypodermic needle (0.4 mm OD). To avoid hyperemia caused by injection trauma (12), the subcutaneous deposit of ^{133}Xe was made at least 2 hr before the measurements started. Radiation from the region was recorded by an external scintillation detector fitted in a wide-angle collimator. The sodium iodide crystal (1.75 × 2 in.) was placed about 15 cm outside the deposit of ^{133}Xe. Radioactivity was registered with a spectrometer connected to a scaler and a linear rate meter (Tri-Carb, Model 3022, Packard), operating a Rikadenki recorder (Model B-341). The standard deviation setting on the rate

TABLE I

EFFECT OF IMMERSION ON ^{133}XE ELIMINATION FROM ADIPOSE TISSUE

Subject	Sex	Age, yr	Height, cm	Weight, kg	Clearance Constant		Change, %
					Dry	Immersed	
C.L.	Male	42	183	78	0.0064	0.0113	+77
P.B.	Male	23	191	80	0.0043	0.0084	+95
G.O.	Male	24	190	81	0.0007	0.0052	+643
K.P.	Female	22	173	60	0.0010	0.0064	+540
B.L.	Male	22	178	73	0.0020	0.0057	+185
M.K.	Male	26	175	63	0.0046	0.0079	+72
G.D.	Male	27	177	63	0.0028	0.0040	+43
K.A.	Male	26	184	74	0.0045	0.0037	−18
L.A.	Male	26	180	82	0.0023	0.0095	+313
M.L.	Male	23	175	66	0.0055	0.0161	+193
S.A.	Male	31	177	63	0.0118	0.0211	+79
C.B.	Female	27	167	55	0.0032	0.0059	+84
Mean					0.0041	0.0088	192*
SD					±0.0030	±0.0052	
Mean blood flow, ml/100 g tissue per min					4.1	8.8	

* = $P < 0.001$; $n = 12$.

TABLE II

EFFECT OF TEMPERATURE ON ^{133}XE ELIMINATION FROM ADIPOSE TISSUE DURING IMMERSION

Subject	Sex	Age, yr	Length, cm	Weight, kg	Clearance Constant		Increase, %
					35 °C	37 °C	
B.L.	Male	22	178	73	0.0057	0.0079	39
M.K.	Male	26	175	63	0.0079	0.0080	1
M.L.	Male	23	175	66	0.0094	0.0165	76
S.A.	Male	31	177	63	0.0159	0.0276	74
U.B.	Male	35	178	63	0.0149	0.0219	47
K.B.	Female	33	170	62	0.0024	0.0137	471
K.P.	Female	22	173	60	0.0048	0.0053	10
K.A.	Male	26	184	74	0.0038	0.0047	24
L.A.	Male	26	180	82	0.0041	0.0052	27
P.B.	Male	23	191	83	0.0089	0.0271	204
E.J.	Male	31	176	57	0.0071	0.0086	21
C.B.	Female	27	167	55	0.0055	0.0098	78
Mean					0.0075	0.0130	89*
SD					±0.0042	±0.0084	
Mean blood flow, ml/100 g tissue per min					7.5	13.0	

* = $P < 0.01$; $n = 12$.

meter was 1%. Pulses corresponding to gamma energies between 60 and 120 keV were counted. Registered activity was usually about 1–10 × 10^5 cycles/min. No correction was made for background activity, since it was always less than 0.3% and usually less than 0.1% of the measured activity. The recorded activity during a 30-min period was plotted semilogarithmically against time. The half time and the clearance constant were calculated from the line, which was usually straight, that could be drawn through the washout curve in each 30-min period. This line was fitted by eye.

Effect of Immersion

About 2 hr after injection of ^{133}Xe, the subjects, wearing trunks, were placed in a canvas pool, where they sat comfortably on chairs (Fig. 1). The pool could be filled with prewarmed water in 2–3 minutes. In the immersion conditions the water level was at the subject's chin. Water temperature was continuously measured with a thermistor thermometer with an accuracy of ± 0.1 °C. The sensing element was placed in the water at the knee level. Pool water was continuously stirred by a stream of air bubbles let in at the level of the subject's feet. The temperature of the water was controlled manually by a water heat exchanger and maintained at 35 ± 0.1 °C. This water temperature is considered to be neutral (8). In the dry conditions the water level was 5 cm above the injection site to keep local environmental conditions, except external hydrostatic load, identical to conditions during immersion with the head above water (6). The water at the injection site was therefore still at 35 °C. A plastic cover was placed over the pool which allowed only the subject's head to protrude to decrease the amount of body cooling during dry conditions. The air temperature measured with the sensing element at chest level was about 28 °C, which is considered to be neutral in dry conditions (3).

Effect of Temperature

In these experiments, subjects were positioned as described, with the water level at the subject's chin. Neutral water temperature was 35 ± 0.1 °C, and warm water temperature was 37 ± 0.1 °C (5), with the sensing element of the thermometer at knee level. Changing between the two temperatures could be done within 10 min. There was a 20-min temperature adaptation period before the registration started.

Results

Statistical calculations were made by the method of paired comparisons and the Student-*t* test. Experimental results of the effect of immersion on ^{133}Xe elimination from adipose tissue are shown in Table I. With the first 6 subjects, dry conditions preceded immersed ones; with the last 6 subjects, the order was reversed, to avoid the slight possibility that the higher clearance rate was caused by hyperemia. However, since the measurements did not start until at least 2 hr had lapsed, it is unlikely that such higher initial clearances were caused by this factor (12). The clearance constants were higher during immersion in all but one subject, with a range from −18% to +643% and a mean increase of +192% ($P < 0.001$).

Adipose tissue blood flow (ATBF) was calculated according to the principles of Larsen et al. (12) and Hansen et al. (10). These calculations assume that ^{133}Xe activity will decline

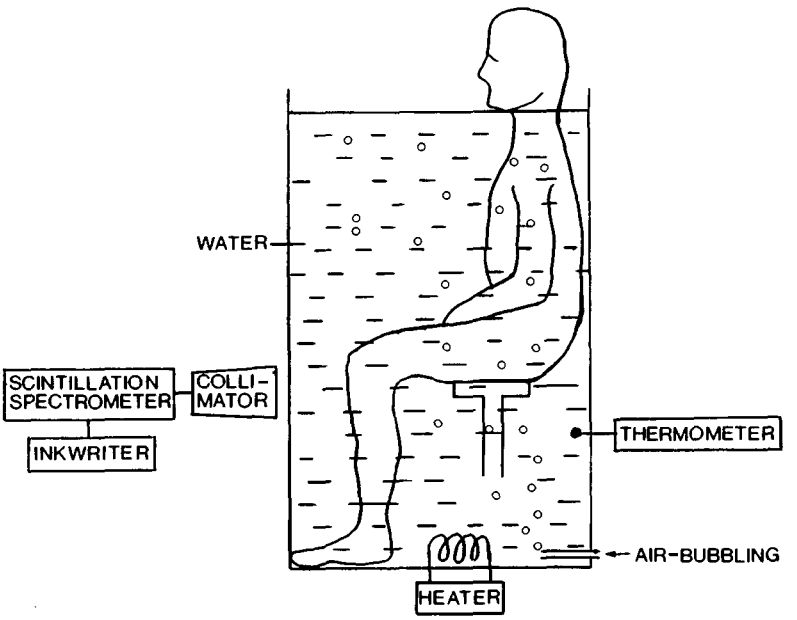

Fig. 1. Experimental arrangement.

monoexponentially with time if equilibrium is obtained between homogenous tissue and the perfusing blood at a constant blood flow. Adipose tissue blood flow may therefore be calculated

$$\text{ATBF} = 100 \times \lambda \times \text{k ml/100 g tissue/min}$$

where λ is the partition coefficient (ml/g) for ^{133}Xe between adipose tissue and blood, $\lambda = 10.0$ (1, 12), and k is a clearance constant that can be determined from the experimental data (ln 2 divided by the ^{133}Xe clearance half time). These calculations gave a mean ATBF of 4.1 ml/100 g tissue per min in dry conditions and 8.8 ml/100 g tissue per min during immersion.

The first 6 experiments started with a water temperature of 35 °C, followed by a period at 37 °C; in the last 6 experiments, the order was reversed. The clearance constants increased in the warm environment in all experiments (see Table II), ranging from +1% to +471%, with a mean increase of +89% ($P < 0.01$). Mean ATBF was 7.5 ml/100 g tissue per min at 35 °C and 13.0 ml/100 g tissue per min at 37 °C.

Discussion

Findings in this study of a higher elimination rate of ^{133}Xe from adipose tissue during immersion and in a warm environment agree with the previous measurements of an increased elimination rate of another inert gas, nitrogen, from all the tissues of the body during similar conditions (3, 5). These findings may be ascribed to changes in blood circulation.

Calculations of ATBF from the ^{133}Xe elimination curves give a mean value of 4.1 ml/100 g tissue per min in dry conditions at neutral temperature. This concurs with the findings of others (10, 11, 12, 15, 16, 18). Any methodological error in absolute flow values caused by using the ^{133}Xe-elimination technique in lean subjects (12) should have been reduced because each subject acted as his own control. The increase in ^{133}Xe elimination, and therefore ATBF, during immersion may be attributed to enhanced cardiac output (2) and presumably to similar mechanisms, such as a decrease of the myogenic basal vascular tone during the decreased transmural pressure in the vessels in immersion (14), which have elsewhere been suggested as the cause of increased muscle blood flow (6). Cardiac output during immersion has been shown to increase about 1800 ml/min (2), of which the total muscle blood flow is about 690 ml/min (6). Assuming a mean of about 13 kg of total body fat in a 70-kg person (7, 13, 19), and that the subcutaneous blood flow in front of the anterior tibial muscle is representative of blood flow of total body adipose tissue (15), the increase in total body ATBF during immersion can be estimated. During immersion the increase in ATBF was 4.7 ml/100 g tissue per min, which means a total increase of about 600 ml/min of the 1800 ml/min increase in cardiac output. Hydrostatic influence during immersion is, however, most pronounced in dependent regions of the body, when the calculation of the total increase in ATBF may represent an overestimation.

Nitrogen elimination studies are made during oxygen breathing, which is known to decrease cardiac output (2), as well as muscle blood flow (6) and ATBF (10). The increase in ^{133}Xe elimination from adipose tissue in immersion, therefore, could be expected to be somewhat lower during oxygen breathing than during air breathing, as the increases in cardiac output (2) and muscle blood flow (6) seem to be.

The increased ^{133}Xe elimination rate and ATBF in a warm environment may be attributed to increased cardiac output and increased blood circulation in the skin and superficial tissues (9, 17), which apparently include subcutaneous fat. The increase in ATBF in a warm environment was 5.5 ml/100 g tissue per min, which means a total increase in ATBF of approximately 700 ml/min, if the same principle of calculation is used. When comparing total ATBF in dry conditions at neutral temperature with that during immersion in a warm environment, the increase will be more than 1 liter/min.

Inert gas elimination rate is an important factor in understanding the development of decompression sickness during ascent. Because nitrogen is five times more soluble in fat than in watery tissues and approximately 70% of the total body nitrogen is dissolved in adipose tissue (7), an increase in gas elimination in this tissue may decrease the risk of decompression sickness or shorten the duration of ascent. This may be especially true during ascent from saturation dives, when the adipose tissues are fully saturated. According to Lundin (13), the elimination half time for nitrogen in adipose tissue amounts to 110–200 min. A more-than-doubled gas elimination rate during immersion, and a more than 3-fold increase during immersion in a warm environment with corresponding increases in ATBF have been found in this study. Theoretically, this should mean that even nitrogen elimination half times in adipose tissue would be shortened during these conditions to less than 50 and 33%, respectively. The preventive effect of denitrogenation on decompression sickness during warm water immersion in human subjects decompressed to altitude (4) demonstrates the importance of this finding.

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MULTIPLE INERT GAS TRANSPORT PATTERNS

G. F. Bond, F. L. Fishback, M. W. Lippitt and R. D. Woodson

Historically, diving physiologists have been concerned with problems of gas transport in the human body. These problems are complex, involve a number of scientific disciplines, and are not likely to be completely resolved in the immediate future. Any explanation of gas transport within the human body must account for the passage of gases across the alveolar membrane, delivery in solution throughout the fimbriations of the arterial tree, passage by diffusion through interstitial fluids or avascular tissues, and, ultimately, their transfer across individual cell membranes. Each of these transitory events must be explained before the total process can be logically interpreted. Similarly, the processes of gas utilization and/or direct passage through the outgoing venous route require investigation, since these events are not necessarily mirror images of the arterial absorptive process.

The metabolic pathways of oxygen and carbon dioxide have been vigorously investigated because these gases are essential components of human metabolism. Additionally, instrumentation for detection and quantification of oxygen and carbon dioxide in alveoli, blood, and tissue has developed rapidly over the past few decades; techniques for the study of transport patterns are readily available to investigators in all parts of the world. Such work is of great importance in diving physiology, but it represents only a portion of the whole problem. This paper will discuss the importance of inert gas transport and describe a pilot study which utilizes currently available techniques to study this phenomenon.

Background

Decompression sickness is still a diving problem. This potentially catastrophic event derives from physiological or physical mismanagement of inert gases within the human body; the syndrome manifests itself in the tissue and, secondarily, in the venous system, but is rarely seen in the arterial system. It is therefore important to pay attention to the uptake and elimination characteristics of any inert gas or combination of gases which might be used by divers during routine operational procedures. At the Naval Coastal Systems Laboratory we embarked on a pilot study to answer some questions about inert gas elimination, and to determine whether current decompression times could be significantly shortened by selecting combinations of multiple inert gases.

Though there is a widespread consensus that, for decompression purposes, only the sum total of the body's dissolved inert gases is important, there is scant but intriguing evidence that such may not be the case. Although Webster (4) argues convincingly for the conventional viewpoint, he also theorizes that some advantage might be obtained if multiple inert gases were used. Workman (6) described an extension of the no-decompression limits developed by the U.S. Navy using a mixture of equal parts of oxygen, nitrogen, and helium; this trimix, however, was never adopted for operational use.

Bühlmann (2) has described alternating helium, nitrogen, and argon to shorten decompression times for very deep dives dramatically; lately, crude neon has been investigated in several research laboratories, with favorable results.

Since the classic work of Behnke and Willmon (1) nearly a quarter of a century ago, very little has been added to our knowledge of inert gas elimination, especially about those inert components which might be used in operational diving. This is understandable, considering the technical difficulty of expired gas analysis. Few investigators have the tenacity and stoicism which repeating the work of these pioneer investigators would require. About eight years ago, however, a compact medical mass spectrometer (Model MSBR, Scientific Research Instruments, Inc., Baltimore) was developed. Almost simultaneously, Teflon-tipped and silicone-rubber-covered probes for intravascular and tissue invasion were developed for use with this particular instrument. The respiratory probe, an older and well-validated instrument, was also easily adapted for use with the mass spectrometer. Woldring (5) has described the mass spectrometer and its associated probes comprehensively.

There are two approaches to the application of multiple inert gas use. Following Keller and Bühlmann, inert gases are alternated during compression and decompression so that no single gas reaches a critical peak of supersaturation; substitution of new inert components provides an adequate driving force for elimination of the dominant, resident tissue gas, without sufficient exposure to become a significant factor in the decompression procedure itself. This method, though generally effective in reducing decompression times, calls for delicately adjusted sequential changes of breathing mixture which are not compatible with operational diving practices. Our study used a different approach, which consisted of delivering a mixture of such proportions that the partial pressure of any one component did not reach a critical limit during a specified dive, although the sum total of all inert pressures might be excessive for the decompression profile of that dive. As demonstrated by Lambertsen (3) and Bond (unpublished observations), it is theoretically possible, according to this theory, to accomplish a saturation dive to remarkable depth with no decompression penalty at all. In practice, this is unlikely, but there are indications that application of the concept may result in appreciable shortening of decompression times.

Because new and reliable equipment for quantification of partial pressures of gases within the venous system was available, we prepared and used a quintimix with a liberal oxygen window and suitable admixtures of argon, neon, helium, and nitrogen, in human and animal subjects, to evaluate and determine individual and composite elimination curves of these gases after no-decompression dives to 60 feet of simulated sea pressure.

Methods and Materials

Mongrel dogs, widely used as hyperbaric subjects, have become especially valuable since Woodson developed the technique of chronic invasion of the pulmonary artery. It may be

possible to achieve simultaneous probe access to the pulmonary artery, the trachea, and the ascending aorta, though animals used only survive for a few weeks (unpublished observations). Such an achievement would allow full visualization of the venous-respiratory-arterial gas exchange. One problem with canine subjects is the requirement that prothrombin levels be maintained between 30 and 50% of normal to prevent coagulation at the heel of the chronically implanted Thomas shunt from the large vascular components, both right and left. This requirement presents a serious physiological problem of prothrombin control, and canine subjects rarely respond predictably to combined dicumarol-aspirin medication. Additionally, bloodstream infections are common in these experimental animals, and some common invasive organisms are resistant to all chemotherapy and broad-spectrum intravenous treatment.

To measure inert gas tensions in mixed venous blood, we utilized both canine and human subjects. Chronic surgical preparations of mongrel dogs consisted of attaching a Thomas shunt to the pulmonary artery with permanent external tubing which could be flushed twice daily with a heparin solution and into which a Teflon-tipped probe could be inserted to read out partial pressures of each component of inhaled gas dissolved in the mixed venous blood; this system was connected to the mass spectrometer machine and results were recorded on a standard strip chart recorder.

In human volunteers, the procedure was considerably simpler, since it was not necessary to maintain a low prothrombin level during these acute experiments. After an initial very small injection of xylocaine at the puncture site, a size-16 trocar was inserted in the brachial vein, and through this, the long catheter was gradually passed to within about two inches of the axilla. The procedure was simple and relatively painless. Aseptic technique was maintained throughout, and final location of the catheter was verified by radiogram before hyperbaric exposure, as shown in Fig. 1.

With this catheter firmly in place, the human subject was moved from the operating room to the recompression chamber, where he was connected directly to the mass spectrometer via a penetration through the hull. For the next half hour, the mass spectrometer alternately read the partial pressures of the subject's venous blood and a calibration gas mixture, which was continuously bubbled through a tonometer. Once the readings were steady, the subject was fitted with a tight oronasal mask through which the appropriate gas mixture was supplied, and a dive to a simulated depth of 60 fsw was made.

Thereafter, continuous recordings of the partial pressures of the venous gases were made; after a preselected bottom time, the subject was brought to the surface without decompression stops. The time on the bottom varied according to previous progress in dives of the series. Upon reaching the surface, the intravenous blood gas recording was continued so that an entire family of elimination curves was obtained, with individual data points for each of the inert gases.

The diving procedure was different for the canine subjects. The dogs were placed in a tightly closed plastic box, through which the appropriate gas mixture flowed, first at a brisk flushing rate, and later at a fixed flow of approximately 7 liters/min, which proved adequate to prevent carbon dioxide build-up and to provide sufficient oxygenation for the subject. Upon completion of the requisite bottom time for each specified dive, the animal was brought to the surface directly, without decompression, the top of the cage was unbolted and removed, and a steady flow of cool, fresh air was introduced into the cage during the surface interval of the experiment. Figures 2, 3, 4, and 5 show the typical elimination curves obtained with humans and with dogs.

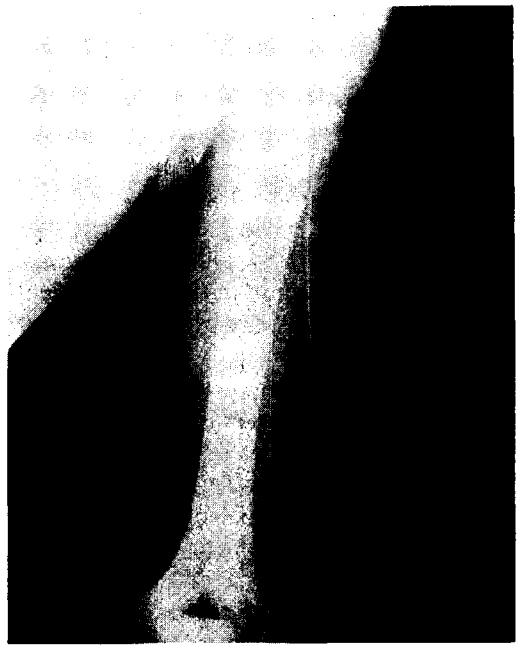

Fig. 1. Placement of gas-sensing catheter in the brachial vein of a human subject.

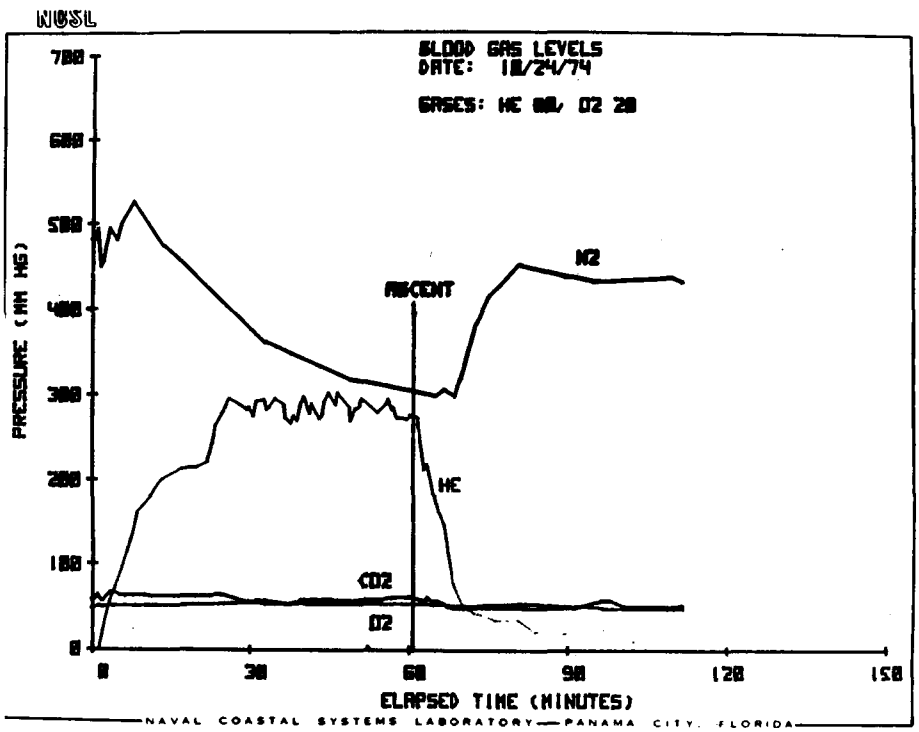

Fig. 2. Typical helium elimination curve determined by sensing catheter in pulmonary artery of a canine subject.

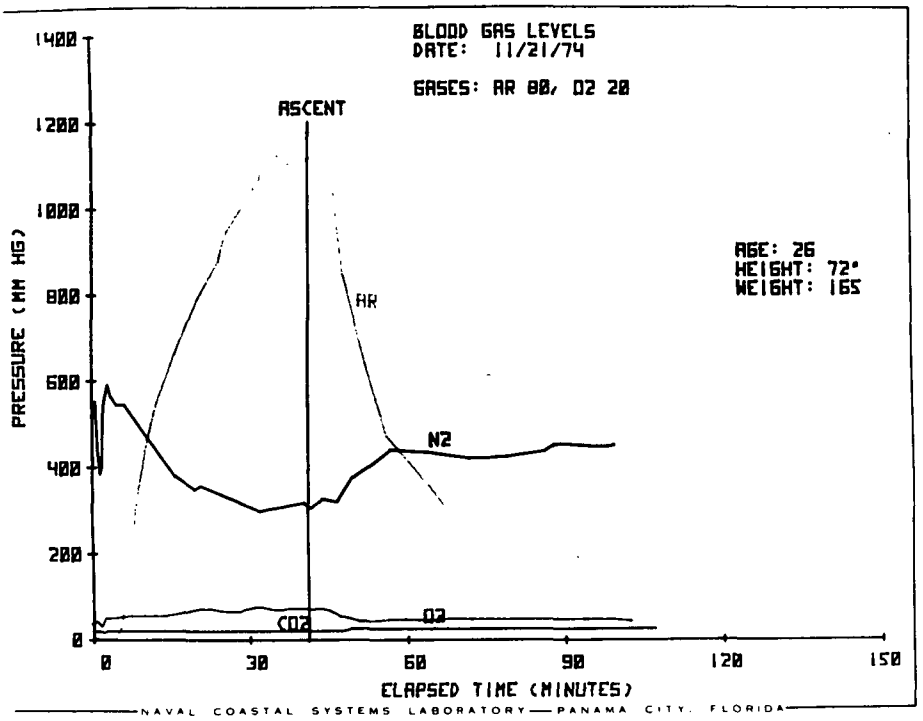

Fig. 3. Typical argon elimination curve determined via sensing catheter in brachial vein of a human subject.

Results

In the first few experiments with chronically prepared canine surgical specimens, we attempted to establish elimination curves for each of the individual inert gases which would later be combined in the final breathing mixture. The simulated depth of 60 fsw was chosen because of the considerable human diving experience at this level, and because this is an appropriate depth for treatment on the short oxygen tables, should such treatment become necessary.

The first two experimental animals did not survive long enough to complete a reasonable series of exposures. In one case, the catheter, which had been passed through the shunt for contrast radiography, was inadvertently advanced as fas as the right ventricular wall, and fibrillation resulted immediately. The second animal succumbed to cardiac tamponade, caused by perforations of the right ventricular myocardium, probably sustained during the manipulations performed prior to arrival at the laboratory.

After the loss of the early experimental dogs, we turned to human volunteers for assistance. In this case, the procedure was much simpler, since no dicoumarization was required, and insertion of the sensing cathether required no more than common surgical prudence. Ultimately, 17 human and 8 canine dives were accomplished, with successive extensions of hyperbaric exposure times beyond the no-decompression limits previously established for compressed air dives. To date, the longest single human exposure has been to 60 fsw for 180 min, with immediate surfacing and no ill effects. The maximum exposures without ill effects to dogs were 240 min at 60 fsw, and 120 min at 100 fsw.

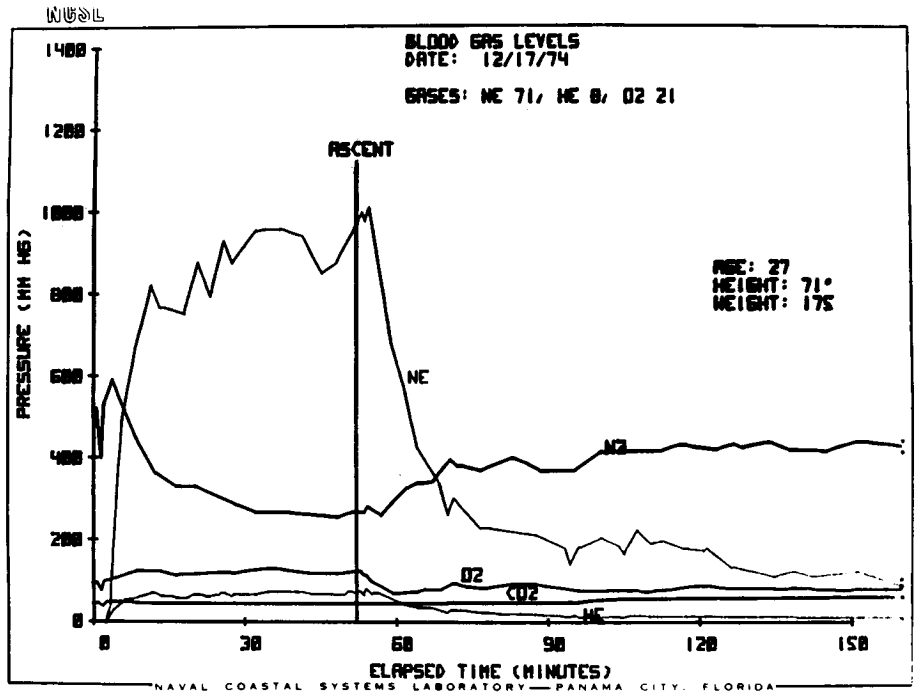

Fig. 4. Typical elimination curves for crude neon (Ne 71%, He 8%) determined from sensing catheter in brachial vein of human subject.

Instrumentation Problems

Discussion of a few of the instrumentation problems encountered in these experiments may be helpful to future investigators. The catheter probes currently available break, although if Teflon-tipped catheters break the event is not hazardous, since it occurs in the stainless steel tubing contained by the tough plastic outer membrane. Also, aberrant mass spectrometer readings generally signal the break, allowing removal of the intravascular catheter. The Silastic-membrane catheter is in common clinical use because of its rapid response to oxygen and carbon dioxide, but since it is relatively impermeable to helium, it cannot be used in inert gas measurements. These catheters are hazardous, because the Silastic outer membrane also breaks when the stainless steel tubing does. Such a break can result in loss of the distal metal fragment in the circulatory tree, which can only be retrieved through open-chest surgery. The degree of catheter probe performance degradation caused by age and repeated sterilizations has not been determined, nor has the exact rate of permeation by the various inert gases, singly or in combination. These considerations led our investigative group to give greater weight to data from the slopes of the inert gas elimination curves than to the absolute values recorded by the entire instrument complex.

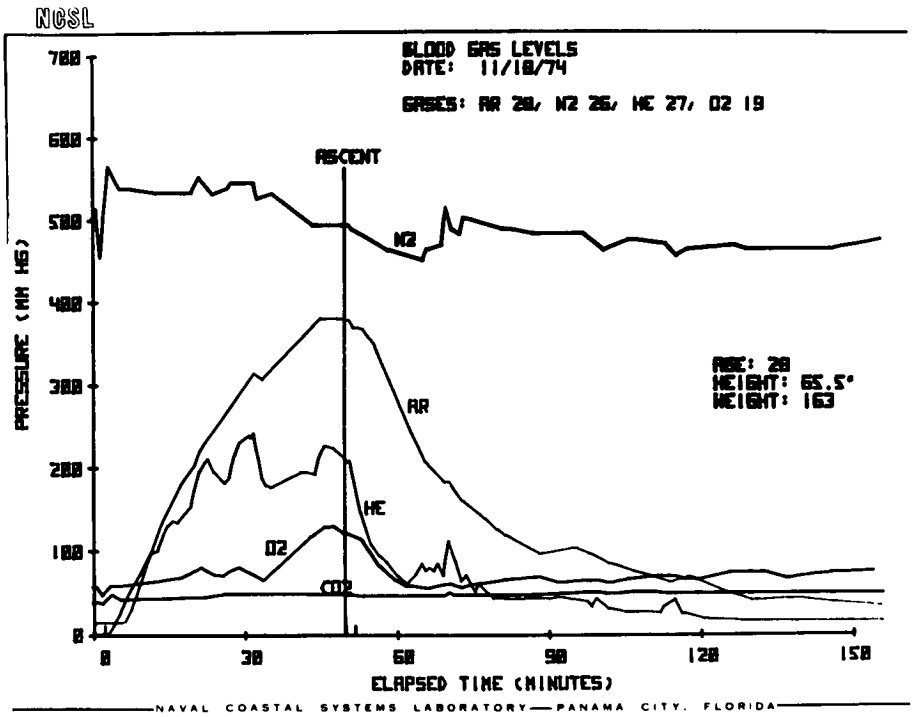

Fig. 5. Typical elimination curves for a helium-argon breathing mixture, determined in a human subject with sensing catheter placed in brachial vein.

Conclusions

This study describes a series of gas elimination curves derived after prolonged compression to 3 and 4 ATA, using the inert gases argon, neon, helium and nitrogen, individually and in combinations. The hypothesis that using several inert gases might lessen the decompression penalty associated with conventional diving practices was also investigated, using a quintimix made up of these four usable inert gases. The use of the mass spectrometer was essential for determination of inert gas elimination following a simulated dive in both humans and animals, and well-calibrated intravenous cathether probes proved vital to data acquisition. Preliminary data show a family of inert gas elimination curves not previously derived in this fashion. It is possible that analysis of these curves, taking other physical and physiological parameters into consideration, may allow current diving procedures to be shortened. Empirically, it seems likely that the use of multiple inert gas breathing mixtures may have considerable value in operational diving.

Application of the multiple inert gas mixture concept to operational diving, however, will require titration and careful study of a great many sets of elimination curves, derived from a large number of human subjects, with proper consideration of individual differences and other parameters peculiar to diving experimentation. After the selection of ideal mixtures for

specific test dives, several years of experimental work will be required before the concept, as applied to operational diving, can be validated.

Even if proven safe and successful, the concept of multiple inert gas use would probably be limited by depth and cost. As depth increases beyond 4 ATA, the percentage of inert components must be decreased to prevent narcosis, which reduces the theoretical efficiency of the systems. In addition, the relatively higher cost of the gas mixtures would probably limit their use to closed-circuit SCUBA and saturation diving systems. However, since these two diving modalities seem to be the wave of the future in Navy diving and commercial undersea manned operations, the prospect of multiple inert gas use deserves earnest consideration.

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DEEP-TISSUE ISOBARIC INERT GAS EXCHANGE: PREDICTIONS DURING NORMOXIC HELIUM, NEON AND NITROGEN BREATHING AT 1200 FSW

C. A. Harvey and C. J. Lambertsen

Investigators at the University of Pennsylvania have observed that counterdiffusion of two inert gases through a two-layer composite of materials having different permeabilities may lead to supersaturation and development of bubbles at the interface between the layers (7). In simulated diving situations, human subjects, surrounded by helium and oxygen while breathing a mixture of oxygen and a heavier gas such as nitrogen or neon, at times experience dermal itching, gross maculopapular skin lesions or a severe vestibular derangement associated with vertigo, nausea, vomiting, and nystagmus (2, 16).

Idicula, Graves, Quinn and Lambertsen (13) demonstrated the cutaneous phenomenon in animals and showed that it could lead to massive, lethal intravascular gas embolization. No lesions were noted when the subjects breathed oxygen and a light inert gas while surrounded by a denser gas. In another experiment, Graves and colleagues offered theoretical insight into this phenomenon, which develops when gases from the capillaries and open spaces adjacent to the skin counterdiffuse across the skin (8).

A related change in the saturation state within the deeper tissues of the body under isobaric conditions is theoretically possible if the inert gases in the breathing mixture are altered. For example, when an individual breathes a mixture containing nitrogen, all body tissues will gradually increase their nitrogen content until they reach equilibrium with the inspired partial pressure of nitrogen. If helium is substituted for nitrogen in the breathing mixture, arterial blood will come into equilibrium with the inspired partial pressure of helium, within the recognized limits of the pulmonary washout curve. A form of counterdiffusion will then take place in the capillaries, as helium enters the tissue and nitrogen leaves. As Keller and Bühlmann noted in 1965 (14), light gases diffuse in or out of the body tissues more rapidly than heavy gases. Thus, the deep tissues may experience transient isobaric inert gas supersaturation in this situation (Fig. 1). Keller and Bühlmann also noted that subsaturation could occur if the inert gases were reversed, so the tissues were first saturated with a light inert gas and heavy gas was then substituted (Fig. 2). This form of deep-tissue isobaric inert gas counterdiffusion supersaturation is transient, but has been demonstrated by Strauss and Kunkle in a gelatin model (20). This could represent a mechanism for primary bubble generation or accelerated growth of pre-existing bubbles. Subsaturation generated by this process might offer significant advantages in treatment of decompression sickness or gas embolism and could shorten decompression times, as Keller and Bühlmann demonstrated by using multiple inert gases (14).

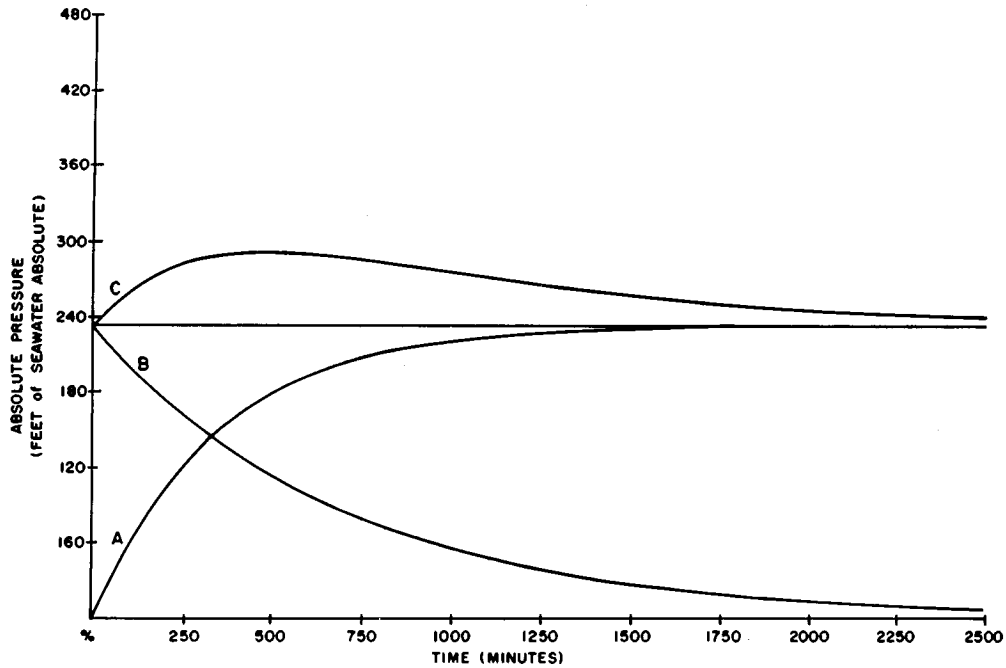

Fig. 1. Supersaturation as a result of isobaric inert gas counterdiffusion in deep tissues. Supersaturation is achieved as total inert gas tension (Curve C) increases when an inert gas with half time of 240 min (Curve A) is substituted for an inert gas with half time of 480 min (Curve B) in breathing mixture. Straight line is total inert gas tension in breathing mixtures.

A series of human exposures to alternating breathing mixtures containing helium, nitrogen, and neon took place during Predictive Studies III at the Institute for Environmental Medicine (16). This experimental dive offered the opportunity to analyze individual and total inert gas tensions generated during the experiment and to correlate the observed presence or absence of decompression sickness symptoms or the isobaric inert gas counterdiffusion syndrome with this analysis, after the actual dive had been completed.

Procedures

During the course of Predictive Studies III, subjects were exposed to normoxic breathing mixtures which contained alternating helium and nitrogen pressures at 100, 200, 300, and 400 fsw, alternating helium and neon pressures at 700, 900, and 1200 fsw, and an alternating helium, neon, and nitrogen cycle at 1200 fsw (Fig. 3). Typical exposure cycles at each of the test depths were later selected for detailed analysis and correlation of changes in individual and total inert gas tensions with observed presence or absence of any symptoms. Periods of alternating breathing gas mixtures (Fig. 4) during the dive often lasted several hours.

Breathing mixtures (Table I) contained, in general, a normoxic (0.20 to 0.21 ATA) oxygen concentration. Very small amounts of neon, at the 400-fsw level, and nitrogen, at the deeper levels, were ignored in the chamber breathing-gas mixture for the purposes of the analysis.

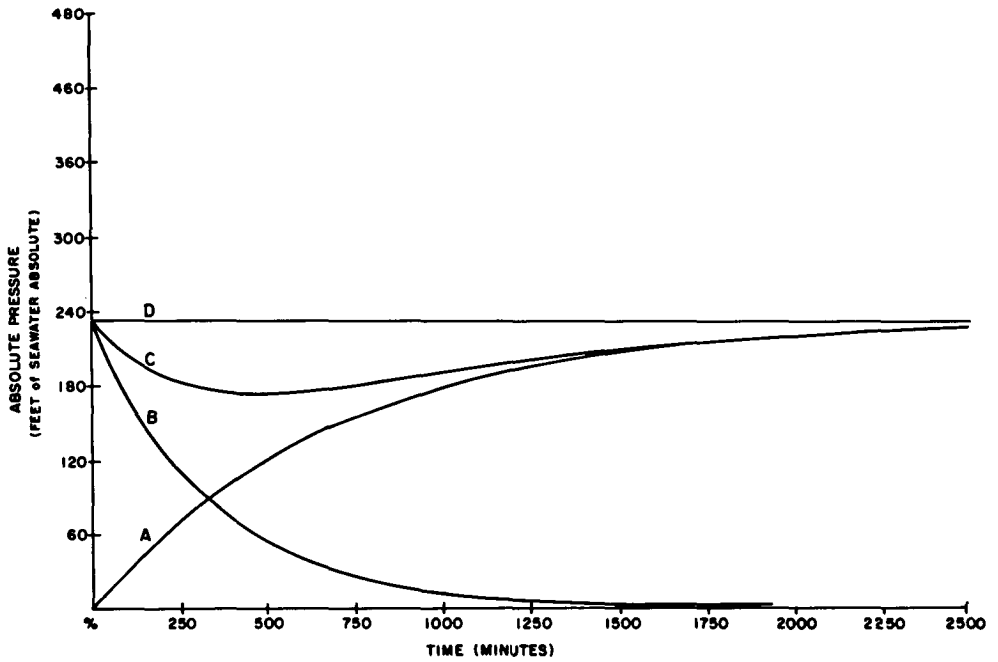

Fig. 2. Subsaturation as a result of isobaric inert gas counterdiffusion in deep tissues. Subsaturation is achieved as total inert gas tension (Curve C) decreases when an inert gas with half time of 480 min (Curve A) is substituted for an inert gas with half time of 240 min (Curve B) in breathing mixture. D is total inert gas tension in breathing mixtures.

Bottom times of one day at 100, 200 and 300 fsw did not allow time for complete equilibration of inert gas tensions in all body tissues, but all tissue compartments were assumed to be saturated with the helium-oxygen chamber mixture at the start of periods when breathing mixes containing another inert gas were employed alternately.

The decompression concepts put forth by Boycott, Damant, and Haldane (3) for analyzing the transport of inert gases within the body are widely accepted. Their model treats the body as a group of discrete parallel mathematical compartments, or half-time tissues, which exchange inert gas in solution with the blood. The blood is, in turn, assumed to be at equilibrium with the inspired gases. Schreiner (19) suggested a 15-compartment concept, based on the Haldane model, which assumes that the body tissues have a wide range of blood perfusion rates and percentages of fat content (Table II). This model further assumes that rates of inert gas exchange in the tissue compartments are functions of gas solubility in the blood and tissue compartments, as well as functions of the blood perfusion rates of the tissue compartments. Because this model offers a rational relationship for establishing half times for several inert gases in a single tissue compartment, Schreiner's 15-compartment approach was chosen to demonstrate changes in total gas tensions that can occur during isobaric counterdiffusion in deep tissues.

Actual computations were carried out using the PADUA (Pennsylvania Analysis of Decompression for Undersea and Aerospace) computer program, which calculates cumulative

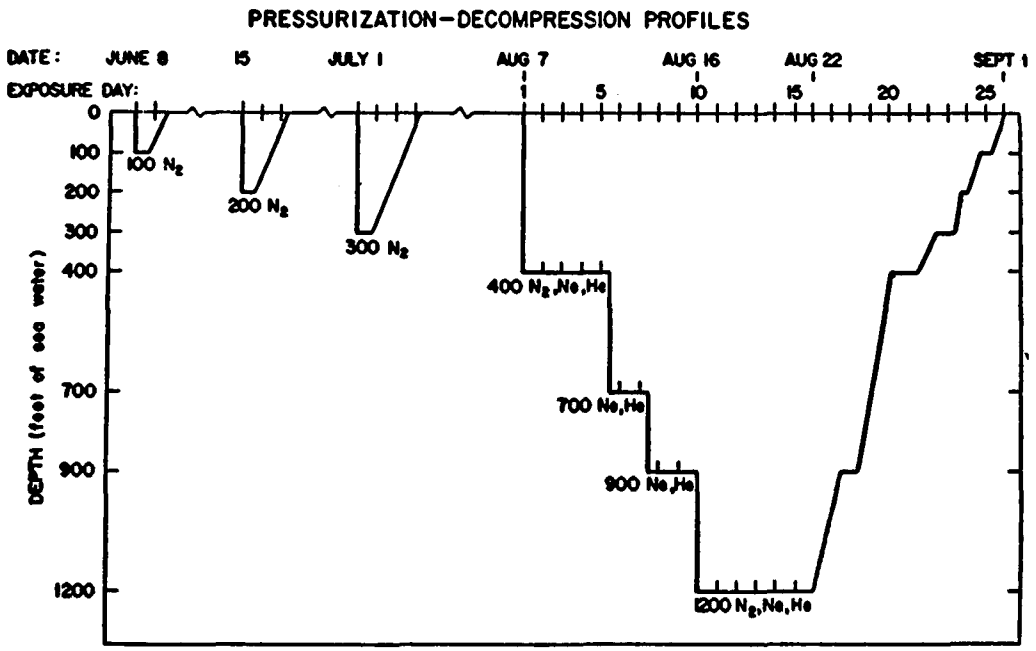

Fig. 3. Sequence of exposures to increased pressure in environmental chamber system. Ambient gas at 100 fsw on June 8 was N_2 with normoxic P_{O_2}. Gases indicated on figure are those breathed during experimental intervals; these gases differed from ambient gas during part of each experimental day. (From C. J. Lambertsen and J. Idicula, A new gas lesion syndrome in man induced by "isobaric gas counterdiffusion." *J. Appl. Physiol.* 39:434–443, 1975).

tissue inert gas tensions by a standard exponential gas exchange formula. For gas exchange during periods that did not involve a change in ambient pressure, the formula takes the form

$$\pi_f = P_I + (\pi_0 - P_I)\frac{1}{2^{t/H}}$$

where π_f = final tissue tension of inert gas; π_0 = initial tissue tension of inert gas; P_I = inspired partial pressure; t = exposure time; and H = half time of the tissue.

The PADUA program was also used to examine theoretical changes in inert gas tensions that could result if varying half-time relationships for inert gas exchanges were considered in a tissue compartment.

The Predictive Studies III program involved four subjects, who breathed the different inert gases alternately. Inert gas tension values taken from one subject for each depth were selected to illustrate the group's experience at that depth.

Analysis

A minute-by-minute analysis of the partial pressures of each inert gas and of the total inert gas reveals patterns of subsaturation and supersaturation in each tissue compartment, similar

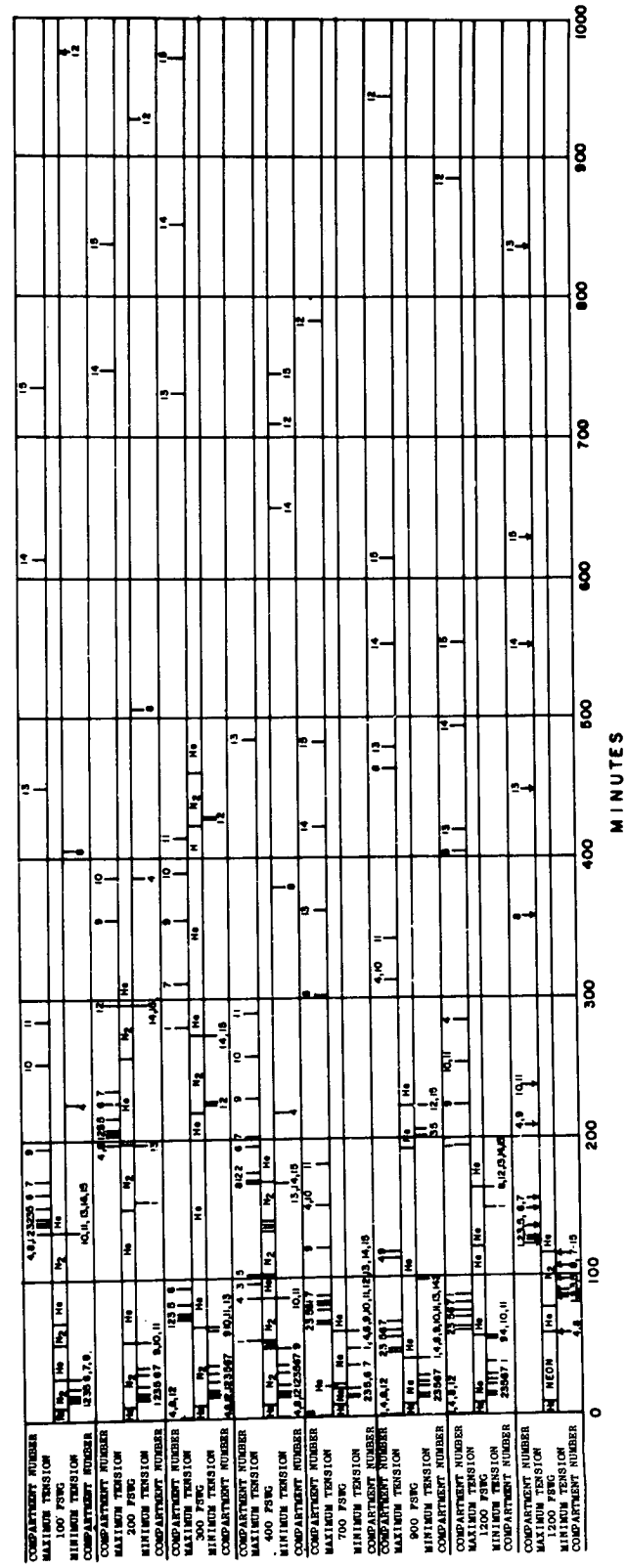

Fig. 4. Relation of maximum and minimum total inert gas tensions in 15 theoretical tissue compartments to sequence of changes in respiratory gases at depths of 100, 200, 300, 400, 700 and 900 fsw, and during 2 separate sequences at 1200 fsw. Helium is principal inert gas in chamber mixture. Small numbers indicate tissue compartment that reached its maximum or minimum total inert gas tension at a particular time. Interval between bars shows duration of breathing inert gas mixture indicated.

TABLE I

BREATHING MIXTURES USED AT 100, 200, 300, 400, 700, 900 AND 1200 FSW; N_2 AND NE MIXTURES DELIVERED BY MASK IN DRY CHAMBER

	He-O_2 Chamber Mixture (Saturation Gas)				N_2 Mixture				Ne Mixture			
Depth, fswg	O_2%	He%	N_2%	Ne%	O_2%	He%	N_2%	Ne%	O_2%	He%	N_2%	Ne%
100	9.9	78.7	11.4	0	5.2	2.5	92.3	0				
200	3.2	83.9	12.9	0	3.0	0	97.0	0				
300	2.1	86.8	11.1	0	2.1	0	97.9	0				
400	1.6	91.6	6.8	0	1.5	43.5	55.0	0				
700	1.0	96.0	0	3.0					1.8	32.9	0	65.3
900	0.7	95.2	0	4.1					1.3	25.7	0	73.0
1200	0.6	97.0	0	2.4	1.0	76.0	23.0	0	1.1	31.5	0	67.4

TABLE II

CALCULATED HALF TIMES OF TRANSPORT OF HE, NE, AND N_2 FOR COMBINATION OF 4 REPRESENTATIVE RATES OF BLOOD FLOW AND 4 LEVELS OF TISSUE FAT CONTENT

Blood Flow, cc/min/cc	Fat Content of Tissue, % 0	30	70	100
0.3	He | Ne / N_2	3 | 3 / 5	3 | 4 / 9	4 | 5 / 12
0.1	7 | 7 / 7	8 | 9 / 15	10 | 12 / 27	12 | 15 / 35
0.03	23 | 23 / 23	28 | 31 / 52	34 | 41 / 89	39 | 49 / 118
0.0085	81 | 81 / 81	99 | 108 / 182	122 | 145 / 315	139 | 171 / 416

Values are minutes. (Adapted from (19)).

to those shown in Fig. 5. Each theoretical tissue compartment demonstrates its own profile, as shown in this tracing of total inert gas pressures for compartments 9 through 15. The time course and degree of maximum supersaturation and subsaturation varied widely from tissue to tissue. Maximum supersaturation and subsaturations in each theoretical tissue compart-

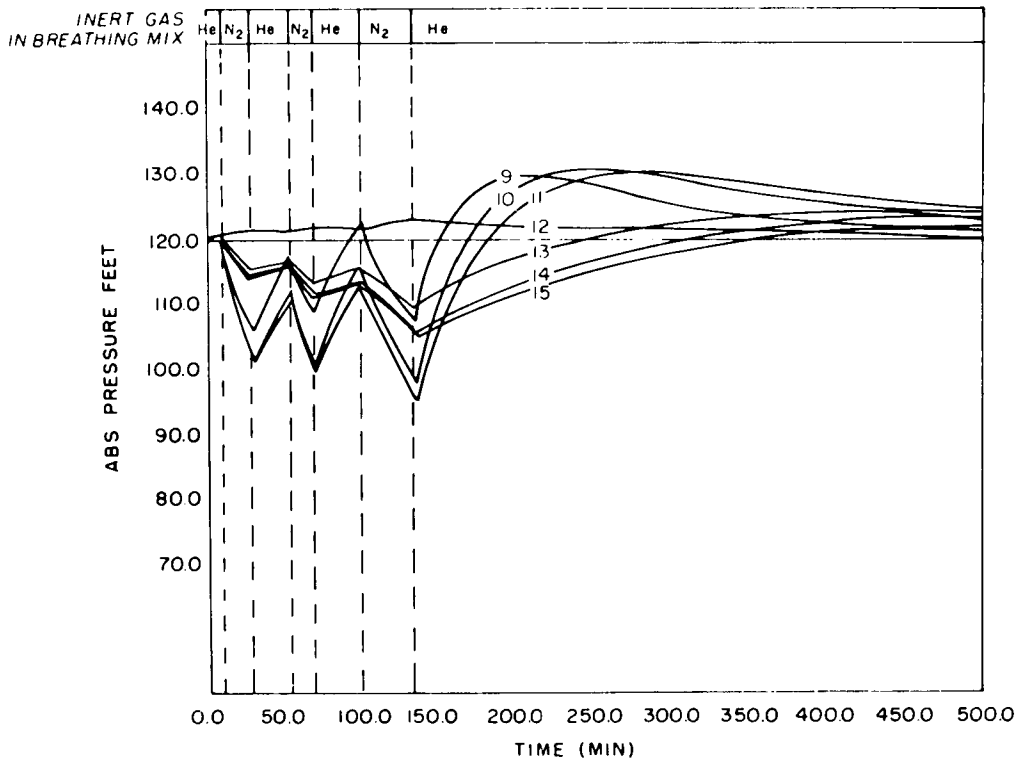

Fig. 5. Total tissue inert gas tensions in tissue compartments 9–15 at 100 fsw related to sequence of changes in respiratory gases. Helium is principal inert gas in chamber mixture. Interval between bars shows duration of breathing inert gas mixture indicated.

ment at depths of 100, 200, 300, and 400 fsw are shown in Fig. 6, and for 700, 900 and 1200 fsw in Fig. 7. The times when maximum deviations occurred in each tissue compartment are indicated in Fig. 4. Most tissues showed a decrease in total inert gas tension during each period of breathing the dense inert gas, as expected, and an increase, often to supersaturation levels, when breathing of chamber gas was resumed. Tissues described as having no lipid content which had gas exchange rates limited only by the blood perfusion rate (tissues 4, 8 and 12 in Table II) showed only slight changes in total inert gas tension caused by minor variations in the oxygen content of the breathing mixes compared to the chamber mixture.

The onset of itching occurred 11 min into the second period of nitrogen breathing and 21 min into the third period of PS III. Skin lesions appeared 4 min into the fourth period. Similarly, at 1200 fsw itching of the forehead occurred 28 min into the first period of neon breathing and became more severe toward the end of the period. Many cycles of alternating the breathing mixtures were not associated with symptoms. The onset of nausea, vomiting and vertigo in the final exposure at 1200 fsw brought the period of breathing 8.6 ATA of nitrogen mixed with helium and oxygen to an end after 39 min.

Itching and skin lesions developed repeatedly during breathing of heavier inert gases, when

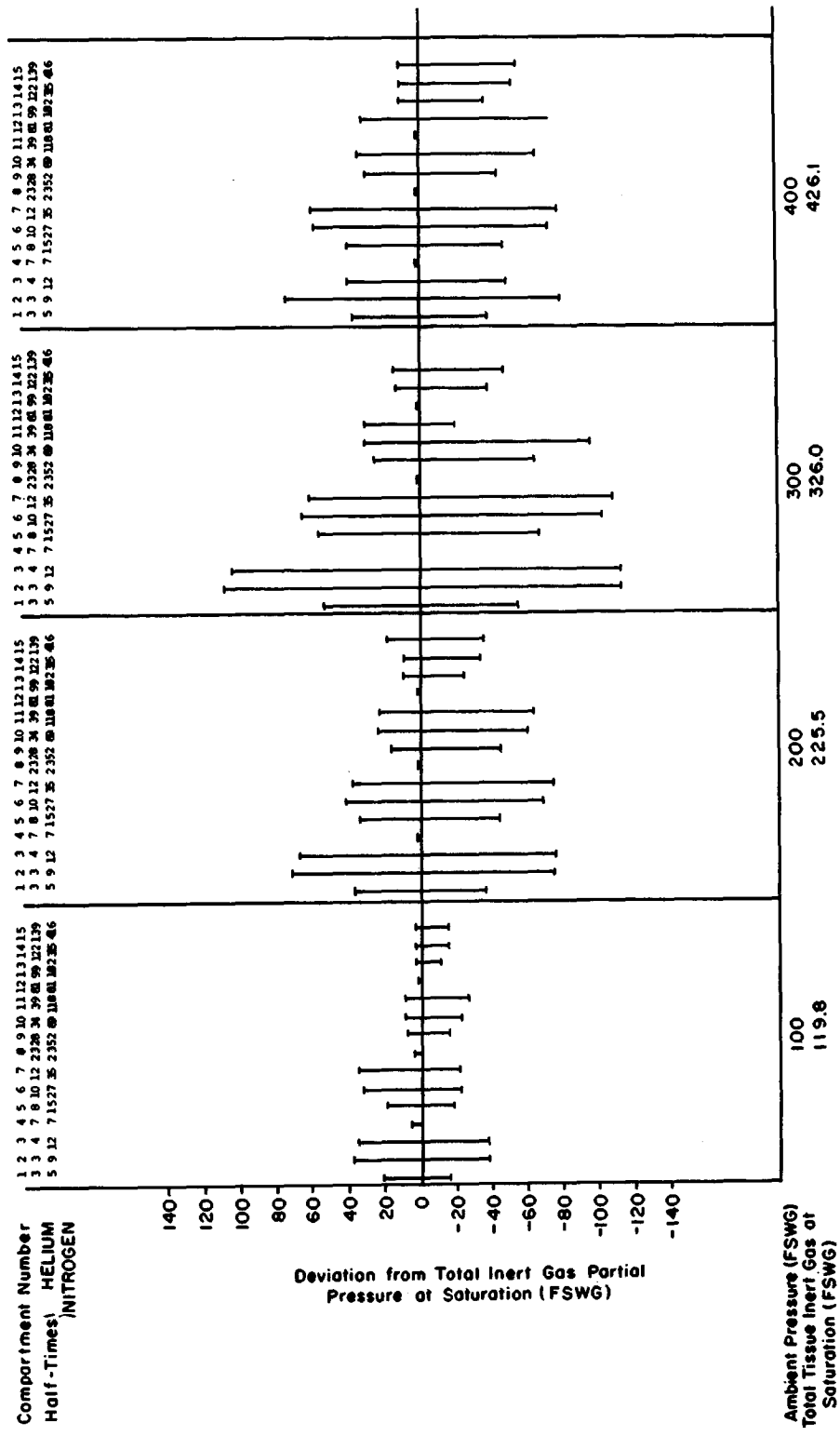

Fig. 6. Maximum deviations of total inert gas partial pressure in each of 15 theoretical tissue compartments as a result of alternating breathing mixtures containing helium and nitrogen at 100, 200, 300 and 400 fsw simulated pressure.

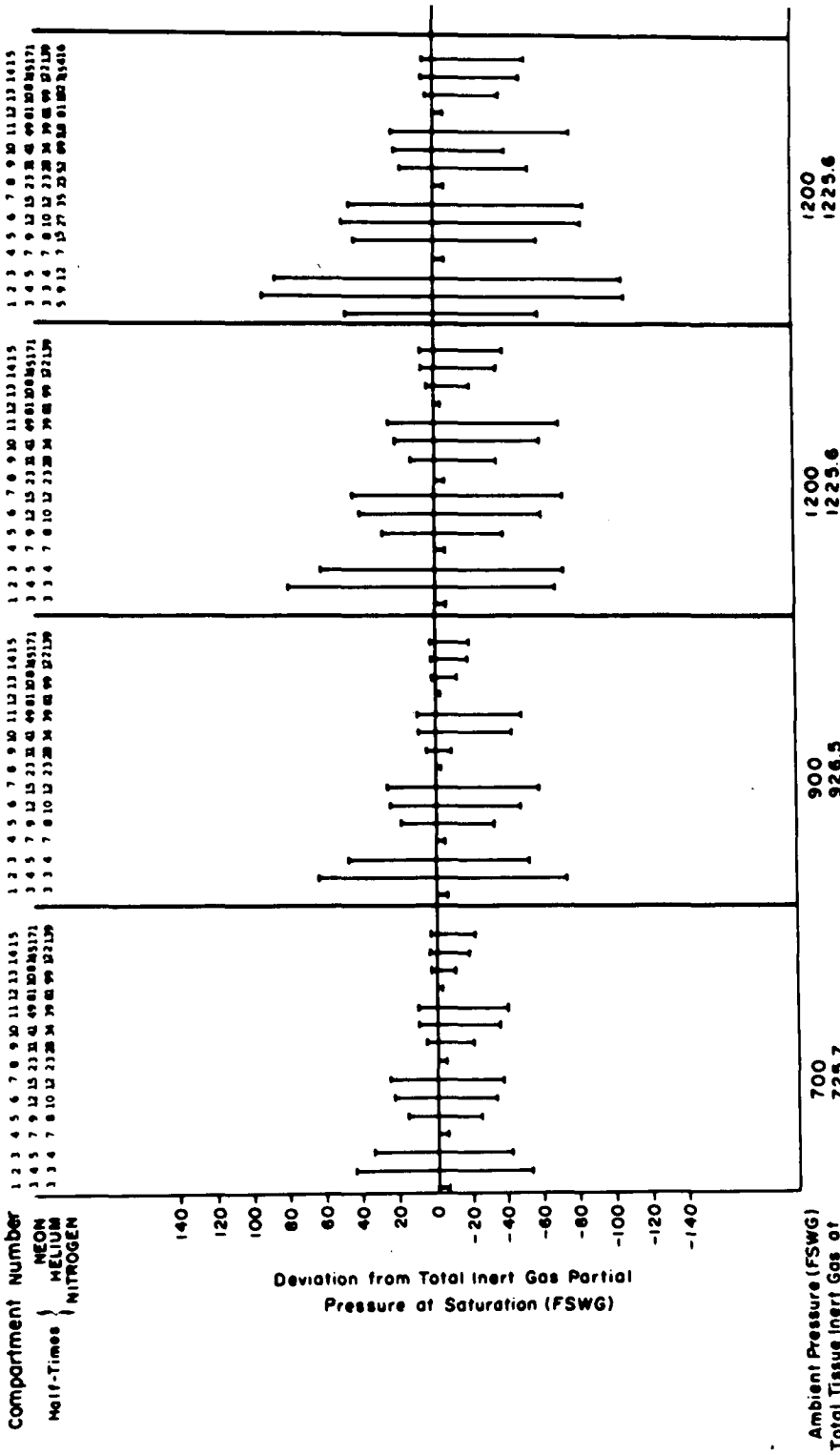

Fig. 7. Maximum deviations of total inert gas partial pressure in each of 15 theoretical tissue compartments as a result of alternating breathing mixtures containing helium and neon at 700, 900 and 1200 fsw and helium, neon and nitrogen at 1200 fsw simulated pressure.

counterdiffusion through the skin was producing an increasing supersaturation in the superficial tissues. The deep tissues, however, were achieving a state of subsaturation at these times. Maximum stresses of supersaturation were achieved in all compartments after the return to helium breathing. It is interesting to note that the vertigo, nausea and vomiting experienced in the final exposure at 1200 fsw continued for some 18 hours or more after breathing of the heavier nitrogen was discontinued. If gas phases generated by the superficial tissue form of isobaric counterdiffusion were responsible for initiating the symptoms, the resolution of these bubbles may have been slowed by the diffusion of inert gas into the bubbles from one or more tissue sites in the body, which theoretically were reaching their maximum supersaturations serially for many hours after the return to helium breathing.

A single episode of nausea and vomiting occurred at the 300-fsw level almost 30 min after completion of the second asymptomatic nitrogen-breathing period. Could deep tissues reaching the peak of their isobaric supersaturation have initiated or caused the growth of previously asymptomatic bubbles that had been generated by the superficial form of isobaric counterdiffusion during the nitrogen-breathing period? This seems unlikely, because the symptoms resolved spontaneously and did not recur later in the day after a third period of nitrogen breathing. Nevertheless, the presence of tissues in a supersaturated state during this isolated incident of nausea and vomiting is worthy of note.

Predictive Studies III involved alternating inert gases in the breathing mixtures for relatively brief intervals. An analysis of the total inert gas tensions achieved after a single change in inert gases for a long period was undertaken. Figure 8 compares two theoretical compart-

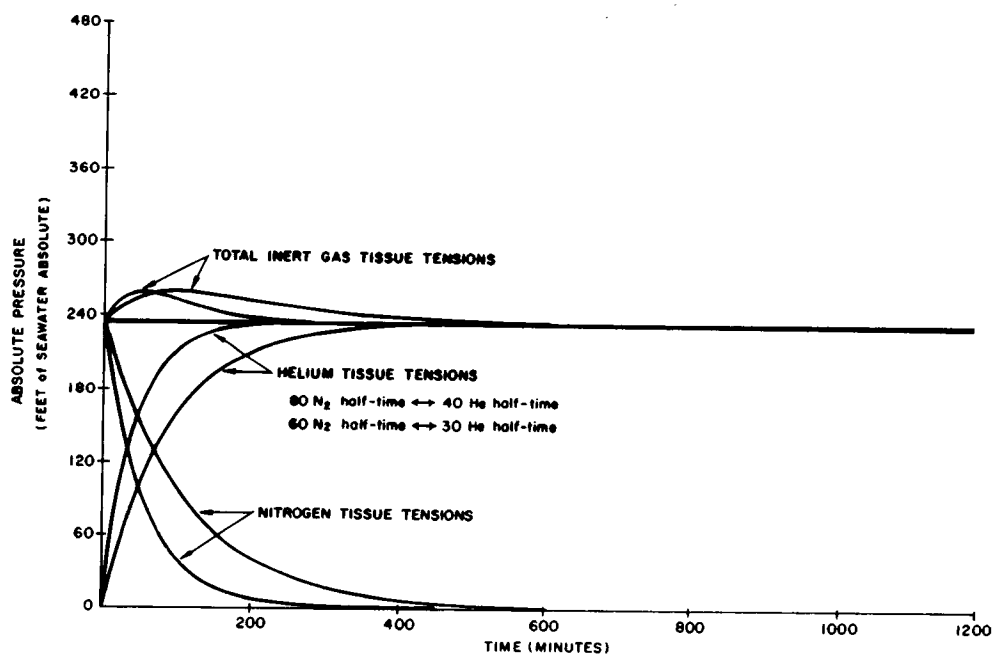

Fig. 8. Comparison of total inert gas supersaturation generated by isobaric counterdiffusion in 2 theoretical tissue compartments when helium was substituted for nitrogen in breathing mixture. Half times for 2 inert gases in each compartment are indicated. Total inert gas tensions in breathing media are indicated by a straight line.

ments in which the ratio of the half times of the inert gases is a constant 2:1 for washout gas/uptake gas, and the actual half times are N_2 = 80, He = 40, and N_2 = 60, He = 30. It can readily be observed that the peak supersaturation achieved was the same in each case, but that supersaturation was achieved more slowly and maintained much longer in the compartment with the slower half times. Figure 9 shows that the supersaturation theoretically achieved increases proportionally as the ambient pressure is increased, using half times for washout gas/uptake gas of N_2 = 480, He = 240 for the tissue compartment. The time to achieve maximum supersaturation is the same at each depth. However, a significant degree of supersaturation may be achieved more quickly and maintained longer if the ambient pressure of the exposure is greater.

The effect of varying the uptake gas in a single compartment can be seen in Fig. 10. Here a washout gas with a half time of 480 min is compared to three uptake gases with half times of 180, 210 and 240 min. The supersaturation achieved with each combination shows an increase as the half time of the uptake gas decreases and the ratio of the half time of the washout gas to the half time of the uptake gas increases. This can be seen to approach a theoretical maximum of twice the ambient pressure (Fig. 11) if very great half-time ratios could be achieved. Conversely, the subsaturation achieved can be seen to approach 0 pressure as the ratio of the washout gas to the uptake gas decreases from one toward zero. The absolute values of the respective half times influence the time course of the deviation, but only the ratio of the half times affects the total amount of the supersaturation or subsaturation.

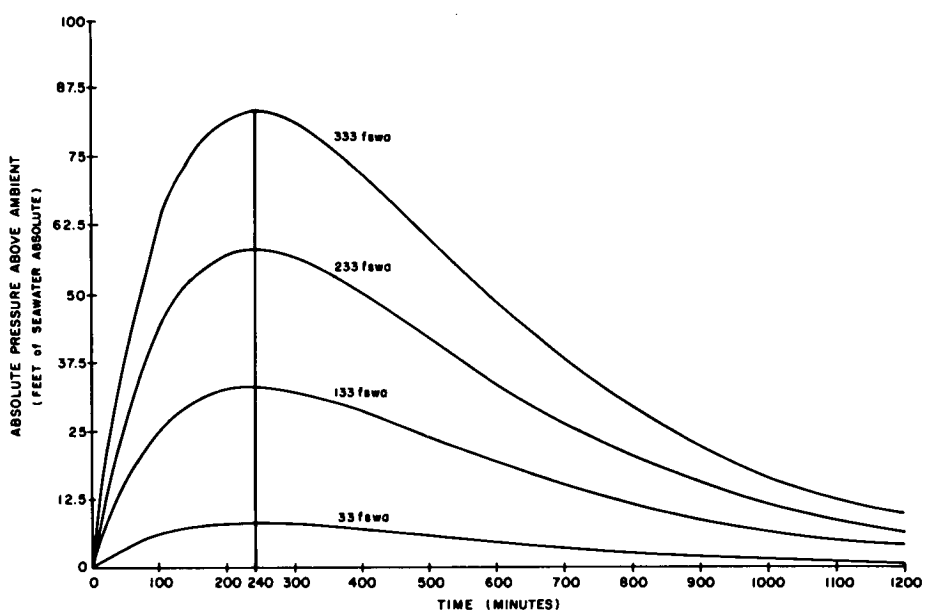

Fig. 9. Supersaturation (absolute pressure above ambient) achieved by isobaric counterdiffusion of N_2 and He in a tissue compartment with half times of N_2 = 480, He = 240 min at 33, 133, 233 and 333 fsw absolute simulated pressure.

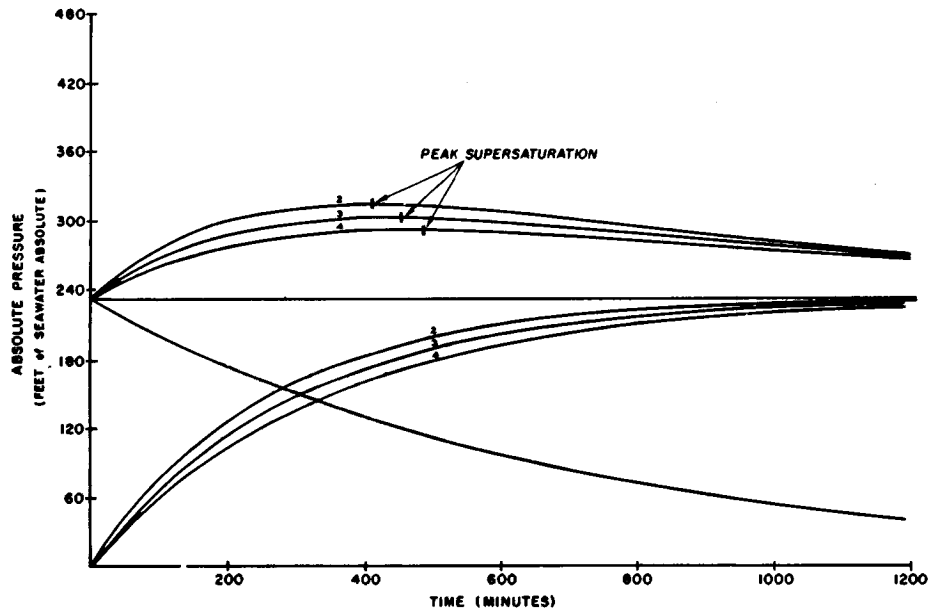

Fig. 10. Comparison of supersaturation achieved by isobaric counterdiffusion when an inert gas with half time of 480 min is replaced by a different inert gas with half time of 180 (Curve 2), 210 (Curve 3) or 240 (Curve 4) min.

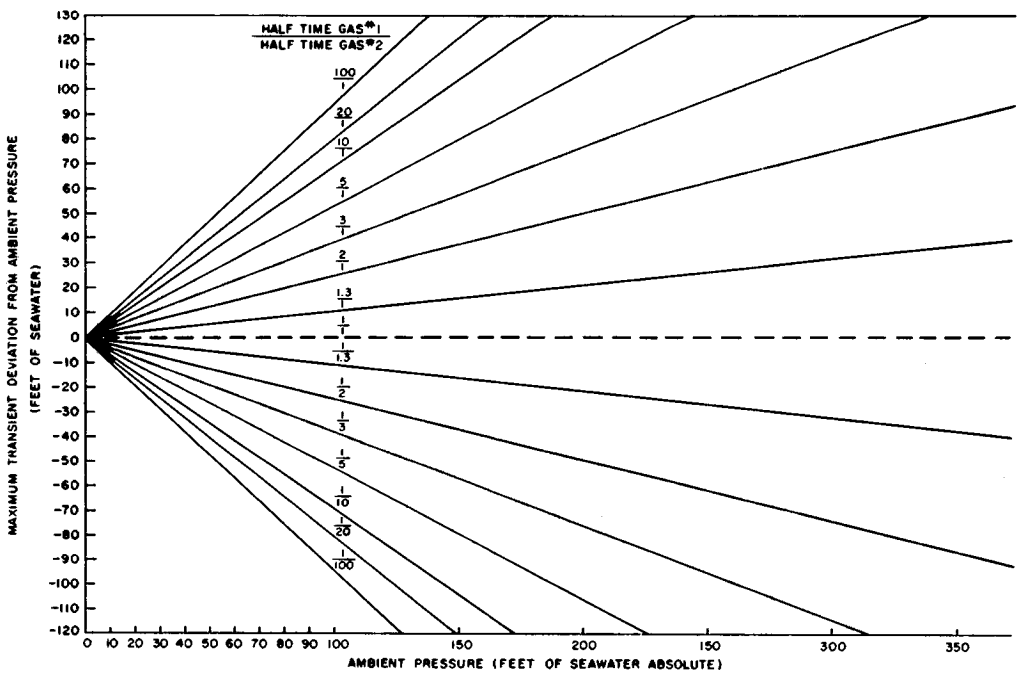

Fig. 11. Maximum transient deviation in total inert gas tissue tension related to ambient pressure of exposure and ratio of half time of gas being washed out to half time of gas being taken up during isobaric counterdiffusion in deep tissue.

Discussion

Results indicate that differential rates of gas uptake and elimination for different inert gases may lead to transient desaturation and excess saturations of deep body tissues without a change in ambient pressure. Several investigators have reviewed and offered alternatives or modifications to the Haldane concepts that have become conventional in establishing decompression procedures (6, 11, 12, 14, 17, 18). Experience indicates that there is a difference in the rates of uptake and elimination of the inert gases used in diving operations (15). The majority of the mathematical models used in computing gas exchanges in the body would predict isobaric transient deviations in total inert gas tensions. If, for example, the difference in exchange rates of inert gases in a tissue is assumed to be caused by their molecular weights and a diffusion-limited system between the tissues and capillaries, transient isobaric counterdiffusion would still produce supersaturation and subsaturation.

Relative half times and decompression characteristics of inert gases have been explored by several investigators (4, 5), but the actual exchange rates for real tissues in the body are not accurately known. The choice for half-time values assigned to theoretical tissue compartments for different inert gases is therefore somewhat arbitrary. There may well be a wide range of half times for an inert gas that corresponds to a single half time for a second inert gas when several tissues are considered, as illustrated in Fig. 10. Several variables affect the total inert gas tissue tensions induced by isobaric counterdiffusion, including the ambient pressure of the experiment, the duration of the periods of breathing inert gases alternately, and the relative partition coefficient between blood and the tissues for each gas. The effects of other variables such as oxygen and carbon dioxide partial pressures, ambient temperature, dry chamber or wet diving, muscular work and a host of other environmental and physiological factors have yet to be considered in relation to deep-tissue isobaric inert gas counterdiffusion.

The changes in total inert gas tensions theoretically analyzed during Predictive Studies III were relatively small, but exposure times to the denser inert gases were relatively short. Behnke (1) and Workman (21) have indicated that the half times of different inert gases may be almost equal in well-perfused (short half-time) tissues. Therefore, exposures of relatively brief duration may not stress the "slower" tissues sufficiently to produce problems.

Keller and Bühlmann (14) have demonstrated the advantages of isobaric deep-tissue counterdiffusion subsaturation in shortening decompression from brief, deep dives. A word of caution is in order for those who would use this technique. Care must be taken to avoid mask breathing of "slow" inert gases while surrounded by a "fast" gas, because symptoms of the isobaric counterdiffusion syndrome may develop in superficial tissues.

Deep-tissue supersaturation from isobaric counterdiffusion might become important in several other diving situations. Individuals suffering from decompression sickness or gas embolism after deep, long dives using nitrogen or neon as an inert gas may require recompression to rather high pressures. If helium–oxygen mixtures are utilized as treatment gases in this situation, the symptoms may be exacerbated, because counterdiffusion produces supersaturation and bubble growth, rather than resolution, in deep tissues.

Deep dives utilizing hydrogen might be safe and feasible if the inert gas shift occurred at depths deeper than the oxygen-combustion zone. This shift to hydrogen might become hazardous if prolonged periods of breathing a slower inert gas at elevated pressures preceded the shift. The utilization of helium, neon or hydrogen to avoid inert gas narcosis during excursions from a nitrogen-based breathing mixture in a saturation diving situation might lead to deep-tissue counterdiffusions that force exceptionally long decompression stops

before returning to the saturation chamber and its breathing mixture (9). Decompression from dives involving the alternate breathing of inert gases must not be undertaken without proper consideration of the total inert gas tissue tensions generated by inert gas counterdiffusion. Utilization of a light inert gas mixture such as helium in a breathing mixture during decompression from elevated pressures where a denser inert gas was utilized may prove particularly hazardous.

The utilization of gaseous anesthetics at elevated chamber pressures in a closed breathing system might prove dangerous, because supersaturation may be generated by isobaric counterdiffusion if high ratios exist for the half times of the inert gas in the tissues of the patient compared to the half times of the anesthetic gas.

The possible consequences of this isobaric counterdiffusion supersaturation producing significant stresses for prolonged periods in the rigid confines of bone marrow must be considered by investigators examining the etiology of, and experimental models for producing, dysbaric osteonecrosis (10).

Summary

Theoretically, it has not been shown that supersaturations and subsaturations generated in deep tissues of the body by alternation of inert gases in the breathing mixtures are responsible for the development of symptoms in the absence of pre-existing bubbles. It is evident that deep-tissue isobaric inert gas counterdiffusion would probably grossly exaggerate the growth of bubbles already generated by decompression sickness, traumatic gas embolism, or the superficial form of isobaric inert gas counterdiffusion.

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DEVELOPMENT AND OPERATIONAL VALIDATION OF ACCELERATED DECOMPRESSION TABLES

William P. Fife, Michael J. Mezzino and Roger Naylor

Commercial divers who make repeated heavy working dives daily often find after a week or more that their tolerance to bends has been reduced. After such repeated exposures, a dive which would initially have been uneventful may produce bends which require treatment. One method of minimizing these bends is bringing the diver to his first decompression stop more slowly. Such an approach, however, also extends the time these divers must remain deeper than the first scheduled stop. The success of this technique appears to contradict the accepted decompression concept, which states that the diver should be brought to the lowest permissible ambient pressure as quickly as possible to produce a high driving pressure for the outward diffusion of dissolved gas.

The traditional concept of shallow first decompression stops was questioned by LeMessurier and Hills (6), who conducted a study of the divers of the Torres Straits. They showed that these divers, some of whom were working at depths in the vicinity of 244 fsw on air, were taking their first stops as deep as 180 fsw. Also, in some instances they surfaced directly from the 30-fsw stop, eliminating the 20- and 10-fsw stops entirely. Bends in these divers averaged about 7%, but varied greatly among diving crews. In one instance, for example, a particular diving crew had a 16% incidence of bends, but most of these occurred on air dives to depths greater than 200 fsw. The astonishing thing is that the incidence of bends in the Torres Straits' divers was not much higher and the symptoms more severe than reported.

These observations must be viewed in light of studies of bubble dynamics, such as those presented by Hills (4). This work showed that the presence of bubbles added a new dimension to Haldane's equation, which could only be accounted for if bubbles developed much deeper than previously supposed. The Haldanian concept of decompression is based on preventing bubbles from occurring in the body. If bubbles do in fact appear, then in addition to inherent unsaturation, the driving force which governs the movement of gas across its surface takes the form

$$P = \frac{2\gamma}{r} + t$$

where P = hydrostatic pressure within the bubble, γ = surface tension, r = radius of bubble, and t = tissue pressure.

This somewhat simplified equation shows that since the radius is dependent upon Boyle's Law, the higher the ambient pressure, the smaller the bubble radius will be. This, in turn, results in a larger internal hydrostatic pressure and therefore, a higher driving pressure.

If the presently accepted decompression tables do, indeed, permit bubbles to develop, ascent to shallow first stops would tend to increase their size to the point where the internal driving pressure is significantly reduced or indeed, may become negative. The possibility that bubbles appear at great depths should not be surprising. Behnke (1) and others (2, 5) have suggested that bubble nuclei are always present in the body.

The appearance of gas bubbles at greater depths does not in itself invalidate the Haldanian concept of outgassing. However, it does make it impossible to use Haldane's equation in its original form. The critical question is to determine at what depth bubbles actually appear.

Procedures

As a working hypothesis, if bubbles do occur at a greater depth than previously recognized, an optimum table should have the following characteristics: (1) Deeper stops, since the objective is to prevent formation of the bubbles, or at least keep them small to produce a high driving force; (2) Reduced time at shallower depths, because if bubbles have not been allowed to develop or grow, the subject would not need to remain for long at shallow stops to treat expanded bubbles which, because of size, had a low driving pressure and therefore, slow outgassing; (3) Reduced total decompression time, because outgassing would be most rapid if bubble formation is prevented, and moreover, if bubbles are formed, they would remain small and, therefore, driving pressure would remain high, hastening outgassing.

In selecting an animal model, the large amount of experimental data which British and United States workers have assembled on the goat and the dog was taken into consideration. However, the commercial pig (Hampshire-Yorkshire cross) was chosen as the experimental subject. Preliminary work showed that though this animal does not respond to decompression exactly as a man does, it does respond similarly in some ways. For example, the pig often develops bends on some of the old U.S. Navy Exceptional Exposure Air tables. Further, if the pig is exposed to the same bounce dive profiles which Hawkins et al. (3) and Roby (7) used on man, it develops bends after an almost identical bottom time exposure.

Initially, the effects of deep stops were examined in a rather arbitrary manner, by slightly modifying the old U.S. Navy Exceptional Exposure Air tables in the 150–300 fsw range until about 50% of the animals suffered bends. Time was then arbitrarily deducted from the shallow stops and added to deeper stops (Table I). It was thus possible to reduce the total decompression time shown in the Navy Exceptional Exposure tables by nearly 50% for the same bottom time without developing bends. This caused us initially to limit the allowable vertical movement between stops to 60 fsw for all of the tables, which, of course, resulted in deep stops.

Table II shows a second experimental table compared to the U.S. Navy table for the same depth and bottom time. At the time these tables were developed, they were called "Sizzlers" because they seemed so fast. However, after they were studied with the computer, it was clear they they were still not optimum. Table III shows two even faster computer-derived decompression profiles for the same depth and bottom time as the table shown in Table II. One of these faster tables caused bends, while the other did not. The differences between these two tables illustrate how small an allowance for error the pig offers as a model:

TABLE I

DEVELOPMENT OF DECOMPRESSION TABLE FOR 200 FSW, 120-MIN BOTTOM TIME

		Experiment Number								
Depth, fsw	USN	1	2	3	4	5	6	7	8	9
200	120 min	.....	.....	.....	120 min	.....	.....	.....	.....	.....
140	—	20	20	20	20	20	20	20	20	20
130	—									
120	—									
110	—									
100	6	30	30	30	30	30	30	30	30	30
90	10									
80	10	40	40	40	40	40	40	40	40	40
70	10									
60	24	70	60	30	25	25	30	25	25	25
50	28									
40	40	100	60	25	5	5	5	10	10	10
30	64									
20	98	5	5	5	5	5	5	5	5	5
10	180									
	473	265 OK	215 OK	150	125	125 (m)	130 (m)	130 OK	130 OK	130 (m)

(m) = mild hit; series demonstrates that 130-min table is borderline for the pig.

TABLE II

AIR TABLES, 200 FSW, 30-MIN BOTTOM TIME, SHOWING ARBITRARY ADJUSTMENT OF DEEP STOPS

Depth, fsw	USN, min	Sizzler, (pig)
200	30	30
180	—	2
160	—	2
140	—	2
120	—	2
100	—	4
80	—	6
60	—	8
40	2	10
30	9	—
20	22	20
10	37	—
	73	56

TABLE III

AIR TABLES, 200 FSW, 30-MIN BOTTOM TIME, SHOWING CRITICAL BOUNDARY BETWEEN SUCCESSFUL AND UNSUCCESSFUL DECOMPRESSION IN PIGS

Depth, fsw	Pig #1	Pig #2
200	30 min	30 min
180	1 min	1 min
160	1 min	1 min
140	1 min	1 min
120	1 min	1 min
100	1 min	1 min
80	1 min	1 min
60	1 min	1 min
50	1 min	1 min
40	1 min	1 min
30	1 min	1 min
20	1 min	3 min
10	3 min	3 min
	24 min	26 min
	Bends	No-Bends

there are only 2 minutes between the occurrence of bends and no bends in these tables. The table shown in Table III was used as the basis for extrapolation to a human table (Table IV), which has been used successfully. When this and other similar tables are used, the divers appear to feel better after decompression than they do after using more traditional profiles.

That bubbles appear at very deep depths has now been supported by more direct evidence from several sources. Rubissow and Mackay (8) exposed guinea pigs to a pressure of 10 ATA and examined them by ultrasonic scanning. They were able to see clouds of microbubbles in limb tissue after what appeared to be an 0.5-ATA ascent. Further, it has been noted that electrical impedance across the limb of a subject changes significantly after decompression equivalent to only 20–30 fsw from the bottom (unpublished observations). This shift in impedance probably indicates the appearance of bubbles in the tissue. As a result of this evidence, the maximum permissible vertical ascent between stops has been reduced from 60 to 20 fsw; our current decompression tables now have this arbitrary 20-fsw limit both for animals and men.

The Pig-to-Man Extrapolation Model which we have formalized follows the familiar continuous system compartmental analysis simulation model. Basically, it supports the traditional Haldane concept with what are believed to be important constraints: (1) vertical ascent between stops should not exceed 20 fsw; and (2) no stop should be less than 1 minute in duration.

In addition, two experimentally derived values are included in the model. One is the permissible surfacing tension, which is the maximum inert gas tension allowed in the tissues at the time the diver leaves the 10-fsw stop. This tension was found in both pigs and men by determining the greatest depth at which the subject could be saturated and then returned directly to the surface without bends. The values are expressed in absolute feet of seawater, and may be seen in Table V. These values serve two purposes in our model: to prevent surfacing until all tissues are below the surfacing tension limit, and to provide a surfacing tension ratio used to extrapolate from pig to man.

Another experimentally derived value is related to the solubility and diffusion rates of helium and nitrogen (5). These data have caused us to use a 3:1 ratio in matching tissue half times for helium and nitrogen.

Our computer program is written to show the decompression stops and to identify the tissue which is controlling at that moment. Among other things, the program graphically prints out the washout curve for each inert gas and tissue compartment.

Discussion

Although these tables are under constant revision, it may be useful to examine several more of them in detail. Initially, it seemed that in the 300–400 fsw diving range, the pig responded to decompression in a manner similar to man. We now realize that this was in part because the contraints placed on the pig were not optimal. Even so, it was possible to develop a 400 fsw, 30-minute bottom time table for man which is now being successfully used offshore in operational dives. This profile may be seen in Table VI. This table shows that the total time at each stop was similar for both pig and man, although the times on air and oxygen were not precisely the same. One difference occurs at the point where the arbitrary 1-min stops change to computer-controlled stops. In the case of man, this point is reached at 230 fsw, while in the pig, the first stop based on calculated tissue limitation falls at 180 fsw. This reflects one of the true differences between pigs and men.

TABLE IV

AIR TABLES, 200 FSW, 30-MIN BOTTOM TIME, SHOWING EXTRAPOLATION FROM PIG TO MAN

Man	Pig
180'- 1 min	1 min
160'- 1 min	1 min
140'- 1 min	1 min
120'- 1 min	1 min
110'- 3 min	
100'- 2 min	1 min
90'- 2 min	
80'- 5 min	1 min
70'- 6 min	
60'- 6 min	1 min
50'- 6 min	1 min
40'-12 min	1 min
30'-14 min	1 min
20'-15 min	3 min
10'-27 min	3 min
Surface	Surface
Total time, 118 min	Total time, 26 min

1 min taken between each stop for human table (see text for explanation).

TABLE V

PERMISSIBLE SURFACING TENSIONS

Mixture	Pig	Man
Air	84.6	49.8
He/O_2	100.3	57.7

Values are feet of seawater, absolute.

TABLE VI

COMPARISON OF TESTED HUMAN TABLE WITH PIG TABLE, 400 FSW, 30-MIN BOTTOM TIME

Man		Pig	
380'- 1 min		1 min	
360'- 1 min		1 min	
340'- 1 min		1 min	
320'- 1 min		1 min	
300'- 1 min		1 min	
280'- 1 min		1 min	
260'- 1 min		1 min	
250'- 1 min			
240'- 1 min		1 min	
230'- 2 min			
220'- 1 min		1 min	
210'- 3 min			
200'- 4 min		1 min	
190'- 4 min			
180'- 4 min		3 min	
170'- 5 min		4 min	
160'- 5 min		5 min	
150'- 8 min		5 min	
140'-10 min		5 min	
130'-11 min		6 min	
120'- 7 min	To Air	6 min	
110'- 8 min		9 min	
100'- 9 min		9 min	
90'-19 min		11 min	
80'-21 min		18 min	
70'-25 min		24 min	
60'16 min	O_2	60'-15 min	O_2
50'- 3 min	O_2	5 min	Air
5 min	Air	50'-22 min	O_2
15 min	O_2	5 min	Air
40'- 4 min	O_2	40'-35 min	O_2
5 min	Air	5 min	Air
19 min	O_2	30'-21 min	O_2
30'- 5 min	Air	5 min	Air
20 min	O_2	21 min	O_2
5 min	Air	5 min	Air
12 min	O_2	20'-28 min	O_2
20'- 7 min	O_2	5 min	Air
5 min	Air	28 min	O_2
20 min	O_2	5 min	Air
5 min	Air	10'-33 min	O_2
20 min	O_2	5 min	Air
5 min	Air	33 min	O_2
10'-20 min	O_2		
5 min	Air	Surface	
20 min	O_2		
5 min	Air	Total time, 415 min	
20 min	O_2	Total O_2 time, 225 min	
5 min	Air		
19 min	O_2		
Surface			
Total time, 450 min			
Total O_2 time, 220 min			

There are several other factors which must be considered in extrapolating from experimental animal dives to man. One is the rapidity with which changes in inert gas mixtures are made. Since it is necessary to flood the chamber with the gas being breathed by the pig, gas changes are gradual. This is not the case with a man using a mask. We believe that even at these relatively shallow depths a sudden shift in inert gas may promote bubble formation. Another factor reflecting a difference between table extrapolation from pigs and men is that, to provide an adequate safety margin for human variability, human decompression was arbitrarily calculated with an additional 15 minutes on the bottom, which calls for a more extended decompression. We do not yet know if this last modification is necessary. Even though our 400-fsw table is faster than many others currently in use, we do not believe we have yet reached the optimum decompression time for this depth and bottom time. Some current work suggests several ways of improving it.

We cannot escape the feeling that most of the currently accepted tables are really treatment tables, designed to resolve bubbles created during ascent to the first stop. This probably accounts in part for the long shallow stops required by most current tables, and may also explain why tables which use deep stops reduce the time at shallow stops. In some instances, if deep stops are used, it is possible to eliminate some of the shallow stops altogether, without the appearance of clinical symptoms of bends. In many instances the additional gas dissolved during deeper stops can be removed from the tissue by shallow stops, which results in shorter total decompression times.

Table VII demonstrates another feature of these deep-stop tables, a 250-fsw, 30-minute trimix excursion dive. On arrival at 80 fsw, the diver may choose to switch to a 50/50 nitrogen/oxygen mixture or continue to the surface with air. On reaching 40 fsw, the diver has another choice, to use oxygen or to continue on air. Since on leaving a certain depth the tissue load is identical to that for the same depth on another gas mixture, it is possible to shift between columns as the need arises. If, for example, a 50/50 mixture is not available, the diver may continue to 40 fsw on air, or he may elect to go to oxygen at 40 fsw. If the diver begins to feel that the oxygen exposure is excessive, he can shift at once to air and continue to the surface.

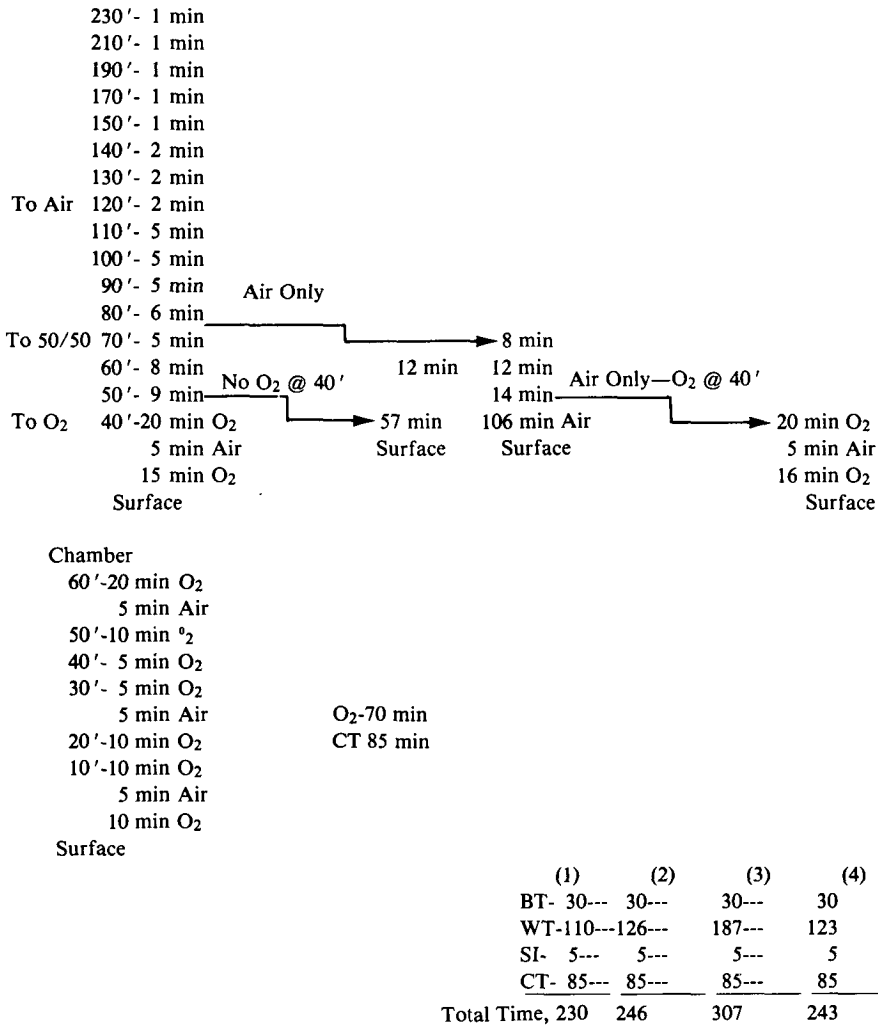
Operational Advantages

Although it is operationally desirable to reduce total decompression time through the use of deep stops, there is another justification for their use. If bubbles occur at such an early point in the decompression profile, their effect on the body must be considered, even though they appear to be clinically asymptomatic. These circulating bubbles may have neurological consequences of a subtle but progressive nature; they may, for example, contribute to the mental deterioration sometimes seen in divers exposed to heavy diving for an extended period of time.

Our approach to the decompression problem has several operational aspects worthy of note. First, we used a portable computer terminal which permits immediate access to the computer program from any telephone. Tables are frequently generated in one of our laboratories 150 miles from the computer, and table testing begins within a few minutes after the table is generated. Within a few minutes after an unsuccessful dive a new table can be generated and testing started. This speed of response permits tables to be altered while a dive is in progress.

TABLE VII

OPERATIONALLY TESTED TABLE, 250 FSW, 30-MIN BOTTOM TIME, MODIFIED TO PERMIT CHANGE OF GAS MIXTURE DURING DECOMPRESSION



	(1)	(2)	(3)	(4)
BT-	30---	30---	30---	30
WT-	110---	126---	187---	123
SI-	5---	5---	5---	5
CT-	85---	85---	85---	85
Total Time,	230	246	307	243

During one dive, a minor problem required a table to be altered during ascent. A new table was created in less than 15 minutes, while the divers remained at a stop.

This capability may be a useful asset to offshore diving. There is a possibility that a bank of tables in the public domain will be available in program form. The portable computer terminal, the size of a portable typewriter, could be carried to the dive site. If alteration of a table was required, or if a different table was needed, it could be received in printout form at the dive site within 15–30 minutes by radiophone.

Another advantage of our approach is that the experimental use of the pig permits rapid development of tables for humans. It is possible to start with an untested table, and to determine a safe table for the animal within a few dives. This table can then be extrapolated to man and validated operationally with a few human tests. In the development of the 400-fsw table shown in Table VI, the initial computer-derived table was generated in just three days. It was tested on the pig and modified to produce a clean dive in less than 7 days. The human tables extrapolated from this work were successfully used operationally within about two months after the initial work was begun.

The theoretical significance of the arbitrary deep decompression stops described in this paper should be considered. They tend to optimize a basic mechanism related to gas movement during decompression. These inert gas dynamics need to be undestood, so that, when properly modeled, valid, optimal deep stops can be generated. Though it is now possible to generate workable deep-stop decompression tables, we believe that such deep stops must satisfy some as yet poorly understood principle. This study has been designed primarily to produce a family of improved, practical decompression tables for both air and gas diving which will increase the comfort and safety of the diver, and which will also meet the rigorous economic and operational needs of commercial diving.

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THEORY AND DEVELOPMENT OF SUBSATURATION DECOMPRESSION PROCEDURES FOR DEPTHS IN EXCESS OF 400 FEET

P. B. Bennett, R. D. Vann, J. Roby and D. Youngblood

It is presently believed that the incidence of decompression sickness in offshore work at depths greater than 400 fsw is 10% or greater. The decompression tables in use are not readily available, and little is known about how they have been computed or how effectively they have been tested.

This research is designed to determine how a man may best be safely decompressed, and then to make this information available. The work was supported by an unusual combination of private, university, government and commercial research funds, and was conducted in pressure chambers in the hospital of a major university medical center so that skilled, specialized treatment would be available immediately if necessary.

Two dives were made per week to 500–600 ft, involving 3 or 4 men, one of whom worked with his arms ($\dot{V}O_2$ 0.60–2.23 liter·min$^{-1}{}_{STPD}$) in standard commercial diving equipment in cold water. At the time of this report, 113 simulated oxygen-helium working dives had been made, for a total of 374 man-exposures at depths of either 500 or 600 ft, with bottom times of 30 minutes and a compression rate of 100 ft/minute. In addition, over 30 man-dives have been made to 1000 ft, with compressions of 30–60 minutes and decompressions of between 4–5 days.

Some criteria were set for testing tables. These included avoidance of: central nervous system (CNS) symptoms of decompression sickness, decompression sickness deeper than 60 ft, and vestibular decompression sickness; optimal use of oxygen without toxicity, minimal use of BIBS (Built-in-Breathing System) and simplicity of table design. Design simplicity was achieved by minimizing the number of gas mixtures and using staged stops rather than linear ascent.

Deciding on the number of successful tests required before a decompression table could be considered satisfactory was difficult. An arbitrary decision was made that 12 or more dives without the occurrence of decompression sickness would be required.

The on-bottom breathing mixture was standardized at 7% oxygen-93% helium. However, a mixture of 7% oxygen, 10% nitrogen, and 83% helium, was also examined; in this mixture, the nitrogen was used to suppress the signs and symptoms of the High Pressure Nervous Syndrome (HPNS) evoked by a compression rate of 100 ft/minute (2).

Schedule Development by Haldane Methods

The program started with the evaluation of an Oceaneering International, Inc. table to a depth of 500 ft, with a 30-min bottom time, including a compression of about 5 minutes. This table will be called the "parent" in this paper (Fig. 1); it was calculated, like the majority of commercial decompression tables, using Haldane theory (9), and a ratio and tissue half-time matrix derived from operational diving experience and such theoretical knowledge as was available.

The table (Fig. 1) used oxygen to the full, as do the majority of commercial tables; 8% oxygen was used at depth, and the concentration of this gas was increased with decreasing depth. Nitrogen was incorporated at 300 ft to increase the helium desaturation gradient, and air was used at 150 ft. Air and oxygen were alternated between 50 ft and the surface. The total time for this table was 666 minutes, with a decompression time of 636 minutes (Table I).

This table had two problems, vestibular decompression sickness at the air change at approximately 160 ft, and type I decompression sickness between 30-50 ft. Initially, in an attempt to avoid being forced into any specific computation hypothesis, the air shift was changed to 130 ft to reduce the risk of the nitrogen causing rapid growth of bubbles formed deep, and the deep stops were lengthened, to produce tables B, C and D. Table B had a 20-min bottom time, as did table A; no decompression sickness occurred in 12 dives on these tables. However, with tables C and D (30-min bottom time) 4 cases of bends occurred, three cases in working divers and one in a tender. When these bends occurred, two of the divers were at 100–110 ft, and the other diver was at the surface. The incident involving the tender occurred at 15 ft.

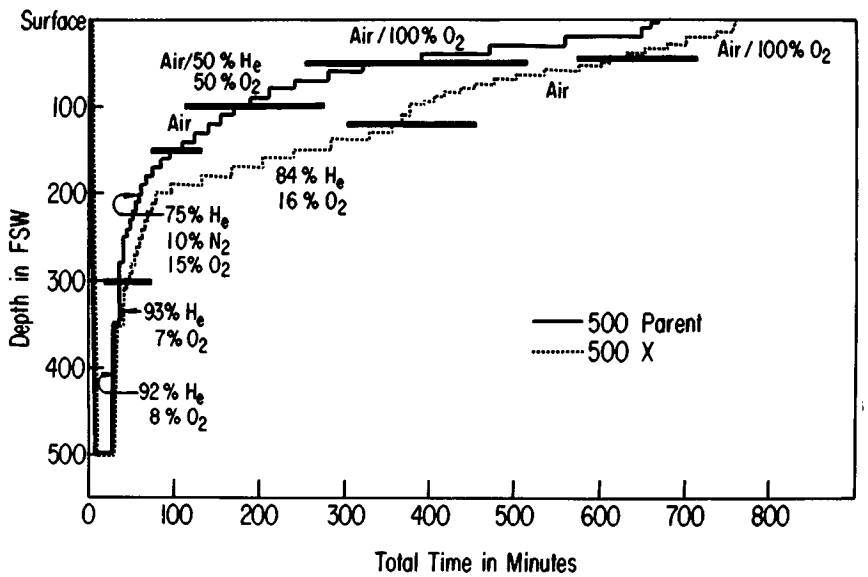

Fig. 1. Decompression profiles for parent table and 500 X-ray, illustrating difference between Haldane (parent) and Haldane-diffusion-nil supersaturation (500 X-ray) methods of computation. Parent produced vestibular bends at air change and leg bends at 30 to 50 ft. Table 500 X-ray produced no bends in 22 experimental dives.

TABLE I

PARENT TABLE, TO 500 FT WITH 30-MIN BOTTOM TIME, USING 6% O_2, 94% HE

Gas	Depth	Travel Time	Stop Time		Elapsed Time
6% O_2/94% He	500–230 at 50 ft/min	3	—		3
or 8% O_2/92% He†	350–280 at 25 ft/min	3	—		6
15% O_2/10% N_2/75% He	280	—	4		10
	280–250	3	—		13
	250	—	2		15
	240	—	2		17
	230	—	3		20
	220	—	3		23
	210	—	4		27
	200	—	4		31
	190	—	4		35
	180	—	6		41
	170	—	10		51
	160	—	↓		61
linear	↓ begin air	—	25 ↓	**Begin slow bleed upon	76
Air decompression	140 at 150 ft	—	15	ariving at 160 ft	91
	130	—	15	Resume staged decompres-	106
	120	—	15	sion at 140 ft	121
	110	—	15		136
50% O_2/50% He mix	100	—	20		156
with air breaks	90	—	20		176
	80	—	30*		206
	70	—	40	(20–20*)	246
	60	—	40	(30–10*)	286
O_2 with air breaks	50	—	70	(20–10*–20–20*)	356
	40	—	80	(20–10*–20–10*–20)	436
	30	—	90	(30*–20–10*–30)	526
	20	—	90	(30*–20–10*–30)	616
	20–0 at 1 ft/min	—	20	(10*–10)	636

Compression schedule: 0–200 at 150 ft/min = 1:20
Stop :30
200–400 at 100 ft/min = 2:00
Stop :30
400–500 at 100 ft/min = 1:00
5:20
Ventilate lungs well at stops

Elapsed time includes stop time; all travel time from first stop is 10 ft/min.

*air time

**Note: 1) Complete 170-ft stop and go to 160 ft in 1 min
2) On arrival at 160 ft begin 25 min of linear decompression at a rate of 1 ft/1.25 min (5 ft in 6.25 min)
3) When crossing 150-ft mark, begin air changeover
4) Continue linear decompression until arrival at 140 ft
5) Resume stage decompression according to schedule, remembering to add 1 hr to 20-ft oxygen stop

†Note: Trimix should be started on board at 350 ft or as soon thereafter as practical

At this time a Haldane-type matrix was applied to the tested decompression tables to see if additional information could be obtained.

Calculations were made of the ratio N_2 + He/ambient (9) for 16 tissue half times: 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540, and 600. These were printed out for each stop on one piece of paper and from this the highest or limiting ratio was selected for each depth to give a back bone of limiting ratios (Table II). The limiting ratios for tables A through O are shown in Table III, with the oxygen toxicity indicator, UPTD or Unit Pulmonary Toxicity Dose (4, 11), total time of the table, number of exposures, and incidents of decompression sickness.

The many variations of stop time used in the different tables will not be discussed in this paper. However, in developing each table, the ratio and the possible need for reduction to a lower value in areas believed responsible for supersaturation leading to decompression sickness were kept in mind.

Assistance was also obtained by using Doppler bubble indicators from probes placed over the heart. In this Haldanian series of tables, a good correlation was obtained between the incidence of Doppler bubble noise and the occurrence of bends.

As bends developed ratios were reduced, and a table with total times of about 600 minutes was recalculated and tested. However, decompression sickness occurred consistently, primarily in the 20-to-50-ft depth ranges. These were almost exclusively limb bends, and occurred without exception in the wet working diver.

By the time table J was reached it seemed apparent that despite many changes in the ratios, decompression sickness occurred with striking consistency in the 20-to-50-ft stops. The one region of consistent ratios which had not been changed was that between 200 and 300 ft, where ratios between 1.13 to 1.14 were evident.

Accordingly, for table J, the ratios from 500 to 200 ft were reduced significantly so that in the 200-to-300-ft depths the ratio was between 1.06 to 1.08. Nine man-dives were made (3 wet) with no decompression sickness at the 20-to-50-ft depth ranges. However, vestibular decompression sickness occurred on the air change between 140 and 130 ft.

The air change was therefore changed to 100 ft, resulting in table 500 L (Table IV). Twelve man-dives were made on this table, with decompression sickness occurring 4 hours after the dive in one subject, and an oxygen convulsion at 60 ft in another. That the table was the longest tested at that time, with the highest UPTD (922), explains the toxicity; the greater time on helium-oxygen rather than air added to the problem by causing a higher cerebral oxygen tension (1).

Attempts continued to achieve a table with the same ratios in a reasonable time, but without decompression sickness or oxygen problems. However, it became apparent that the oxygen had to be reduced significantly or there would be no margin for the application of an oxygen treatment, if required, without causing oxygen toxicity. If the surfacing ratio was to be maintained, at the very least, in the 1.7 region, which by Haldane standards seemed remarkably high, the only way to achieve such a table would be with a time well over 1000 minutes. A change of hypothesis was needed. We therefore decided to use a concept involving diffusion and nil-supersaturation.

Diffusion and Nil-Supersaturation

During the early 1950's, Hempleman (5) noted that for dives requiring no decompression, the dive depth was inversely proportional to the square root of the bottom time. This would

TABLE II

TISSUE HALF TIMES AND RATIOS OF MATRIX FOR PARENT TABLE

	5	10	20	30	45	60	90	120	180	240	300	360	420	480	540	600
0	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
500	1.28	1.15	0.86	0.68	0.52	0.43	0.32	0.26	0.20	0.17	0.15	0.13	0.12	0.12	0.11	0.11
350	1.43	1.36	1.07	0.86	0.66	0.54	0.41	0.33	0.25	0.21	0.19	0.17	0.16	0.15	0.14	0.13
280	1.18	1.28	1.13	0.95	0.75	0.63	0.48	0.39	0.30	0.25	0.22	0.20	0.18	0.17	0.16	0.15
250	1.05	1.20	1.12	0.97	0.79	0.66	0.51	0.42	0.32	0.27	0.23	0.21	0.19	0.18	0.17	0.16
240	1.04	1.20	1.15	1.00	0.82	0.69	0.53	0.44	0.34	0.28	0.25	0.22	0.20	0.19	0.18	0.17
230	1.01	1.18	1.16	1.03	0.85	0.73	0.56	0.47	0.36	0.30	0.26	0.23	0.22	0.20	0.19	0.18
220	0.99	1.16	1.18	1.06	0.89	0.76	0.59	0.49	0.38	0.32	0.28	0.25	0.23	0.21	0.20	0.19
210	0.97	1.13	1.18	1.09	0.92	0.80	0.63	0.52	0.40	0.34	0.29	0.26	0.24	0.23	0.21	0.20
200	0.96	1.11	1.19	1.11	0.96	0.84	0.66	0.55	0.43	0.36	0.31	0.28	0.26	0.24	0.23	0.22
190	0.95	1.09	1.20	1.14	1.00	0.88	0.70	0.59	0.45	0.38	0.33	0.30	0.27	0.26	0.24	0.23
180	0.94	1.06	1.19	1.16	1.03	0.92	0.74	0.62	0.49	0.41	0.36	0.32	0.29	0.27	0.26	0.25
170	0.91	1.00	0.14	1.15	1.06	0.96	0.79	0.67	0.53	0.44	0.39	0.35	0.32	0.30	0.28	0.27
160	0.91	0.96	1.10	1.13	1.08	0.99	0.84	0.72	0.57	0.48	0.42	0.38	0.35	0.32	0.30	0.29
150	0.85	0.91	0.04	1.10	1.09	1.02	0.88	0.77	0.61	0.52	0.46	0.41	0.38	0.35	0.33	0.31
140	0.84	0.88	1.00	1.07	1.09	1.04	0.92	0.82	0.66	0.56	0.50	0.45	0.41	0.38	0.36	0.34
130	0.84	0.87	0.97	1.05	1.09	1.07	0.97	0.87	0.71	0.61	0.54	0.49	0.45	0.42	0.39	0.37
120	0.85	0.87	0.96	1.04	1.10	1.10	1.01	0.92	0.77	0.66	0.59	0.53	0.49	0.46	0.43	0.41
110	0.85	0.88	0.96	1.04	1.11	1.12	1.06	0.98	0.83	0.72	0.64	0.58	0.53	0.50	0.47	0.44
100	0.56	0.64	0.78	0.91	1.03	1.08	1.06	1.00	0.87	0.76	0.68	0.62	0.58	0.54	0.51	0.48
90	0.54	0.58	0.70	0.82	0.97	1.04	1.07	1.03	0.92	0.81	0.74	0.67	0.63	0.59	0.55	0.53
80	0.86	0.83	0.83	0.88	0.99	1.06	1.11	1.09	0.99	0.89	0.81	0.75	0.70	0.65	0.62	0.59
70	0.52	0.58	0.66	0.74	0.86	0.94	1.02	1.02	0.95	0.87	0.80	0.74	0.69	0.65	0.61	0.58
70	0.85	0.81	0.80	0.84	0.93	1.01	1.09	1.10	1.04	0.96	0.88	0.82	0.77	0.72	0.69	0.65
60	0.50	0.53	0.60	0.67	0.77	0.86	0.97	1.01	0.98	0.92	0.86	0.80	0.75	0.71	0.68	0.65
60	0.80	0.74	0.74	0.77	0.86	0.95	1.07	1.12	1.09	1.03	0.96	0.90	0.85	0.80	0.76	0.73
50	0.05	0.18	0.37	0.49	0.63	0.76	0.92	0.99	1.01	0.97	0.92	0.86	0.82	0.78	0.74	0.71
50	0.60	0.48	0.49	0.55	0.65	0.76	0.91	0.98	1.00	0.96	0.91	0.86	0.82	0.78	0.74	0.71
50	0.03	0.12	0.24	0.34	0.48	0.60	0.78	0.87	0.93	0.91	0.87	0.83	0.79	0.76	0.72	0.70
50	0.84	0.70	0.58	0.58	0.64	0.73	0.89	0.09	1.04	1.03	0.99	0.94	0.90	0.86	0.83	0.79
40	0.05	0.17	0.29	0.36	0.47	0.58	0.76	0.88	0.97	0.97	0.94	0.91	0.87	0.84	0.80	0.78
40	0.60	0.48	0.43	0.45	0.51	0.60	0.76	0.87	0.96	0.96	0.94	0.90	0.87	0.84	0.80	0.78
40	0.03	0.12	0.21	0.28	0.38	0.47	0.65	0.78	0.89	0.91	0.90	0.87	0.84	0.81	0.78	0.76
40	0.60	0.45	0.38	0.38	0.43	0.51	0.66	0.78	0.88	0.91	0.89	0.87	0.84	0.81	0.78	0.76
40	0.04	0.13	0.22	0.28	0.37	0.47	0.66	0.80	0.95	0.99	0.99	0.97	0.94	0.91	0.89	0.86
30	0.77	0.70	0.58	0.53	0.52	0.56	0.68	0.80	0.93	0.97	0.98	0.96	0.93	0.91	0.88	0.86
30	0.04	0.17	0.29	0.33	0.38	0.44	0.59	0.71	0.86	0.92	0.93	0.92	0.90	0.88	0.86	0.84
30	0.60	0.48	0.43	0.43	0.44	0.48	0.60	0.71	0.86	0.92	0.93	0.92	0.90	0.88	0.86	0.84
30	0.01	0.07	0.18	0.25	0.33	0.40	0.57	0.71	0.91	1.00	1.03	1.03	1.02	1.00	0.98	0.96
20	0.77	0.70	0.57	0.52	0.50	0.52	0.61	0.73	0.90	0.98	1.01	1.02	1.01	0.99	0.98	0.96
20	0.04	0.17	0.28	0.32	0.36	0.41	0.52	0.65	0.83	0.93	0.97	0.98	0.98	0.97	0.95	0.93
20	0.60	0.48	0.43	0.42	0.42	0.45	0.54	0.65	0.83	0.92	0.96	0.98	0.97	0.96	0.95	0.93
20	0.01	0.07	0.18	0.26	0.33	0.39	0.53	0.68	0.91	1.04	1.11	1.14	1.14	1.14	1.13	1.11
10	0.77	0.56	0.47	0.48	0.51	0.57	0.72	0.89	1.18	1.35	1.44	1.48	1.48	1.48	1.46	1.44
0	0.19	0.28	0.33	0.38	0.44	0.50	0.66	0.84	1.14	1.31	1.40	1.44	1.46	1.46	1.44	1.43
0	0.19	0.28	0.33	0.38	0.44	0.50	0.66	0.84	1.14	1.31	1.40	1.44	1.46	1.46	1.44	1.43

500 ft for 30 min, helium-oxygen

TABLE III

HALDANE-TYPE TABLES

	Depth		Parent	A	C/D	E	F	Depth	G	H	I	J	L	N	O
He/O_2	500		1.28	1.27	1.27	1.27	1.27	500	1.34	1.34	1.27	1.09	1.01	1.01	1.01
	450												1.26	1.26	1.26
	400											1.18			
	350		1.43	1.32	1.32	1.32	1.32	330	1.25	1.22	1.32		1.15	1.15	1.15
	300											1.16	1.13	1.13	1.13
	290											1.15	1.10	1.10	1.10
	280		1.28	1.14	1.14	1.14	1.14	305	1.22	1.21	1.14	1.12	1.08	1.08	1.08
	270			1.14	1.14	1.14	1.14				1.14	1.10	1.06	1.06	1.06
	260			1.13	1.13	1.13	1.13				1.14	1.08	1.06	1.06	1.06
	250		1.20	1.13	1.13	1.13	1.13	255	1.16	1.15	1.13	1.06	1.06	1.06	1.06
	240		1.20	1.13	1.13	1.13	1.13				1.13	1.06	1.06	1.06	1.06
	230		1.18	1.13	1.13	1.13	1.13	235	1.16	1.16	1.13	1.07	1.07	1.07	1.07
	220		1.18	1.14	1.14	1.14	1.14				1.13	1.07	1.07	1.07	1.07
	210		1.18	1.14	1.14	1.14	1.14	215	1.17	1.17	1.14	1.07	1.07	1.07	1.07
	200		1.19	1.15	1.15	1.15	1.15	200	1.16	1.16	1.14	1.07	1.07	1.07	1.07
	190		1.20	1.15	1.15	1.15	1.15	190	1.15	1.15	1.15	1.07	1.07	1.07	1.07
	180		1.19	1.14	1.14	1.10	1.05	180	1.15	1.15	1.15	1.07	1.06	1.06	1.08
	170		1.15	1.11	1.11	1.06	0.99	170	1.10	1.12	1.12	1.08	1.06	1.06	1.07
	160*	(Vestibular)	1.13	1.11	1.11	1.02	0.96	160	1.11	1.11	1.10	1.08	1.06	1.06	1.07
	150*	(Vestibular)	1.10	1.10	1.10	1.01	0.95	150	1.11	1.11	1.10	1.08	1.07	1.07	1.09
	140		1.09	1.09	1.09	1.00	0.95	140	1.09	1.09	1.09	1.08* (Vestibular)	1.07	1.07	1.09
	130		1.09	1.09	1.09	1.02	0.97	130	1.07	1.12	1.10	1.07	1.07	1.07	1.08
	120		1.10	1.11	1.10	1.05	0.99	120	1.07	1.12	1.09	1.07	1.08	1.08	1.09
	110		1.12	1.13	1.12	1.09	1.02	110	1.09	1.12	1.08	1.06	1.08	1.08	1.08
	100		1.08	1.08	1.14*	1.11	1.05	100	1.09	1.11	1.09	1.05	1.08	1.08	1.08
	90		1.07	1.07	1.16	1.15	1.09	90	1.11	1.11	1.08	1.05	1.08	1.08	1.08
	80		1.11	1.11	1.18	1.18	1.14	80	1.13	1.12	1.09	1.06	1.08	1.08	1.09
	70		1.10	1.10	1.19	1.20	1.18	70	1.16	1.15	1.09	1.09	1.08	1.08	1.08
	60		1.12	1.13	1.24	1.25	1.18	60	1.11	1.10	1.10	0.99	0.98* (O_2)	1.09	1.09*
O_2/Air	50*		1.04	1.05	1.13	1.15*	1.12	50	1.14	1.03	1.02*	1.04	1.00	1.06	1.06* (O_2)
	40		0.97	1.00	1.06	1.09	1.08	40	1.03*	1.02*	1.01	1.04	0.98	1.09	1.09
	30*		1.03	1.03	1.09	1.13	1.14	30	1.06	1.07	1.08	1.03	1.15	1.26	1.22
	20		1.14	1.10	1.15	1.19	1.29*	20	1.24	1.25	1.26	1.19	1.20	1.31	1.59
	16											1.30	1.31	1.45	
	15			1.38	1.27*	1.33		15							
	10		1.48		1.48		1.63	10	1.60	1.61					
	8											1.57	1.58	1.74	
	5							5							
	Surface		1.46	1.56	1.62*	1.96	1.66	Surface	1.60	1.61	1.63	1.75	1.76*	1.74	1.76*
Total time			636	575	585	624	749		629	570	617	742	796	971	849
O_2, UPTD			838	755	697	721	986		783	788	819	842	922	847	886
DCS, O_2 toxicity				—	4	1	1		1	1	1	1	1	—	3
No. exposures				12	20	12	6		3	3	6	9	12	3	21

TABLE IV

500 LIMA EXPERIMENTAL DIVING SCHEDULE ONLY

Gas	Depth	Stop Time		Ascent Elapsed Time (includes Stop Time)	Total Time of Dive
7% O_2/93% He	500	30		0	30
	at 50 ft/min (1 min)				
	450	1		1	31
	at 25 ft/min (4 min)				
	350	5		6	36
	at 20 ft/min (1 min)				
	330	2		8	38
	at 20 ft/min (1 min)				
	310	3		11	41
20% O_2/80% He	300	3		14	44
	290	3		17	47
	280	3		20	50
	270	3		23	53
	260	3		26	56
	250	4		30	60
	240	4		34	64
	230	4		38	68
	220	5		43	73
	210	5		48	78
	200	5		53	83
	190	8		61	91
	180	10		71	101
	170	10		81	111
	160	10		91	121
	150	15		106	136
	140	15		121	151
	130	20		141	171
	120	20		161	191
	110	30		191	221
Air	100	40		231	261
	90	50		281	311
	80	60		341	371
	70	90		431	461
	60	60	(25*-10-25*)	491	521
	50	70	(10-25*-10-25*)	561	591
	40	90	(30-25*-10-25*)	651	681
	30	35	(10-25*)	686	716
	20	60		746	776
	at 1 ft/min to surface			766	796

* = air time

Decompression schedule: 0-500 ft at 100 ft/min = 5 min (Duke University, 17 June 1974). Safe nonsaturation diving capability to 1000 ft; 500-ft depth, 30-min bottom time (including compression); gas = 7% O_2, 93% He. Total dive time: leave surface at time 0; arrive bottom at 5 min; leave bottom at 30 min. Four 3-man dives (12 exposures) were made, with 1 case of bends in wet diver 4 hr postdive, and 1 oxygen convulsion at 60 ft.

prevail in cases where diffusion rather than blood flow or perfusion was the process limiting gas transport in tissues susceptible to pain-only decompression sickness. With the aid of a digital computer, Wittenborn (8) later analyzed the diffusion of dissolved gas between blood and a slab of tissue of finite thickness and was able to show that upon return to the surface after a no-decompression dive, the same volume of gas in excess of ambient pressure was present in the tissue regardless of the depth of the dive. Hills (6) introduced the concept of zero supersaturation, which states that the tension of dissolved gases in susceptible tissue is never allowed to exceed the ambient pressure. By combining Wittenborn's gas transport model with Hills' safe ascent criterion, an algorithm to use in the computation of decompression schedules can be constructed.

An important characteristic of this algorithm is that only one constant is required for each inert gas used on a dive. Thus, a decompression schedule for a dive employing helium/nitrogen/oxygen mixes requires only two arbitrary constants.

In theory, the inert gas constants are not at all arbitrary and can be found from experimental measurement, for example from the no-decompression limits determined for air by Van der Aue (7) and for 80/20 helium/oxygen by Duffner (3) and Workman (10). Figure 2 presents these data and a theoretical approximation of this information. However, since both the air and helium/oxygen data were gathered from different groups of men and because certain important information was not included in the publication, it has been possible to estimate only a wide range for the inert gas constants.

There is a second way in which the constants can be determined from experimental measurement. The algorithm predicts that if the inspired oxygen partial pressure is held constant during decompression from a saturation dive, the rate of ascent to the surface will be constant. Thus, the maximum safe rate of ascent for an inert gas at a given inspired oxygen partial pressure defines the value of the inert gas constant.

A large number of tables were generated on this basis, using different combinations of diffusion and nil decompression constants for both helium and nitrogen (Table V). Calculations of the generated table (Table VI) were also made by Haldane methods for comparison with past tables; the surfacing ratio and the depth of the first stop, as shown in Table V, were particularly noted.

The first tables, P and Q, produced decompression sickness after the dive, with decompression from the last stops at 25 and 30 ft at a rate of 5 ft/min to the surface. For table R, the helium constants were therefore changed to values considered very safe, but which would clearly produce a very long table. We intended to adjust the constants to decrease the time until decompression sickness occurred.

Using this basis, tables R and S did not produce decompression sickness, but table T again caused decompression sickness 8 hours after the dive.

Since these tables involved decompression to the surface from the 25-ft stop at a rate of 5 ft/min (a feat impossible with Haldanian methods of decompression), we thought that the common problem of postdecompression decompression sickness might be resolved in this series if we used less drastic decompression from the last stop.

Accordingly, the surfacing ratios were decreased and alternating air and oxygen was used (instead of air alone); the air change was shifted from 60 to 65 ft, and pure oxygen was used from 30 ft. However, the postdive decompression sickness continued, and unlike our experience with the Haldane tables, both tenders and divers were affected.

As with the previous Haldane tables, a study of the data shown in Tables V and VI indi-

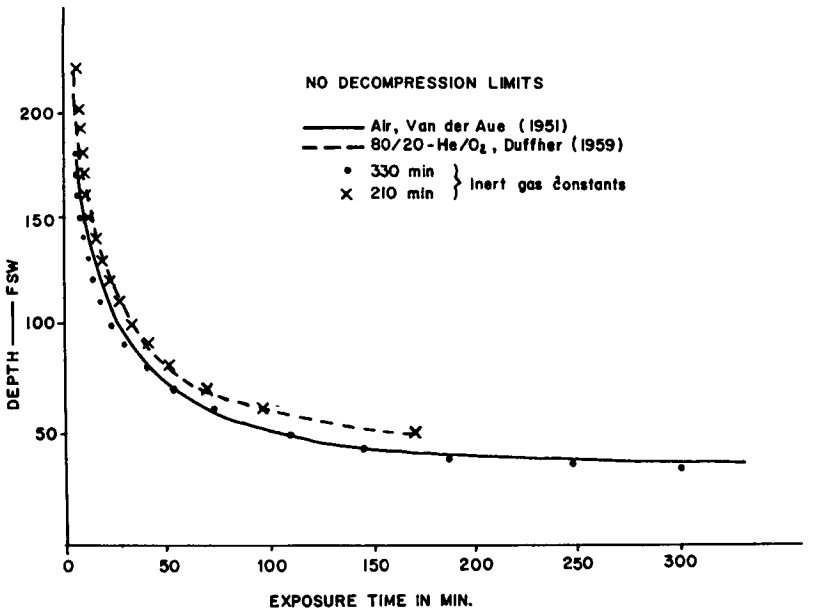

Fig. 2. No-decompression limits for air and heliox, determined by Van der Aue (1951) and Duffner (1959).

cated that one variable had not been changed unduly in the series from P to W: depth of the first stop, which occurred between 220 and 210 ft. The two tables which gave no trouble both had a first stop at 310 ft; these tables consisted of the deep and shallow halves of two unsatisfactory tables. For table X-ray the decompression in the early phase (to 200 ft) was Haldanian in character, but the ratios were lower (Table VI), giving a slower ascent and deeper first stop than did the parent table. The latter part of table X-ray was calculated on diffusion and nil/supersaturation principles. This would conform to the hypothesis that perfusion factors are involved initially in desaturation of gas, while diffusion is involved in the later stages. This seems realistic since once the blood has removed gas from easily accessible tissues with a good blood supply (short half times), diffusion is required to desaturate gas from areas poorly perfused by blood.

Twenty-two man-dives were made with table X-ray without decompression sickness (Table VII), including two from the Johnson-Link submersible off the Bahamas; also, the UPTD of 780 did not produce oxygen toxicity problems. This table is now being used by North Sea divers.

The total time of 757 minutes compared very favorably with the U.S. Navy time for the same depth and time (1017 minutes) and Ocean Systems' MK8A (989 minutes). These tables use considerable amounts of oxygen in the last stops, and some decompression sickness does occur, although to what extent is not known. A variation of table X-ray which uses only air and thus dispenses with BIBS and the use of oxygen is also available.

In conclusion, on the basis of these data, it appears that Haldanian tables generate gas early in the decompression due to too short a time during the deep stages and too shallow a depth

TABLE V

DIFFUSION/NIL-SUPERSATURATION TABLES

Schedule	Depth, First Stop	Last Stop to Surface	Surfacing Ratio	He Constants	N_2 Constants	Total Time	O_2, UPTD	Bends Incidence
Poppa 1	220	25 Air	2.41	42/190	34/190	915	721	All 3 divers, no bends, no bubbles
Poppa 2	220	30 Air	2.49	42/190	34/280	880	714	TC, knee pain 4 hr postdive; DM, mild shoulder 1½ hr postdive
Quebec	250	30 Air	2.76	42/190	32/330	826	735	DF, both knees 5 hr postdive CM, onset depth 7 ft, right knee
Romeo	250	25 Air	2.22	39/230	32/330	1017	823	6 clear
Sugar	250	25 Air	2.38	40/210	32/330	976	772	3 clear
Tango	250	25 Air	2.49	41/190	32/330	865	710	DF, knee pain 8 hr postdive, 2 clear SN, knee pain 8 hr postdive, 2 clear
Utah	220	25 Air and O_2	2.34	41/190	32/330	863	713	JB, 2-3 ft, ieft knee (wet), 8 clear
Victor	220	25 Air and O_2	2.36	41/210	32/330	833	713	CM, 10 ft, both elbows (wet), 5 clear
Victor (2)	210	25 Air and O_2	2.43	41/210	32/330	856	742	JM, 6 ft, both knees (wet), 10 clear DF, both knees 8 hr postdive
Victor (3)	210	20 Air, O_2, Air	2.30	41/210	32/330	875	787	JM, 1 hr postdive, left knee, 5 clear
Whiskey	210	15 Air, O_2, Air	2.22	41/210	32/330	688	712	JB, 42 min, right knee, 1 clear DM, 3½ hr, both knees (wet)
X-Ray	310	10 Air, O_2, Air	2.24	41/210	32/330	757	780	20 clear

TABLE VI

HALDANE ANALYSIS OF TABLE V

	Bends		No Bends		Bends				No Bends
Depth	P	Q	R	S	T	U	V	W	X
500	1.18	0.93	1.18	1.15	0.99	0.99	0.99	0.99	0.01
380	1.17	1.07	1.12	1.15	1.15	1.15	1.15	1.15	1.08
370	1.17	1.08	1.12	1.15	1.15	1.15	1.15	1.15	1.08
360	1.17	1.10	1.12	1.15	1.15	1.15	1.15	1.15	1.09
350	1.17	1.12	1.13	1.15	1.15	1.15	1.15	1.15	1.10
340	1.17	1.13	1.13	1.15	1.15	1.15	1.15	1.15	1.12
330	1.17	1.14	1.14	1.16	1.16	1.16	1.16	1.16	1.14
320	1.18	1.15	1.16	1.18	1.18	1.18	1.18	1.18	1.16
310	1.19	1.16	1.18	1.19	1.19	1.19	1.19	1.19	1.16
300	1.20	1.17	1.19	1.20	1.20	1.20	1.20	1.20	1.13
290	1.21	1.18	1.20	1.21	1.21	1.21	1.21	1.19	1.10
280	1.22	1.19	1.21	1.23	1.23	1.23	1.23	1.17	1.08
270	1.24	1.21	1.23	1.24	1.24	1.24	1.24	1.16	1.06
260	1.25	1.22	1.24	1.25	1.25	1.25	1.25	1.16	1.07
250	1.26	1.19	1.23	1.24	1.24	1.27	1.27	1.15	1.08
240	1.28	1.20	1.22	1.20	1.20	1.28	1.28	1.15	1.09
230	1.30	1.20	1.21	1.18	1.18	1.30	1.30	1.17	1.11
220	1.28	1.15	1.14	1.13	1.13	1.28	1.28	1.19	1.11
210	1.25	1.00	1.09	1.08	1.08	1.27	1.27	1.20	1.12
200	1.26	0.96	1.03	1.03	1.03	1.28	1.28	1.21	1.12
190	1.24	0.94	1.01	1.01	1.01	1.24	1.27	1.21	1.06
180	1.05	0.94	0.99	0.99	0.99	1.06	1.09	1.08	0.96
170	0.95	0.94	0.98	0.98	0.96	0.97	1.00	0.97	0.94
160	0.93	0.95	0.98	0.98	0.95	0.94	0.95	0.93	0.93
150	0.92	0.96	0.95	0.94	0.94	0.94	0.92	0.92	0.93
140	0.93	0.97	0.94	0.94	0.95	0.94	0.93	0.93	0.94
130	0.94	0.99	0.95	0.95	0.95	0.95	0.93	0.93	0.95
120	0.96	1.00	0.96	0.96	0.97	0.96	0.95	0.97	0.99
110	0.97	1.03	0.98	0.98	0.99	0.98	0.96	1.03	1.04
100	1.00	1.05	1.00	1.00	1.00	1.00	0.97	1.05	1.07
90	1.02	1.08	1.02	1.02	1.03	1.02	0.99	1.10	1.11
80	1.05	1.11	1.95	1.05	1.07	1.05	1.02	1.14	1.15
70	1.09	1.15	1.08	1.08	1.10	1.09	1.06	1.17	1.19
60	1.12	1.19	1.18	1.18	1.19	1.18	1.14	1.20	1.23
50	1.24	1.13	1.24	1.28	1.31	1.29	1.25	1.24	1.27
40	1.31	1.39	1.27	1.31	1.36	1.34	1.36	1.31	1.35
35	1.38	1.46	1.30	1.35	1.40	1.37	1.14	1.36	1.39
30	1.45	1.01	1.31	1.36	1.72	1.39	1.41	1.42	1.44
25	1.85		1.69	1.76		1.72	1.75	1.50	1.51
20								1.60	1.77
12 or 15		2.76	2.29	2.38	2.49	2.34	2.38	1.84	
10	2.41							2.22	1.95
5									2.24
S									
Total time	915	826	1017	976	865	863	833	688	757
O_2, UPTD	721	735	823	772	710	713	742	712	780
Problems	1	2	—	—	2	1	1	2	—
No exposures	6	3	6	3	6	8	6	3	20

Marked areas indicate probable areas of supersaturation when compared with Tables R and S.

TABLE VII

500 X-RAY (O_2 MODIFICATION)

Gas	Depth	Stop Time	Ascent Elapsed Time (includes Stop Time)	Total Time of Dive
7% O_2/93% He	500	30	0	30
16% O_2/84% He	50 ft/min (2.4 min)			
at 300 ft on BIBS	380	3.6	6	36
until chamber	10 ft/min (7 min)			
makeup	310	1	14	44
16% O_2/84% He	300	3	17	47
	290	3	20	50
	280	3	23	53
	270	3	26	56
	260	3	29	59
	250	3	32	62
	240	3	35	65
	230	3	38	68
	220	4	42	72
	210	4	46	76
	200	4	50	80
	190	16	66	96
	180	36	102	132
	170	34	136	166
	160	36	172	202
	150	39	211	241
	140	42	253	283
	130	46	299	329
Air	120	23	322	352
	110	13	335	365
	100	8	343	373
	95	20	363	393
	90	11	374	404
	85	13	387	417
	80	16	403	433
	75	18	421	451
	70	22	443	473
	65	26	469	499
	60	33	502	532
	55	41	543	573
	50	27	570	600
100% O_2 with air breaks*	45	10	580	610
	*40	5	585	615
	40	15	600	630
	*35	5	605	635
	35	15	620	650
	*30	10	630	660
	30	15	645	675
	*25	10	655	685
	25	15	670	700
	*20	20	690	720
	20	15	705	735
	*10	10	715	745
	10	10	725	755
	at 5 ft/min to surface		727	757

*Air Breaks. Decompression schedule (Duke University): 500-ft depth, 30-min bottom time (including compression); descent from 0–500 ft at 100 ft/min = 5 min; leave surface at time 0; arrive bottom at 5 min; leave bottom at 30 min.

TABLE VIII

500 JULIET EXPERIMENTAL DIVING SCHEDULE ONLY

Gas	Depth	Stop Time		Ascent Elapsed Time (includes Stop Time)	Total Time of Dive
10% O_2/90% He (begin O_2 makeup)	500 at 50 ft/min (2 min)				
	400 at 25 ft/min (2 min)			2	32
	350 at 25 ft/min (1 min)	1		5	35
	325 at 25 ft/min (1 min)	1		7	37
20% O_2/80% He	300	1		9	39
	290	2		11	41
	280	3		14	44
	270	3		17	47
	260	3		20	50
	250	3		23	53
	240	4		27	57
	230	4		31	61
	220	5		36	66
	210	5		41	71
	200	5		46	76
	190	6		52	82
	180	7		59	89
	170	8		67	97
	160	10		77	107
	150	10		87	117
	140	15		102	132
Air	130	20		122	152
	120	20		142	172
	110	30		172	202
	100	40		212	242
	90	50		262	292
	80	60		322	352
	70	60		382	412
100% O_2 with air breaks	60	60	(25*-10-25*)	442	472
	50	35	(10-25*)	477	507
	40	70	(30-25*-10-25*)	542	577
	30	55	(10-25*)	602	632
	20	90		692	722
Air	at 1 ft/min (20 min)				
	Surface			712	742

*Air breaks.

500 Juliet schedule (Duke University, June 5). Safe nonsaturation diving capability to 650 ft; 30-min bottom time (including compression). Descent from 0-500 ft at 100 ft/min = 5 min. Leave surface at time 0, arrive bottom at 5 min, leave bottom at 30 min. Ascent rates: 500-400 ft, 50 ft/min; 400-300 ft, 25 ft/min; 300-20 ft, 10 ft/min; 20-0 ft, 1 ft/min. Total exposures on this table were 9 (three 3-man dives). One vestibular case occurred at 130-ft air change, in a wet diver.

for the first stop. Such tables are made effective in practice by adding considerable amounts of oxygen near the surface to treat the bubbles generated deep as they expand during the last 30 feet to the surface. In the Haldane/diffusion-nil supersaturation methods for table X-ray, because of the very low Haldane ratios used in the head of the table, the time before the air change at 130 ft (299 minutes) is tripled compared with tables which resulted in vestibular decompression sickness, such as table J (Table VIII) and the parent table (Table I). The total decompression times are remarkably similar, however, because the times close to the surface are shorter than they are for Haldane-type tables, since at least theoretically there are no gas bubbles to treat. The Doppler measurements appear to confirm this statement, as do the extremely high surfacing ratios and the fact that wet working divers, using the Haldane tables, were preferentially affected by decompression sickness because such divers take on more gas than resting divers do. In the nil-supersaturation tables where perfusion was not the problem, decompression sickness occurred randomly, as would be expected, between working and non-working divers, and no bubbles were heard in the precordial Doppler.

Incidence of Decompression Sickness

In the 15 months of this program, 113 dives were made to 500 and 600 ft, for a total of 374 man-exposures. During these experimental dives, 55 cases of decompression sickness occurred, giving an incidence of 14.6%. Except for five of these cases, only the knee or lower leg was affected. The U.S. Navy Treatment Table V was used primarily, but for cases occurring deeper than 30 ft special procedures developed at Duke Medical Center were used. All of the treatments were successful.

As a result of this work, a decompression-sickness-free table, 500 X-ray, has been developed and is now in use.

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DEVELOPMENT AND TESTING OF HELIOX DIVES IN EXCESS OF 100 METERS

P. Cabarrou, K. G. Müller, H. D. Fust, H. Oser, H. Krekeler, and U. Finkeldey

For the depth range between 100 and 200 meters, various decompression profiles have been developed from different models (1, 2, 4, 5, 6, 9, 14). For a given depth and bottom time, partially tested profiles whose decompression times differ by a factor of 2 are available (Fig. 1). We distinguish between type m profiles, which have medium pressure reductions and long decompression times, and type f profiles, with fast pressure reductions and short decompression times. Both type m and type f profiles are equivalent with respect to the avoidance of compression and decompression symptoms and oxygen toxicity. For a relative evaluation of these profiles, the question arises of which criteria, in addition to lack of symptoms, have to be considered. Here the medical requirements—no intermediate bubble formation, no supersaturation and no essential change of blood physiological factors—and the practical requirements of a safe, simple, cost-effective and time-efficient dive must be considered.

The requirement that there be no intermediate bubble formation is quite difficult. Ultrasound bubble detection has shown that even during symptom-free dives, intermediate bubbles may occur (3, 8, 10, 13) and that there is no correlation between the occurrence of barely detectable bubbles and symptoms (7); it is doubtful whether this requirement is justified physiologically, or that meeting it would lead to dives which are appropriate for field use. During the development of our profiles, we have therefore disregarded this criterion.

Achieving the nil-supersaturation requirement depends on the theoretical model used. Profiles which have been calculated with a nil-supersaturation concept, e.g., by Hills (6), may show supersaturations in other models. As long as models do not describe physiological reality, the nil-supersaturation requirement is not a physiological one, but must instead be regarded as an empirical method of calculating decompression profiles.

Calculation of Profiles

We allow supersaturations in our model which are described by a set of M values for isolated tissues. We introduced a generalized Workman model with three typical sets of representative compartments (11). With this model no decision has been made about the actual

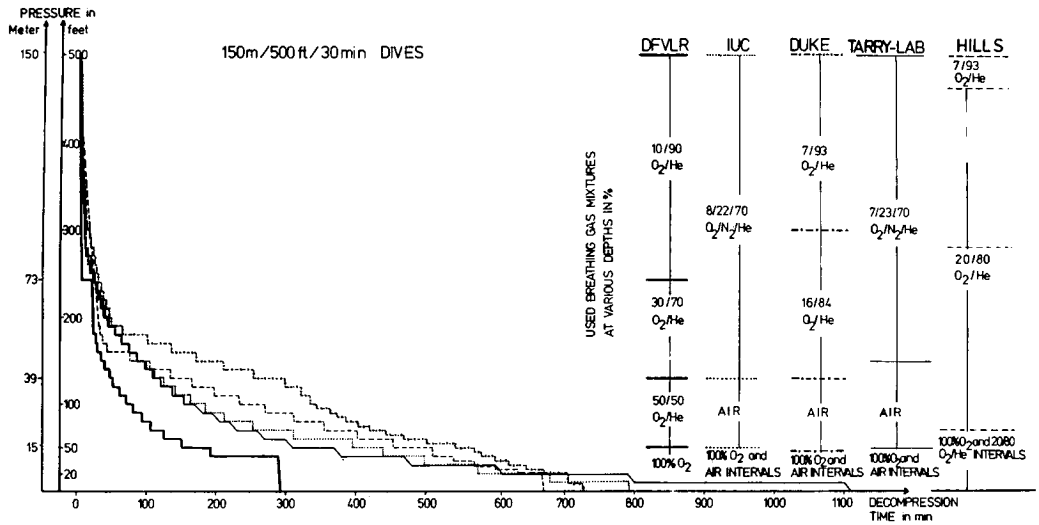

Fig. 1. Time-pressure profiles of Access dive series; dashed lines show planned decompressions.

mechanism of gas transport, especially in regard to diffusion or perfusion. Our choice of compartments is demonstrated in Fig. 2, where each compartment is characterized by its half times for helium and nitrogen. Set I was introduced by Schreiner (12) and can be explained on the basis of a perfusion mechanism. In Set II, only those compartments which show identical half times for the gases helium and nitrogen (He and N_2) are taken into account. Set III can be derived on the basis of a diffusion mechanism, and the ratio of the half times of He and N_2 was taken as 1:2.7 (see Bühlmann (2)). In a qualitative discussion, we simply refer to: fast tissues (τ_H (He) $\leq$ 20 min); medium tissues (25 min $\leq \tau_H$ (He) $\leq$ 75 min); and slow tissues (τ_H (He) $\geq$ 80 min), instead of compartments.

With regard to type f profiles, we distinguish three phases of a profile: the initial decompression phase, where the fast tissues are stressed by the high values of the supersaturation and the slow tissues will still be loaded; the medium decompression phase, where the tissues with medium half times are controlling; and the final decompression phase, where the slow tissues control predominately.

A specific profile was developed in the following way. First, parameters for compression rate, bottom phase and breathing mixtures were defined, and then the parameters of the first stop were selected. With these data a computer program calculated the first proposal on the basis of the M values. Changes were then introduced: (1) to smooth the profile (gaps between adjacent half times produce irregularities in the profile); (2) to redistribute the oxygen breathing time with respect to an optimal inert gas exchange; and (3) to insert air breathing intervals into the oxygen breathing time.

In this way we developed profiles using four breathing mixtures of 90/10, 70/30, 50/50, 0/100 helium/oxygen. Only helium was used as the inert breathing gas, and the problem of switching from helium to air during the decompression was avoided. This meant that we also gained the advantage of using helium exclusively as the inert gas, in case recompression therapy was necessary.

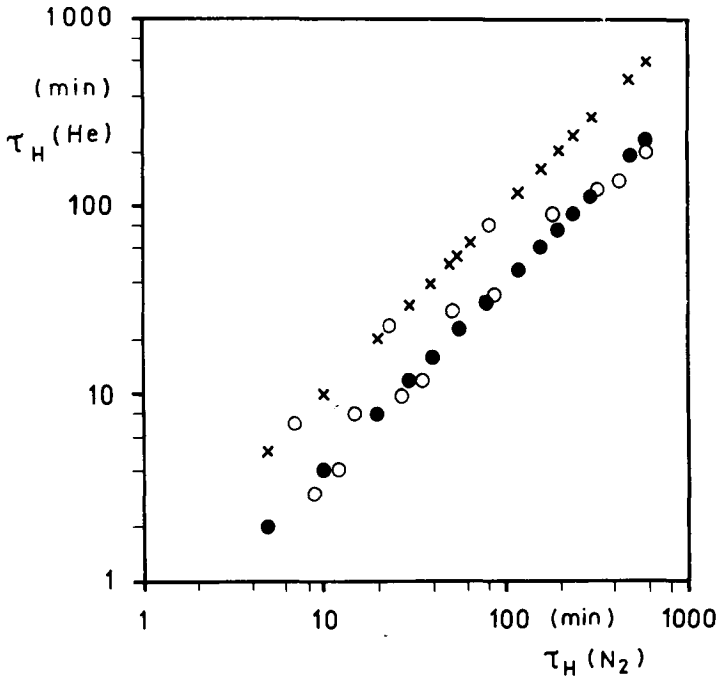

Fig. 2. Half times (τ_H) for He and N_2 for 3 sets of compartments: o = set I (perfusion); + = set II; ● = set III (diffusion).

Test of the Profiles

Table I presents a survey of our dry chamber and wet pot dives. Tests led to the following findings: the compression rates of approximately 30 to 50 meters/min led to a considerable transpiration because of the compression heat, and initially to a light tremor or dizziness. There were, however, no signs of the High Pressure Nervous Syndrome (HPNS) or vertigo. Those subjects who performed second or third dives no longer experienced any tremor or dizziness.

During the decompression we did not experience any sort of central nervous system symptoms or spinal hits or, as has been frequently reported, vestibular bends, eighth nerve disease (END), or ruptured round windows. There is a big advantage in not changing gas, an event frequently reported in connection with bends. Despite a rather sustained high oxygen partial pressure during the whole decompression, we had no clinical symptoms of oxygen toxicity, such as finger numbness or pulmonary irritation. In case decompression sickness treatment was needed, there was obviously no oxygen overloading to lead to oxygen-toxicity-related symptoms. When recompression was necessary, we were able to get the subject completely symptom-free in a few minutes using the short 60-ft oxygen therapy table.

From the practical point of view, diving time versus decompression time compared favorably to any available standard decompression profile for these depths and time. In terms of field requirements, our 150-meter, 30-min table is as safe and, provided breathing

TABLE I

SURVEY OF 110 METER/60 MIN, 135 METER/60 MIN, AND 150 METER/30 MIN TEST DIVES

Date	Depth, m	Compression Time, min	Bottom Time, min	Decompression Time, min	Number of Subjects	Remarks
11.11.74	110	6	54	347	2	no DCS
12.11.74	110	3	57	265	2	1 bends (type I), 45 min after surfacing
13.11.74	135	6	54	433	2	1 bends (type I), 7 hr after surfacing
15.11.74	135	4	56	434	2	2 bends (type I), 30 min after surfacing
16.11.74	135	3	57	526	2	no DCS
18.11.74	135	5	55	452	2	no DCS
19.11.74	135	3	57	428	2	no DCS
2.12.74	150	4	26	319	2	no DCS
3.12.74	150	3	27	314	2	no DCS
5.12.74	150	5	25	300	2	no DCS
21. 1.74	150	5	25	300	2	no DCS wet pot
23. 1.74	135	4	56	428	2	no DCS wet pot
24. 1.74	135	5	55	428	2	wet pot, 1 bends (type I), after surfacing
25. 1.74	150	5	25	300	2	no DCS wet pot
2. 4.75	150	3	27	286	2	no DCS

mixtures are premixed, even simple; this table is also cost-effective, because of its short duration. Despite the long BIBS time of approximately 6 hr, it should be mentioned that our divers did not consider wearing the mouthpiece a real discomfort. With respect to repetitive dives, our hematology study showed that an interval of approximately 72 hr between dives was sufficient. Every subject underwent an intensive pre- and postdive medical checkup. During the dive the electroencephalogram, electrocardiogram, and respiratory gas were monitored. There was always a breathable mixture in the chamber during the entire dive. A survey of the standard test conditions is given in Table II.

Discussion

In contrast to the profiles of other groups, the Deutsche Forschungs-und Versuchsanstalt für Luft- und Raumfahrt type f profiles show the following characteristic features: (1) use of helium-oxygen mixtures only, and avoidance of nitrogen, except for short air intervals at shallow depths; (2) fast compression rate (30–50 meters/min); (3) medium ascent rate to the first stop (9 meters/min); (4) extreme initial pressure reduction to the first stop ($\simeq$2:1); (5) recreation phase at the first stop (15 min); (6) high values of the oxygen fraction (30% for 73 m $\geq$ D $\geq$ 42 m; 50% for 39 m $\geq$ D $\geq$ 18 m; 100% for 15 m $\geq$ D $\geq$ 0 m); and (7) short decompression times.

On this basis a series of slightly modified profiles for 110 meters/60 min, 135 meters/60 min and 150 meters/30 min dives has been developed and tested successfully in a dry chamber and a wet pot (Table I). In the case of the 150-meter, 30-min dive, there were no clinical symptoms of compression disorders, decompression sickness or oxygen toxicity in 12 man-dives. Applying our model to greater depths and bottom times has been only partially

TABLE II

STANDARD TEST CONDITIONS

Chamber compressed with air	
Chamber temperature:	
Immediately after compression	23–26°C
During bottom time	20°C
Decompression to first stop	14–15°C
Between decompression and surface	20°C
Breathing equipment:	
Compression phase, bottom time and decompression until 15-meter stop: Draeger deep diving unit SMS I (semi-closed-circuit)	
Between 15 meters and surface: BIBS (overboard dump), Draeger SAA I, with modified snorkel mouthpiece	
Work load during bottom time:	
In dry chamber, 80 watts on bicycle ergometer	
In wet pot, nut and bolt exercises, weight lifting	
Oxygen consumption at bottom:	
To calculate tables, an oxygen consumption of 0.6 liters/min at rest and 1.7 liters/min (average) was assumed during work load	
Measured values during tests ranged from 0.6 liters/min to 0.95 liters/min at rest and 1.6 liters/min to 3.0 liters/min during work load	

successful. In some of these tests, cases of decompression sickness (type I symptoms) occurred, but there have been no symptoms of type II vestibular decompression sickness, central nervous system symptoms or spinal disorders.

In conclusion, type f profiles show the advantages of short decompression times, no vestibular or central nervous system decompression sickness, easy treatment in case of decompression sickness, and nonstressful decompressions. There may possibly be problems with high compression rates, high oxygen partial pressures, high initial pressure reduction leading to silent bubbles, and relatively long BIBS time which might limit this model's application to depths in excess of 200 meters.

Another upper pressure limit for the application of type f profiles may occur when, during the first decompression phase, medium tissues govern. This series of successful profiles for 135 meters/60 min and 150 meters/30 min, each slightly modified, may provide a starting point for the introduction of type f standard tables.

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SHORT-DURATION DIVES TO 1000 FSW USING BOTH RAPID COMPRESSION AND SATURATION-EXCURSION DECOMPRESSION

R. W. Hamilton, Jr. and D. J. Kenyon

To perform work at depths approaching 1000 feet of seawater (fsw)[1] using current saturation techniques requires one day for pressurization and equilibration, and 7 to 10 days for decompression. The saturation approach is effective for jobs requiring as much as two hours of work, but may involve excessive time and resources if only a few minutes of work need to be done. Our laboratory explored methods of diver access to 1000 fsw for short-duration work periods, using the technique of excursions from saturation at intermediate pressure in combination with direct, rapid compression from surface pressure. Rapid compression was used to reduce the duration of exposure to pressure, and hence the decompression obligation. Rapid compression, however, is known to invoke the High Pressure Nervous Syndrome (HPNS), especially if it is used deeper than 1000 fsw. We sought to determine how well rapid compressions could be tolerated, to compare effects on diver performance of direct compression versus excursions from intermediate depth, and to assess the benefits of added nitrogen. Further, we hoped to avoid problems with hyperbaric arthralgia by minimizing the duration of exposure to high pressure. We also had the opportunity to study the extent to which CO_2 accumulates in the lungs during compression.

The series of dry chamber simulated dives called Access was conducted to study these questions and to evaluate decompression techniques. Details of the decompression plan used[2] are presented elsewhere (8, 9, 12).

Methods

Figure 1 shows the general outline of the project, which consisted of three dives, each preceded by two short exposures (200 fsw) for training and securing base-line data. All three

[1] Equivalent pressures:

200 fsw	= 61 msw	= 7.2 bars abs.
500	= 152	= 16.4
600	= 182	= 19.4
800	= 244	= 25.6
1000	= 305	= 31.7

[2] Detailed profiles and logs of all dives in the Access series have been deposited with the International Decompression Data Bank, 14 Medical Labs Building G2, University of Penna., Philadelphia, PA 19104.

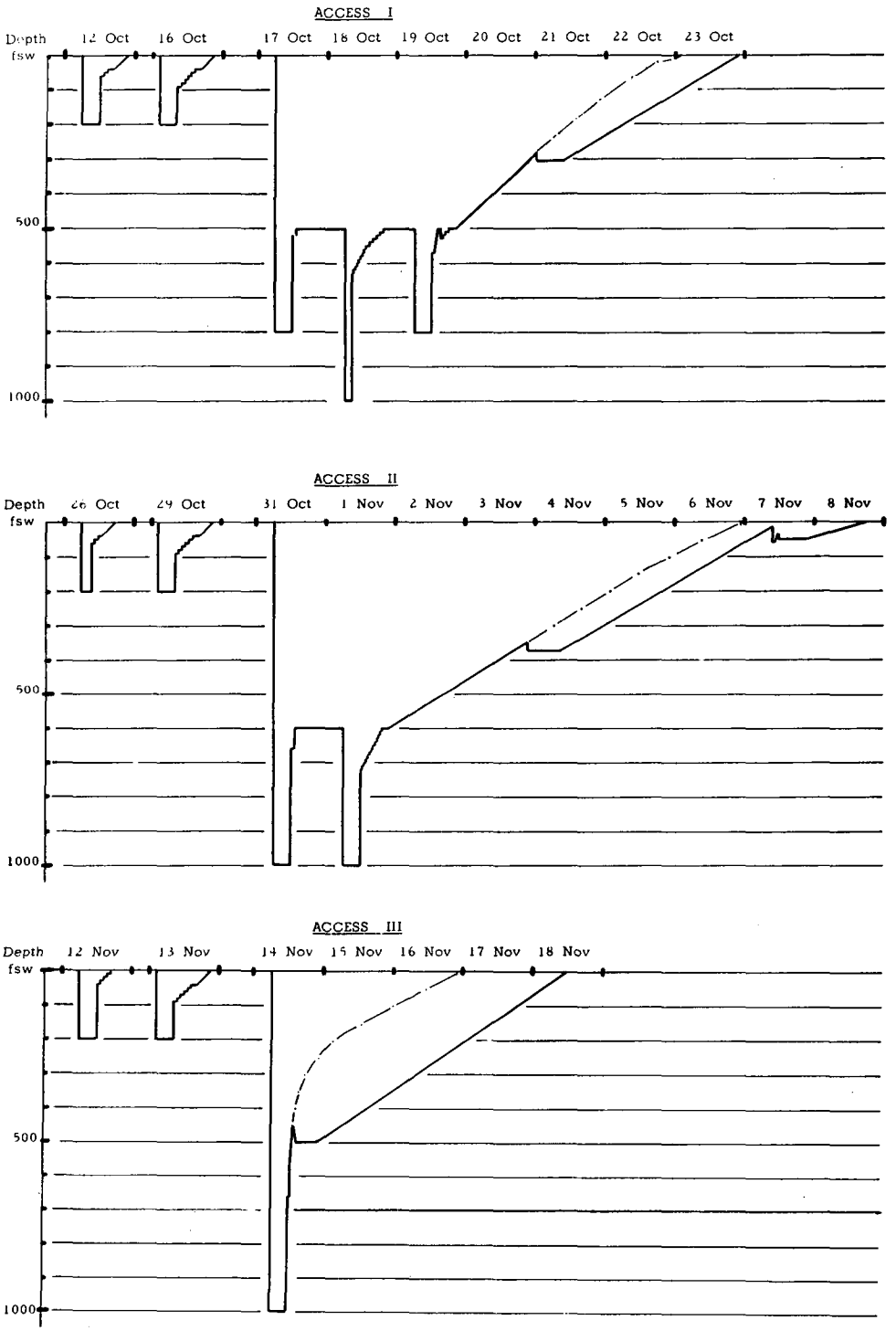

Fig. 1. Time-pressure profiles of Access dive series; dashed lines show planned decompressions.

Access dives involved direct compression from the surface to the working depth, 800 fsw in the first dive and 1000 fsw in the other two. In Access I, divers were compressed to 800 fsw, then decompressed to a holding depth of 500 fsw, from which depth excursions were made to 1000 and 800 fsw on successive days. In Access II, 600 fsw was used as the holding depth, with an excursion to 1000 fsw. After direct compression to 1000 fsw in the third dive, an attempt was made to follow an accelerated decompression profile to the surface; decompression sickness forced extension of the decompression time from a planned 2½ days to 4 days.

During compression and bottom time each diver occupied one of two interconnected spherical chambers, approximately 1520 mm (5 ft) in diameter. One sphere was equipped for voice and performance measurement, and the other contained a bicycle ergometer. These chambers were connected to a larger deck decompression chamber equipped with bunks and minimal sanitary facilities. Except for the 10-min, 1000-fsw excursion in Access I, all bottom times consisted of a carefully rehearsed sequence of activities lasting 30 minutes. Each diver performed a short exercise period on a bicycle ergometer for 7 minutes at 120 watts, and the divers switched chambers halfway through the experimental bottom time. The exercising subject wore a KMB-8 mask, which had not been modified for this depth.

When selecting the breathing gas mixtures, we had to consider both nitrogen and oxygen components at both working depth and storage depth. Helium made up the balance in all mixtures.

For oxygen, operational procedures developed by Ocean Systems, Inc., called for high oxygen (over 0.5 bars but not over a P_{O_2} of 1.2 bars) during bottom work. Oxygen partial pressure during level saturation has traditionally ranged between 0.3 and 0.6 bars. A single oxygen value of 3.5% was therefore usable at all depths involved in these dives.

The nitrogen value was selected to mitigate the effects of the High Pressure Nervous Syndrome, and therefore both narcotic effect and density of the mixture had to be taken into account. On the basis of empirical observations in air diving, a nitrogen pressure of 4 bars was chosen, equivalent to about 135 fsw (40 msw) of air. Experiments in progress at Duke University by Bennett and colleagues (2) had shown that a P_{N_2} of 5.8 bars causes excessive narcosis at 720 fsw (256 msw); this finding reinforced our choice. The rationale for the use of nitrogen to offset HPNS is well covered in the report of the Duke experiments (2).

The gases used are shown in Table I. During decompression the P_{O_2} was 0.4 bars, with occasional variations in the range between 0.3 and 0.5 bars.

Following established laboratory practice, the divers were compressed slowly to 40 fsw (12 msw) while breathing pure oxygen; this allowed checking of all systems before timing began. On departing 40 fsw, the chambers were pressurized with premixed gas at the rate of 100 fsw/min (3 bars/min). A stop at 500 fsw for one minute was made in Access I and II, and one-minute stops at 500 and 800 fsw were made in Access III. Excursions were made from the storage depths at the same 100 fsw/min rate, with no stops.

Expired breathing gas from one of the divers was sampled during compression via a small plastic catheter taped to his face and inserted 10 to 20 mm into one nostril. The sample was bled to the outside of the chamber and vented to atmospheric pressure in a 5-cc syringe barrel. From there it was drawn into an LB-1 infrared analyzer (Beckman) and recorded as a continuous tracing on an oscillograph. Calibration standards were produced with a Woesthof gas mixing pump (Calibrated Instruments, Ardsley, NY). The breath-by-breath tracings showed typical sloping plateaus except when the diver was talking or breathing by mouth. End-expiratory values were read from the peaks. When the diver was breathing from the

TABLE I

PARTIAL PRESSURES OF SELECTED O_2 AND N_2 PERCENTAGES AT VARIOUS DEPTHS

	Holding Depth, fsw		Working Depth, fsw	
	500	600	800	1000
Bars	16.3	19.4	25.5	31.7
Oxygen 3.5%	0.6	0.7	0.9	1.1
Nitrogen 13%	2.1	2.5	3.3	4.1

KMB-8 mask, the same sampling method was used except the sample was drawn from the oronasal cup inside the mask. For predive tests at sea level, both samples and calibrating gases were drawn directly into the analyzer pickup head.

External breathing resistance (pressure) was measured in the exercising diver by means of a differential pressure transducer connected by a catheter to the oronasal cup of the KMB-8 mask.

Vertex-occipital (C_2-F_2, ground A_1) EEG recordings were made during compressions and working periods. Finger tremor was measured at the end of each compression with a Bachrach tremor transducer (1, 2), modified to provide a performance score. To use this instrument, the diver presses on a lever with his middle finger against a force of 50 grams (low) or 500 grams (high), in an attempt to maintain an indicator needle within specified limits. A counter determines the proportion of the time the needle is kept within limits during each 30-second trial. Recordings were made of the tremor pattern for visual inspection, but no spectral analysis was performed. The tremor test was begun immediately on arrival at maximum pressure.

A standard Purdue Pegboard test was used to measure finger dexterity. Each diver installed as many pieces in 4-part assemblies as possible in 45 seconds, using both hands. Critical flicker fusion (CFF) was determined, after compression, with a device which introduced flicker into a portion of the evenly lighted field seen in a hand-held eyepiece (Biviator, Grechen, Switzerland). Five trials were used for each CFF determination. The subject adjusted the frequency for a barely detectable flicker and the investigator recorded the score.

Results and Discussion

All compressions were carried out as planned. Total compression time (departing from the 40-fsw safety stop) was 8.5 minutes to 800 fsw in Access I, 10.1 minutes to 1000 fsw in Access II, and 12.0 minutes to 1000 fsw in Access III.

These rates are, as far as we can determine, the fastest ever performed to 25 and 31 bars. Keller and Bühlmann (10, 11) took 25 minutes to reach 31.5 atm, and the Duke experiments mentioned earlier took 30 minutes.

The time course of end-expiratory carbon dioxide tension and the pressure profile are both shown in Fig. 2 for the three long compressions. Similar results were seen in the excursions.

Investigators have long believed that CO_2 may accumulate during compression. This could be caused by Valsalva maneuvers or breath-holding while equalizing, but could also occur

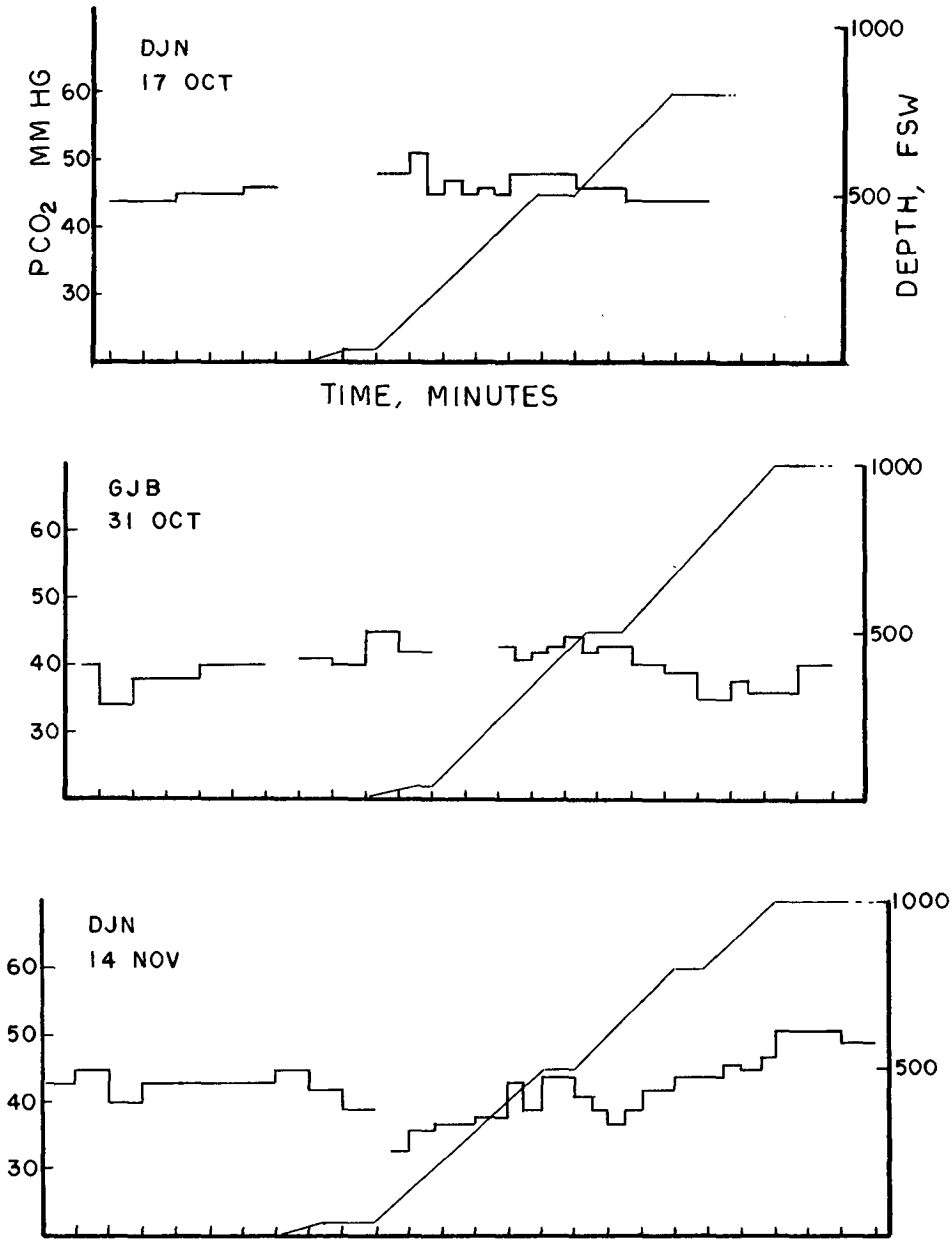

Fig. 2. End-expiratory CO_2 curves (heavy line) during compression to 800 and 1000 fsw; thin line is pressure profile.

during normal breathing. During compression, additional gas is taken into the lungs over and above normal ventilation. This additional volume will equal total lung capacity each time pressure is doubled, or an average of about 5 liters per minute during compression to 500 fsw.

From the data in Fig. 2, it is clear that there was no important increase in peak end-expiratory CO_2 during pressurization, at least in these two subjects (diver *DJN* was monitored in both Access I and III). Cabarrou (5) has reported a slight increase.

Figure 3 shows the peak end-expiratory CO_2 values for diver *DJN* at various times. Data for the other divers were similar, rarely falling outside the normal range of 35–45 mmHg at rest but occasionally exceeding 60 mmHg during exercise. There did not appear to be a definite regression of peak CO_2 on depth; the CO_2 was as likely to reach high values in exercise at 200 fsw (air or He-O_2) as at 600, 800 or 1000 fsw. It is significant that rather high levels of CO_2 were reached and tolerated by divers at these pressures and with this breathing equipment.

Peak breathing pressures seemed to be a matter of individual breathing pattern. The slow, steady breathers, usually the experienced divers, showed lower pressure excursions, ± 5 to 10 cmH_2O, and those with more abrupt patterns showed greater pressures, up to 20 cmH_2O. The predictable correlation between breathing pressure and peak CO_2 was seen in all cases. These measurements reflect the effects of the complete system rather than individual responses; the KMB-8 mask used is not generally considered adequate for these depths without modification.

One subject, not a trained diver, was unable to complete the exercise period at 1000 fsw while breathing by mask, but in both cases finished exercising after removing the mask. Neither his CO_2 nor breathing pressure were excessive.

The EEG recordings showed essentially normal tracings in all situations, with the single exception of some occasional slow wave activity on reaching maximum pressure. There were not enough EEG changes to assess the difference between compression from the surface and excursions. The electrode location used in these experiments was not the best one for observing slow wave activity, and the depths attained were not great enough for classical EEG manifestations of HPNS.

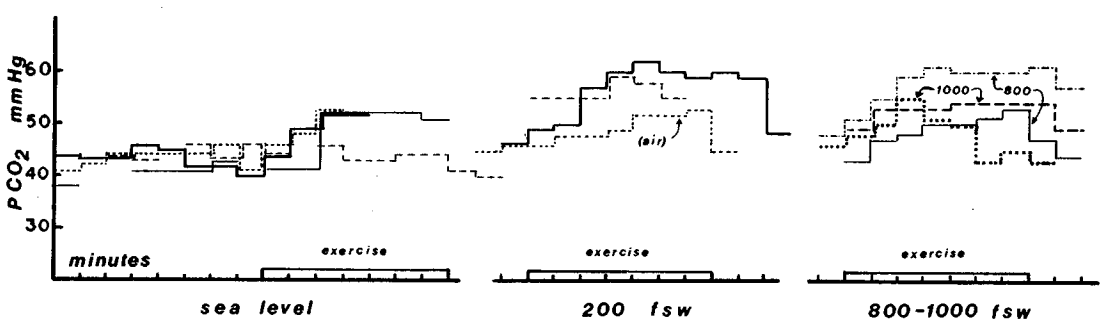

Fig. 3. Time course of end-expiratory CO_2 sampled from KMB-8 mask, subject *DJN*. Exercise was on bicycle ergometer at 120 watts. Air was breathed at sea level and once at 200 fsw, as shown. Each style of line represents a single day's experiment.

The counter attachment to the Bachrach tremor transducer produces a numerical score that correlates quite well (inversely) with amplitudes of finger tremor as recorded with an oscillograph. It is a performance score rather than the objective profile provided by power spectral analysis, for example, and as such it is dependent on both motivation and training. Results of these measures are given in Table II. Variances were large, and statistical analysis was therefore probably not justified. From an examination of the scores, however, certain trends can be seen. Clearly, the high force is more difficult to track than the low. There is little difference among scores at sea level, 200 fsw and the holding depths of 500 and 600 fsw, but there is a distinct decrement after compression to 800 or 1000 fsw. Compression from the holding depth appears to cause less decrement than from sea level.

TABLE II

RELATIVE PERFORMANCE SCORES ON THE BACHRACH TREMOR TRANSDUCER

	RH		*DJN*	
Access I	Low	High	Low	High
SL 16 Oct.	715	568	898	566
200 16 Oct.	733	640	788	660
SL 17 Oct.	732	658	672	800
800 17 Oct.	728	509	680	550
500 18 Oct.	680	488	720	648
1000 18 Oct.	574	480	685	580
500 19 Oct.	668	528	769	700
800 19 Oct.	750	495	755	680

	GJB		*TCS*	
Access II	Low	High	Low	High
SL 29 Oct.	699	444	770	541
200 29 Oct.	718	340	738	365
SL 31 Oct.	655	443	688	516
1000 31 Oct.	300	266	478	273
600 1 Nov.	498	568	748	500
1000 1 Nov.	498	338	515	347

	MF		*DJN*	
Access III	Low	High	Low	High
SL 13 Nov.	700	499	757	470
200 13 Nov.	720	438	720	399
SL 14 Nov.	679	443	738	417
1000 14 Nov.	565	278	550	260

Pooled Purdue Pegboard scores produced the data shown in Fig. 4. Results correspond to the tremor performance scores, showing only a slight difference between sea level scores and scores on the 200-fsw dives and during saturation, and a substantial (18%) reduction following compression to maximum depth from sea level. As before, the decrement on compression from saturation appears to be less than that on compression from sea level.

Examination of the CFF results revealed good reproducibility among the five trials in each determination, some day-to-day variation (a tendency for CFF to increase) but no changes as a result of compression. One diver was unable to complete the test after direct compression to 1000 fsw; he complained that staring at the lighted background caused bright flashes and "sparklers" to appear. Another admitted after the dive that he felt he was guessing; his score however, was normal.

Seki (14) and Seki and Hugon (15) found good day-to-day negative correlation of flicker sensitivity with saturation depth and general fatigue in several of the very deep Comex experiments. This decrement was reduced when trimix was used; this may partially explain our failure to observe changes. Seki's observations related to long-duration stays at much greater pressures, and thus were not strictly comparable to ours.

The subjective observation of flashes, sparklers and visual paresthesia was noted by several subjects, along with intensification of sound, fuzziness of peripheral vision, confusion, disorientation, and other more traditional signs and symptoms of HPNS. Pain in the distal joint of both thumbs bothered one diver throughout his pressurization. Other arthralgia symptoms, such as ankle pain and substernal pain during deep breathing, were felt by some, but not all, divers in the minutes after compression; these symptoms generally persisted throughout the saturation phase and were not exacerbated by subsequent compressions. This experience only partly supported our initial premise, that hyperbaric arthralgia could be avoided by completing the work in less time than the time pain normally takes to develop (4). Pain was present but not debilitating; the concept worked, but not as well as had been hoped.

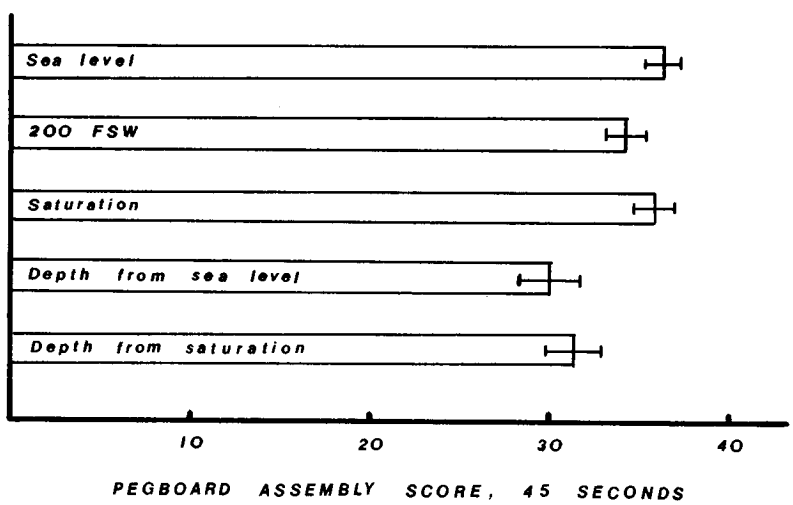

Fig. 4. Means and SE of pegboard scores; scores of all subjects tested in each condition were pooled.

The experienced divers reported euphoria equivalent to that at 200–250 fsw on air, which was subjectively greater than the calculated nitrogen equivalent used. All agreed that the level was close to the optimal level which would give the maximum HPNS relief without excessive narcosis.

Divers who could crack their knuckles at normal pressure were unable to do so right after compression (4).

Access I divers felt that the initial adiabatic cooling of decompression exacerbated their HPNS symptoms, and that this was relieved considerably when the chamber heat was turned on. This relief coincided with continued decompression; response was too quick to have been associated with a change in core temperature.

Quite predictably, the Access divers experienced a substantial number of effects immediately after the rapid compressions to 800 and 1000 fsw from sea level, and they were all unable to perform for the first few minutes. In contrast, however, most subjects felt that the HPNS effects of the excursions—from 500 fsw to 800 and 1000, and from 600 fsw to 1000—were benign, and that in those cases they could have gone to work immediately with little performance decrement. They felt, however, that the euphoria and other narcotic effects were about the same in both cases. While the subjects felt much better subjectively on excursions, their performance as measured by the finger tracking task (tremor transducer) and Purdue Pegboard was not significantly different for the two conditions.

Conclusions

The Access experience provided a useful perspective on short-duration diving in the 1000-foot depth range, and makes some generalizations about alternatives to full saturation possible.

Compression in 10–12 minutes appears to be physiologically tolerable. However, this procedure caused a significant temporary disorientation and decrement in performance. After such a pressurization, a recovery period of 5–10 minutes may be necessary before a diver feels safe to enter the water and go to work. Annoying but tolerable joint pain (hyperbaric arthralgia) can be expected to develop within minutes in some individuals, and most divers will experience varying degrees of tremor and mild euphoria. Effective operational utilization of direct compression to these depths might be achieved by the use of trimix (about 4 atmospheres of nitrogen at maximum depth), pressurization rates of 100 ft/min with several 1- or 2-min stops between 500 and 1000 fsw, and use of divers who are not unusually sensitive to HPNS or hyperbaric arthralgia. The Access concept assumes that work periods will be no longer than one to two hours, followed by a decompression of at least several hundred feet.

A much less stressful technique is to approach the working depth from saturation at an intermediate depth. Compression back to working depth from a saturation decompression can also be made if subsequent dives are needed while decompression is underway.

The feasibility of the Access concept of saturation-excursion diving has been verified and extended to greater depths by the Predictive Studies IV program at the University of Pennsylvania (6). No-stop excursions involving lesser changes in depth have been used successfully for some time by the US Navy (7), and the Coraz program of the French diving company COMEX uses a similar approach (13).

ACKNOWLEDGMENT

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PART VII. INERT GAS TRANSPORT

DISCUSSION

H. R. Schreiner, Chairman

Dr. Schreiner: I would very much like Dr. Hills to step forward to give us his views on what we have heard so far this afternoon.

Dr. Hills: I must take Dr. Harvey to task on one point. I would quite agree that supersaturation by counterdiffusion is eminently feasible and is a most ingenious concept, but you cannot get supersaturation by counterperfusion, and Dr. Harvey went on with the Haldane tissues, which are surely normally interpreted on a perfusion basis. So isn't this rather incompatible with counterdiffusion supersaturation, and aren't you really disproving Haldane yet again?

Dr. Harvey: I'm afraid I'm stuck in that position. I would certainly agree that if perfusion is the only limiting factor it is impossible to have different inert gas exchanges. And yet I think we have demonstrated that there is a different rate of exchange for gases within the body. If you look at the perfusion-limited model as also having diffusion characteristics with a radical diffusion into the tissues based on molecular weight differentials at the capillary level, that might be one explanation. If you look at perfusion and the ratios of gas solubility in blood to gas solubility in tissue, as Dr. Schreiner did, then differential rates within a single tissue become possible. It is certainly necessary to modify the pure perfusion-limited model.

Dr. Hills: As long as you don't take a pure Haldane type of approach, that is okay.

Dr. Harvey: I would be quite interested in what your model would produce in this situation. I haven't analyzed that.

Dr. Hills: I would agree with you entirely, but I'm not an advocate of Haldane.

Dr. Schreiner: I think that we have to consider that the theoretical interpretation of what really went on in these divers involves a partition of inert gas between blood and tissue, and clearly this partition will differ among different gases. So as not to prolong this discussion, I am prepared to meet with Dr. Hills privately to work out why it is possible to use partition coefficients to explain this transient increase in different inert gases in a given body compartment.

Dr. D'Aoust: I would like to compliment all of the authors on some very interesting papers, and make another comment that the calculations are lovely, but measurements are better—you know where my favoritism lies. I think it might be of general interest now to mention that we have recently gotten data that clearly show the importance and the inseparability of both the ascent criteria issue and the inert gas elimination rate. We clearly showed that decompression itself imposes an asymmetry on the elimination of nitrogen. Whether this involves all separated gas or a physiological response to partially separated gas remains to be seen. The upshot of this is very much in line with what Dr. Spencer suggested, that it is time to pull in our horns with respect to the ascent criteria and concentrate on a few tissues, say 20 to 80 minutes, where I believe much of the action is.

Dr. Schreiner: Dr. Bühlmann, having pioneered the use of multiple inert gases, would you care to comment?

Dr. Bühlmann: First, I wish to express my compliments to the speakers. I have three comments. First, until today, we had no experience with heart catheterization in the pressure chamber. Second, analyzing our decompression accidents with bends, we calculate inert gas pressure equalization for nitrogen with a longest half time of 635 minutes, and for helium, 240 minutes. Third, concerning the use of multiple inert gases and the phenomenon of counterdiffusion: using a sufficient decompression profile, we have never seen skin bends or inner ear trouble after switching from helium-oxygen to air. By exceeding the empiric supersaturation factors, however, we have been able to provoke skin bends if the diver is breathing only oxygen-helium in a chamber filled with the same gas mixture or with air.

Dr. Harvey: Dr. Bühlmann, if I can respond quickly. On our pre-SHAD dive which Dr. Adams will report later, we used a 480-minute tissue and I bent two divers out of two on the first try after a two-day saturation at 50 feet. We have retreated to a 640-minute nitrogen tissue in our calculations also.

Dr. Evans: I have a question for Dr. Balldin. Your last slide showed the effect of increasing the water temperature from 35 to 37° on the elimination of ^{133}Xe from the fat pad in the leg, and you said that the mean increase caused by this 2 °C temperature rise was 89%. Most of the increases varied between 1 and 74%, though one subject had an increase of 471%. Have you considered the possibility that on this occasion the injection of xenon was not into the fat but into the adjacent muscle, the effect this would have on the mean of the remaining values, and whether the increase that remains is then significant?

Dr. Balldin: After each experiment, I let the subject do muscle work with the leg. There was no increase in the elimination rate after this muscle work, so I am practically sure that I didn't inject into the muscle.

Dr. Linaweaver: From the practical side of the house: we reported a case just recently that happened during a deep nitrogen-oxygen dive while we were monitoring the pulmonary artery with ultrasonic Doppler; at one of the stops the master diver called a fire drill which required the divers to go on a semi-closed-circuit helium-oxygen mixture. Bubbles immediately appeared in the pulmonary arteries of the divers we were monitoring, and we got a central nervous system hit. As a result of this incident, we have recommended that the Navy Diving Manual, which recommends the use of helium-oxygen during serious conditions, be changed.

Dr. Vorosmarti: Two questions, one for Dr. Spencer and one for Dr. Harvey. As you will see from our paper later this afternoon, we don't follow any kind of mathematical models at all during decompression any more. I was completely lost in Claude's paper, but I would like to know how many times you computed theoretical supersaturations during your experiments and how many times you had symptoms?

Dr. Harvey: Every time we went off it, we were saturated with helium at the start of each depth experiment, or close to it. The calculations included minute-by-minute analysis while we were on the heavier inert gas and when we returned; we had cycles when we were on inert gas, back on the chamber gas, and so on. So we computed minute-by-minute through all of those cycles. Supersaturation was reached often if the tissues had short enough half times between the cycles of breathing, and there was always supersaturation in every compartment following the final return to the chamber atmosphere. Some of them took quite a long time to develop, however, because of the slow half times of the tissue. So we always reached supersaturation when we switched inert gases like that.

Dr. Vorosmarti: How many times did you have symptoms, though?

Dr. Harvey: We had no symptoms. The inert gas tissue tensions that we calculated did not violate the M values which one would have expected for starting decompression or moving up at that depth. I do not believe that the supersaturation was significant enough for these brief exposures to produce problems. When we had the nausea and vomiting episode, which we thought was generated by counterdiffusion through the skin and through the ear, perhaps into the inner ear, it took 18 hours to resolve; we were also calculating supersaturation as existent within deep tissues during that time, and it may very well have slowed down resolution of those symptoms.

Dr. Vorosmarti: Dr. Spencer, you showed on that one slide that Dr. Schreiner asked about that 62% of these divers showed bubbles and 19% had bends. Have you seen bends without bubbles in any of your divers?

Dr. Spencer: No, we have never seen bends without prior detection of bubbles.

Dr. Vorosmarti: Then I would like to ask you how you grade?

Dr. Spencer: The grading is based on something similar to a clinical method for murmurs. Grade I is an occasional bubble, just clearly recognizable but coming along quite sporadically. Grade IV means the entire heart cycle is filled with a continuous, loud, frothing sound, where you cannot differentiate one bubble from another. Grade II and III lie in between. The difference between II and III would be whether or not all heart cycles have bubble signals in them, and Grade II would be signals in most but not all cycles.

Dr. Lundgren: Mr. Chairman, I would like to make a brief comment to the laconic answer that Dr. Balldin gave to Dr. Saltzmann's question. I would like to stress that there is good reason to believe that the enhancement of inert gas elimination during immersion is to the very largest extent a result of increased circulation. Fahri and Yokoyama showed several years ago that for the ventilation to have an appreciable effect on gas elimination from the blood and hence from the tissues, it has to be quite soluble gases. Nitrogen is poorly soluble, and still we have a very marked enhancement of nitrogen elimination. Admittedly, xenon is a good deal more soluble, but it does not approach the soluble gases where you really see the effects, say ether and similar gases. Furthermore, if a change in ventilation and/or ventilation-perfusion is a factor, then turning to the earlier work by Saltzman and his colleagues, if anything, immersion seems to make ventilation-perfusion adaptation worse; they have shown a slight increase in alveolar arterial oxygen difference during immersion.

Dr. Saltzman: I agree, and that was one point I hoped would come out and I'm glad Dr. Lundgren said it. My question this time is directed to Dr. Harvey. If I understand your elegant presentation adequately, the change from a light to a heavy breathing gas during decompression would be associated with less danger. Empiric experience during decompression of divers who commonly switch from heliox to air shows an increased incidence of vestibular hits. Would this contradict your calculations?

Dr. Harvey: I would think not, unless the diver breathes a heavier gas by mask and is left surrounded by the helium; then one could get the same kind of vestibular problems we had during the 1200-foot dive at Penn. If there were light inert gas trapped in the middle ear, I would be suspicious that this might counterdiffuse in while the heavier inert gas was going through the circulatory system and perhaps get into the vestibular system and produce a gas phase there. I can't back that up, but it is possible.

Dr. Saltzman: So this would, in your mind, represent a surface exchange effect?

Dr. Harvey: Yes.

Dr. Wissler: This question is addressed to Dr. Harvey and may pertain to the previous question. Have you considered what happens to the sum of the partial pressures in blood when a diver is saturated on a light gas and then switched to a heavy gas? Since the light gas will diffuse into blood from tissue more rapidly than the heavy gas diffuses in the opposite direction, the end-capillary total pressure may exceed the ambient pressure.

Dr. Harvey: I would think blood would equalize so rapidly in the lungs and at the tissue level that probably you would end up with an ambient total pressure in there, but I am not certain of that. Rapid tissues tend to be much closer in their half times, but blood of course would be a very rapid tissue.

Dr. Behnke: This is addressed to Dr. Schreiner. I can state that in the 30's it was routine in diving to breathe helium-oxygen at deep depths, switch to air during decompression, and then to oxygen. At no time were there any vestibular hits, nor did we observe the phenomenon of counterdiffusion. I believe that in measurements of gas transport, we are in an early stage. If we could declare a moratorium on all calculations for a year or two, and then get down to the serious business of making measurements of gas transport following tissue equilibration with ambient gas pressure, we would be able to determine desaturation time of the slow tissues. During decompression, retarded elimination of gas, i.e., indicative of 'slow' tissues, may reflect the presence of bubbles, and we are then talking about resolution of gas from bubbles rather than transport of gas from tissues in the dissolved state.

I would like to see decompression carried out so as to eliminate percutaneous diffusion of inert gas by placing an individual surrounded by oxygen in a suit so that he could be immersed in water. This would permit exercise under conditions favorable to increased gas transport. The answer to two questions could be ascertained: 1) After the nitrogen in air is in equilibrium with body tissues, how long does it take for subsequent elimination of the previously absorbed nitrogen during the course of oxygen inhalation? 2) To ascertain if the nitrogen elimination follows the same course as during oxygen inhalation, these test conditions should be repeated substituting helium-oxygen for oxygen. I think that such quantitative data would be in the direction of a rather firm basis for the calculation of decompression tables.

Mr. Krasberg: Just a comment on switching to nitrogen: operationally, we find that you can do this on dives to about 380 feet, but beyond that you had better beware. If you postulate that the inner ear has a half time of a few minutes, and then make a switch that occupies maybe 20 or 30 minutes, it can still be done safely.

Dr. Harvey: The isobaric switch where you get counterdiffusion across the skin takes a good bit of time to develop. It is not a quick phenomenon.

Mr. Krasberg: I believe it is in the inner ear. Onset of symptoms after a step-change switch is very rapid. If you take a half hour to make the switch, you won't have any trouble.

Dr. K. H. Smith: I would like to direct this question to Dr. Fife. What kind of pigs did you use? What age were they?

Dr. Fife: They are the standard commercial meat pig, which is a Hampshire Yorkshire breed. We have tested these pigs all the way from about 35 to 40 pounds on up to 100 pounds, and we seem to see quite a bit of consistency between them. So what we usually do is get these pigs in when they are about 40 pounds, and then we start to use them. We have a nice working arrangement because pigs are very tractable and probably have more intelligence than dogs. Therefore these pigs get to be quite well known. You can tell when they feel well or disgruntled, and this helps when you are trying to decide whether they have the bends or not. In fact, they become quite friendly and put their forelegs up on the pen to be petted. But then when they get to be a hundred pounds or over they are too big for our chamber, and so they go to market; I don't have to buy any pig food and I don't have to buy the pigs. We find consistency even though the weights do vary during the time we have the pigs with us; we have the same pigs for about six months.

Dr. K. H. Smith: Six months?

Dr. Fife: Yes, although we only use them for about four. But we have to keep them until they are ready for market. They are in our chamber for about 4 months altogether; if you don't overfeed them they don't grow as rapidly as they normally would.

Dr. K. H. Smith: A standard commercial swine will reach 220 pounds at 180 days.

Dr. Fife: Ours don't grow quite that fast because we have them around in the chamber for several months.

Dr. K. H. Smith: What signs of decompression sickness do you use to determine whether a pig has decompression sickness or not?

Dr. Fife: We use all white-skinned pigs because the first sign of decompression which usually appears is skin bends. This is kind of cyanotic coloration that appears usually on the flank and sometimes on the shoulder; when we see this, we realize we could have problems. But almost invariably, skin bends appear first, followed sometimes by crippling (limping) or even, once in a while, convulsions or obvious respiratory problems.

Dr. K. H. Smith: One last question. I didn't see numbers of replications mentioned anywhere—how many dives with the pig would you make before you would determine whether a table was satisfactory?

Dr. Fife: This varies; sometimes we do just three or four when we can get a very sharp line of demarcation, but usually we will run sometimes for 8 or 10 dives before we really zero in. We can usually "bracket in" rather quickly on these pigs; we can sometimes bracket with as few as four pigs, and then we go back and do some more. But we usually don't find much change after we decide where the boundary line is.

Dr. Lundgren: Now that I have heard how you end your pigs, I feel tempted to leave the goats; goat bacon will never make it on the meat market. I have another question which, if you can confirm it, really makes the pig very interesting. You showed a table where there was a difference at around 25 minutes of exposure of only two minutes between a bend experiment and a non-bend experiment. This was established in two different animals, pig #1 and pig #2, I believe, and you pointed out that this illustrated the good reproducibility of provoking bends in pigs. Were these experiments really representative so that you can draw the conclusion that a two-minute difference makes the difference between a healthy pig and a bacon pig?

Dr. Fife: This does not represent pig #1 and pig #2, but profile #1 and profile #2. On the other hand, these pigs do show this rather sharp line of demarcation between bends and no bends. For example, if we vary some of the deeper stops by as little as one minute, we can get a bends or no-bends profile consistently.

Dr. Fife: I have goats, too. In fact, Dr. Hills is the one who convinced me to have them, and for some things I suppose they are ideal. It is mostly a matter of what you are accustomed to using; maybe that is more important than whether it is a pig, a goat, or a dog.

Dr. Lundgren: Yes, but the reproducibility is nothing like what you have here.

Dr. Fife: Reproducibility in the pig is to my mind far more consistent than in the dog, and I have some evidence of that if you would like to talk about it.

Dr. Bennett: I just wanted to add a point about the accurate reproducibility of the pigs to decompression sickness. You remember Workman and Reeves worked with dogs that also had this one-foot differential between bends and no bends. It may be that dogs and pigs are very good and the goat is the wrong animal model. We have used goats because Haldane did, but Haldane's theory now appears to be wrong as well.

Dr. Madsen: Dr. Spencer and Dr. Fife asked about the whereabouts of these very slow tissues, and I think I can add some information. We have made several hundred determinations on blood flow in subcutaneous adipose tissue in young normal humans. We find an average value of about five milliliters per 100 grams of tissue per minute. However, there is a very big variation in this average, and I would say that about 5% of these people have flow rates down to 1 milliliter per 100 grams per minute; from this we calculate nitrogen half times of 300 minutes, and in between we see even lower values. This shows at least in some of these persons that we have very slow half times in adipose tissue.

Dr. Bennett: Are these normal people?

Dr. Madsen: Yes, normal young people. In obese people you will find the flow rates even lower.

Dr. Mackay: When we do a decompression by monitoring bubble size ultrasonically, we see some time delay effects that suggest comments about delay in nucleation phenomena and also in diffusion phenomena. The practical consequence is that if you want to decompress a subject, you can't simply run him through decreasing pressures as rapidly as possible until you see the first bubble and then maintain that bubble size constant by adjusting the ambient pressure through decreasing values. That is because as soon as you see the bubble it will rather explosively increase in size. It is then almost impossible to adjust the ambient pressure to maintain the size at some reasonable level. The surrounding tissue supersaturation, among other things, simply pumps it up beyond control. So, in fact, if you are going to do something like this you have to bring the pressure down in almost any moderate fashion until you see the first bubble and then, by adjusting ambient pressure, you can maintain the bubble at a threshold size and con-

tinue from there. I believe that this observation does relate to some of the thinking we have heard about. As a separate matter, I would ask Dr. Fife, as an anatomist, what would you guess would be the characteristic time of the lens of the eye, which has no blood supply?

Dr. Fife: I think at that point I like Dr. Hills' diffusion concept. Of course, the question which you have to ask is—suppose it is the lens of the eye that has a 600- or 1,000-minute tissue half time—what does this have to do with bends in the knee, for example?

Dr. Hills: I was most interested in Dr. Bennett's paper in which he uses the zero supersaturation principle, but for the first five or ten minutes of the decompression goes to Haldane and other multi-tissues. I think that you can also invoke perfusion in just the same tissue as you ultimately use for the nil-supersaturation diffusion model because, after all, at the very start, uptake must be perfusion-limited, until you have some actual gradient which is sort of counteracting or decreasing your gradient and therefore increasing the relative contribution of diffusion to the resistance as times goes on. So I think you could still have one tissue and yet have uptake largely perfusion-limited at the start. Would you like to comment on this?

Dr. Bennett: The only comment is that I agree with you; that is all I can say.

Dr. Schreiner: I would like to ask for the record a question each of Dr. Bennett and Dr. Fife, because both of these speakers have presented decompression tables for which operational experience exists. Would you be prepared to enter into the record of these *Proceedings* the quantitative, operational experience you have had with these tables?

Dr. Bennett: From my own point of view, yes. I know of some four or six dives that have been made, man-dives that is, in the North Sea. In two of those dives, the tables were not run correctly, which is always a problem if you send tables out to the field. Eight minutes was knocked off the top of the table at the point we think is most critical. There is actually only one dive with an unadapted diver which was run to the schedule that produced post-dive bends five hours after the dive. The first ascent is at 50 feet per minute, and we have advised changing that to 30 feet per minute; we have also suggested one or two other smaller adjustments in terms of the total time close to the surface, to reduce the surfacing ratio. I think that in reality the move in the deeper area is the one that will make the difference. These tables are still being used and evaluated in the field, though.

Dr. Fife: I wonder if I could ask Mr. Naylor, who took the 410-foot table offshore, if he would discuss the number of dives he made.

Mr. Naylor: I'm trying to do some fast adding. I would like to talk about a family of tables and not just one specific depth, because the first job we went out on we ranged from about 320 to 400 feet, and I made nine dives. I don't remember the depths. These are what we call bell tables. On the air tables we generated with this system, I think there were over 200 dives, and these were from 180 to 220 feet.

Dr. Fife: Are you implying bend-free dives or total dives?

Mr. Naylor: On the first run we were having problems. I think we had too much oxygen and during that period I flew in and we generated another table with less oxygen time. Notice that we have cut that down considerably. The air tables haven't been bends-free. I had one Table 5 hit the first time out. On the second deep work effort, I had a hit at about 190 feet, and it looked like a central nervous system hit. I had no choice but to treat it as such. Whether it was a serious case or not I don't know; I went on a Table 7 for that one.

Dr. Fife: He did that to be on the safe side; the actual systems don't suggest that it was a central nervous system hit. But how many times have these tables been used now?

Mr. Naylor: I've got about 14 dives. These are two-man dives. One man came out clean and the other man has a problem. The air dives have had no trouble. I came in from two dives last week at 250 feet with this same family of 250 feet, 30 minute tables, and I only had two dives on this job. We were locating the distance between a jacket and the stingray line; I had no problems with that one.

Dr. Fife: There is one thing which we have to watch. If these divers come up with no evidence of bends but these bubbles are indeed occurring, which they seem to be doing, I think we are going to have to watch for subtle effects—for example, the personality changes which sometime occur in divers who have done extended, hard diving for several years.

Mr. Naylor: I might add that as far as the bends go in the chamber dives we made, the experimental dives, I had some problems, but nothing past a Table 5 or 5A.

Dr. Bennett: These were when tables were being developed, though.

Mr. Naylor: I think we were in trouble there. We were trying to come up from 40 feet; and offshore, I can get a man up and down in a chamber, in what we call excursion diving, in less than three minutes. I was trying to take five minutes on these experimental runs to cover myself in an offshore situation and we were getting hit coming out, and then I also had some delayed bends which came on 18 hours later that were treated on Table 5.

Dr. Schreiner: Thank you very much; this input from the real world is sobering and useful.

Dr. Bennett: A further piece of information is that we did do two man-dives off Florida, and they were very successful. In the laboratory we were diving twice a week, and we changed the team of divers once every three months; they came in, had full medicals, and started diving. They dived regularly for three months, with a week or so in between. In the field situation, in the particular dives that have been done, the divers had not been diving for quite awhile, and they were suddenly called upon to go to 600 feet without any form of work-up. Experience at the RNPL showed that in deep oxygen-helium diving, it was a very good thing to work up the divers; some form of adaptation to decompression sickness may be one of the reasons why when you go from the laboratory to the ocean you sometimes have to adjust the table a little to make it work.

Dr. Hills: I have a question for Dr. Oser. When he was giving his presentation, he dismissed the zero supersaturation principle as non-physiological. Before this principle was invoked, we used tissue probes and did prove that there was an unsaturation. Would you please say why you think that the zero supersaturation principle is non-physiological?

Dr. Oser: I'm afraid I cannot answer that question. The rationale for not putting it in a physiologic background has been proposed by Professor Müller, who is doing the calculation for our tables, and he is not around.

Dr. D'Aoust: If you won't answer that, I will. I won't say it is non-physiological, I think it is non-real. This whole problem of evaluating these models depends very much where you are in that decompression curve. We can prove you can measure supersaturation first in a stirred liquid system that you have saturated. It is a matter of how long it takes to decay. With a little thought, in our case 20-20 hindsight, we can measure a decay time for 100 feet of supersaturation of 35 minutes; this is an approximate thing. On the other hand, 10 feet of seawater will hang around in exactly the same system for an hour and a half. Obviously, there are a lot of things to consider here, such as initial delta P and the gas solubility of the concentration. With a very slow time constant a no-supersaturation model could perhaps be quite realistic. With a fast tissue, it might be less realistic. But it seems to me that on all these curves we have been seeing there are places where these different models will fit, and if you study that it becomes a self-fulfilling prophecy. I think we really do have to try to make some measurements.

Mr. Krasberg: I've got a model that is very similar to that used by Dick Vann and Dr. Hills; it has some other elements of perfusion in the fast tissues. Over the past three years we have had several hundred exposures, 450 to 600 feet, with bottom times up to 90 minutes. We have had seven hits; three of these were artifacts caused by improper decompression by the diving supervisor. So it is possible to do relatively rapid bounce dives as safely as saturation dives. A typical decompression time is 16 hours for a 550 fsw-40 minute dive.

Part VIII. PROGRESS IN SATURATION AND EXCURSION DIVING

REVIEW OF VERTICAL EXCURSION DIVING UNDER SATURATED CONDITIONS

James W. Miller

The number of individuals involved in diving-related activities has been increasing steadily for several years. This increase is reflected in scientific diving as well as in recreational and commercial diving.

Significant achievements are being made toward working at great depths, particularly in the offshore industries. However, much work remains to be performed in relatively shallow water, i.e., less than 300 fsw.

For both scientific and working purposes, the need for extended bottom times is becoming more apparent. Further, increased costs dictate that the most economical procedures consistent with safety and availability of breathing gases be used, and that sophisticated systems and exotic breathing gases be used only when absolutely necessary. In addition to extended bottom times, divers need maximum flexibility while carrying out both horizontal and vertical excursions. The former have been aided significantly by the use of various swimmer propulsion systems and are primarily an engineering problem. The latter, however, require additional basic and applied physiological research. Most investigations relating to vertical excursions have used the surface (one atmosphere) as the basis for downward excursions. There are no-decompression dive tables, decompression dive tables, repetitive dive tables, and so forth, all based on the diver's being saturated on air at the surface. There is now a need for similar data using higher ambient pressures as a point of departure (or storage depth).

For these reasons, a renewed interest in air and nitrogen-oxygen saturation diving has occurred during the past three or four years. Investigations during this period have shown that it is possible to use air and nitrogen-oxygen breathing mixtures in situations which were previously thought to require the use of more exotic breathing gases (6).

Since 1962, several laboratory and open-sea programs have been carried out in which significant vertical excursions have been made from saturation storage depths exceeding one atmosphere. Only those programs which used air or nitrogen-oxygen as the breathing gas will be discussed.

Figure 1 summarizes these data by identifying the program, the breathing gas used, the saturation depth, and the maximum vertical excursion depths and times.

Figure 1 shows that the shallowest air saturation depth to date is the 26-fsw program of

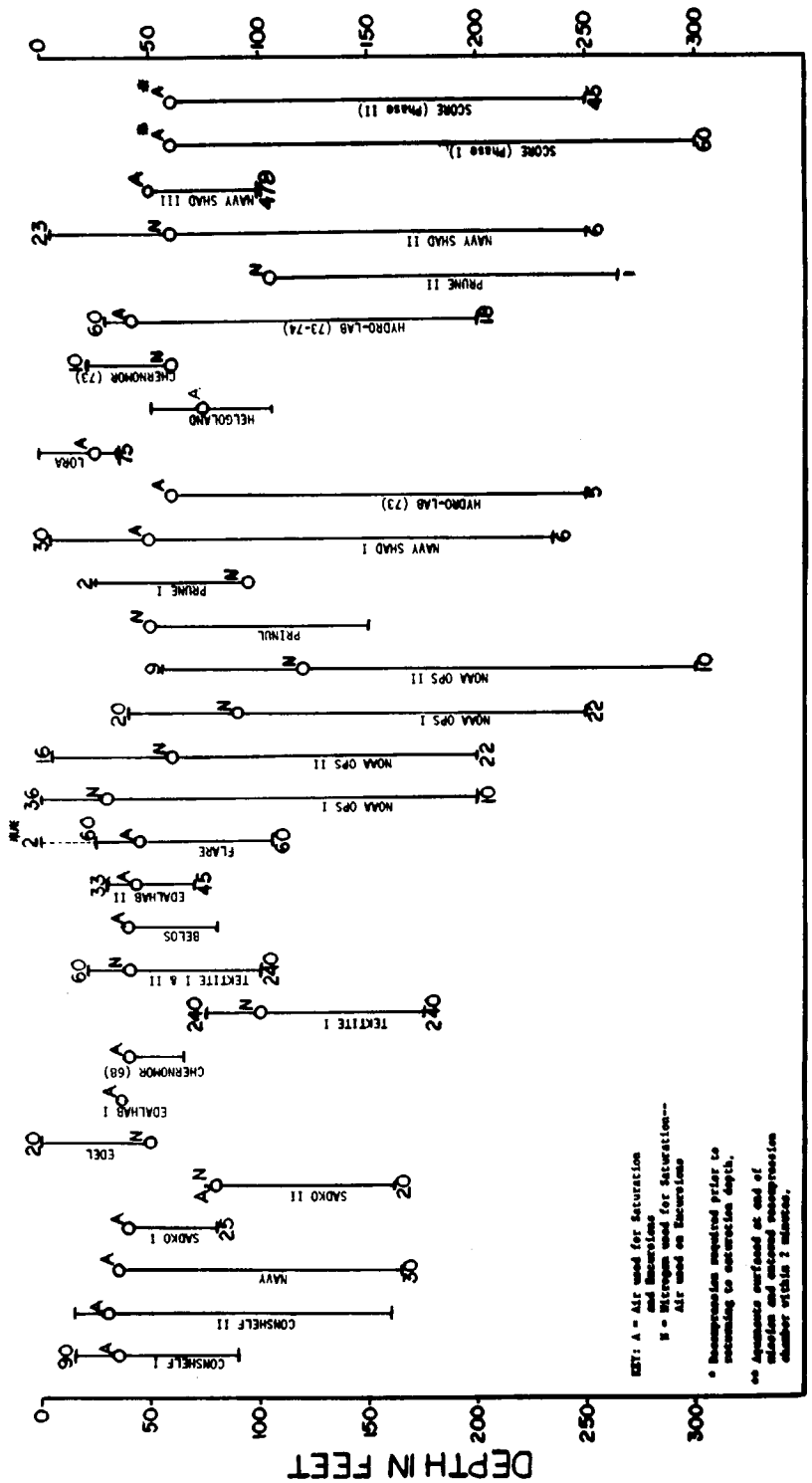

Fig. 1. Vertical excursions and maximum times to depths of 300 fsw, 1962–1975; circles = saturation or storage depth; horizontal bars and numbers show maximum excursion depths and times.

LORA in 1973 (3). The most significant feature of the LORA program is that the two aquanauts surfaced with no decompression after 24 hours' saturation at a depth of 26 fsw. The deepest saturation on nitrogen-oxygen is 120 fsw, which was achieved in the NOAA-OPS program (4). During this program, the subjects made excursions up to a depth of 5 fsw and down to 300 fsw from saturation depths of 30, 60, 90, and 120 fsw.

The first program designed specifically to study vertical excursions from air saturation was reported in 1967 by Larson and Mazzone (5). After a series of dry chamber studies using dogs, a similar series of studies was conducted using 13 human subjects. Air was breathed during both the saturation and excursions. The saturation depth was 35 fsw, with excursions to depths of 165 fsw.

Table I (taken from Larson and Mazzone, reference 5) shows the number of excursions and the results of both the animal and human series of experiments.

Prior to Tektite I, a study was conducted to determine the safe surface interval between direct surfacing from the 42-fsw saturation depth on a normoxic breathing mixture and the onset of decompression sickness (2)—in other words, an upward excursion to the surface from 42 fsw (49 fsw air equivalent depth).

The experiment also was designed to develop treatment tables for use when bends occur after an accidental surfacing. Six subjects were saturated in a normoxic atmosphere at 42 fsw. They were decompressed to the surface in one minute. Two subjects each remained at the surface for 10, 15, and 20 minutes, respectively, prior to recompression to 42 fsw. No symptoms were noted for those subjects who remained on the surface for 10 or 15 minutes. After 19 minutes, one of the subjects in the 20-minute group developed serious neurocirculatory symptoms which dissipated rapidly upon recompression to 60 fsw. The study concluded that, "A surface interval of 15 minutes after accidental surfacing before recompression to 60 feet was accepted as safe against the liability of dysbarism" from a saturation depth of 42 fsw (2).

The excursion limits used during the 42-fsw Tektite program were 4 hours, both for excursions to 25 fsw and 100 fsw, breathing air. While several upward excursions to such depths were made, the seafloor topography did not permit downward excursions beyond a depth of 65 fsw.

TABLE I

DESCRIPTION AND RESULTS OF ANIMAL AND HUMAN EXCURSION SERIES

Animal Series		Human Series	
Saturation exposures	7	Saturation exposures	15
Excursions		Excursions	
165/30	13	165/30	6
135/60	8	135/60	4
117/90	4	117/90	1
109/120	4	109/120	2
105/150	4	105/150	2
100/240	3	100/240	2
Total	36	Total	17
Cases of decompression sickness	0	Cases of decompression sickness	0

In 1972 the Florida Aquanaut Research Expedition program (FLARE) was conducted off the southeast coast of Florida. During this program 25 marine scientists, in teams of three, spent periods of five days on the seafloor at depths ranging from 42-45 fsw.

The depth limit for upward excursions was 20 fsw. No-decompression schedules were established for downward excursions by modifying the U.S. Navy Standard Air Table No-Decompression Limits and Repetitive Group Designation Table for no-decompression dives.

The most notable aspect of this program relating to vertical excursions was the manner in which decompression was achieved. Because the habitat (*Edalhab*) was not a pressure vessel, the aquanauts had to utilize a small decompression chamber located on the deck of the support ship *Lulu*. The entire procedure, which called for the aquanauts to ascend one at a time from the habitat to the deck decompression chamber, required only two minutes. Because of transient reduction of pressure to sea level during transfer from habitat to deck chamber, a 40-minute period of oxygen breathing was carried out in the habitat just prior to ascent to surface. As an added safety factor, a 30-minute period of oxygen breathing at 50 fsw was carried out in the deck chamber as soon as the last aquanaut was recompressed to saturation depth.

The 25 excursions to the surface described above were achieved without incident and became routine by the end of the mission. The advantages of this procedure with respect to cost, simplicity, and lack of need for a diving bell, are obvious.

Hydro-Lab

The Hydro-Lab Underwater Research Program is a long-range scientific and educational program located at Grand Bahama Island. It is centered around the Hydro-Lab, an 8 × 16 foot cylindrical habitat that operates at either ambient or surface pressure.

As of May 1975, the Hydro-Lab system has saturated 293 diver-scientists at the hatch depth of 42 fsw. The average saturation time was 6 days for each team. Another 24 divers have been saturated at a depth of 60 fsw for 5–7 days; the total number of diver-scientist saturations is 317. The shortest dive was 24 hours, and the longest, 13 days. There have been over 90 separate missions.

Prior to the availability of the NOAA-OPS vertical excursion profiles in 1973, excursions from Hydro-Lab beyond a depth of 90 fsw were not permitted. This was a safe practice since the excursion profiles now allow a 5-hour excursion to 90 fsw from a 40-fsw saturation and unlimited time from a 60-fsw saturation.

Excursions from 42 Feet

Sixty-nine no-decompression and four decompression excursion dives were made from a 42-fsw saturation depth to depths in excess of 130 fsw. Figure 2 illustrates the excursion profile used by 25 different aquanauts in making excursions to a depth of 130 fsw in July and August 1974. These excursions required each aquanaut to swim approximately 3,000 feet, round trip, and involved a 33-minute bottom time. No symptoms of bends occurred during any of these excursions. According to the NOAA-OPS tables, the maximum bottom time for a 130-fsw, no-decompression excursion is 70 minutes; the lack of bends is therefore not surprising. Longer bottom times were not attempted because the divers were college students participating in an advanced scientific diving-training program.

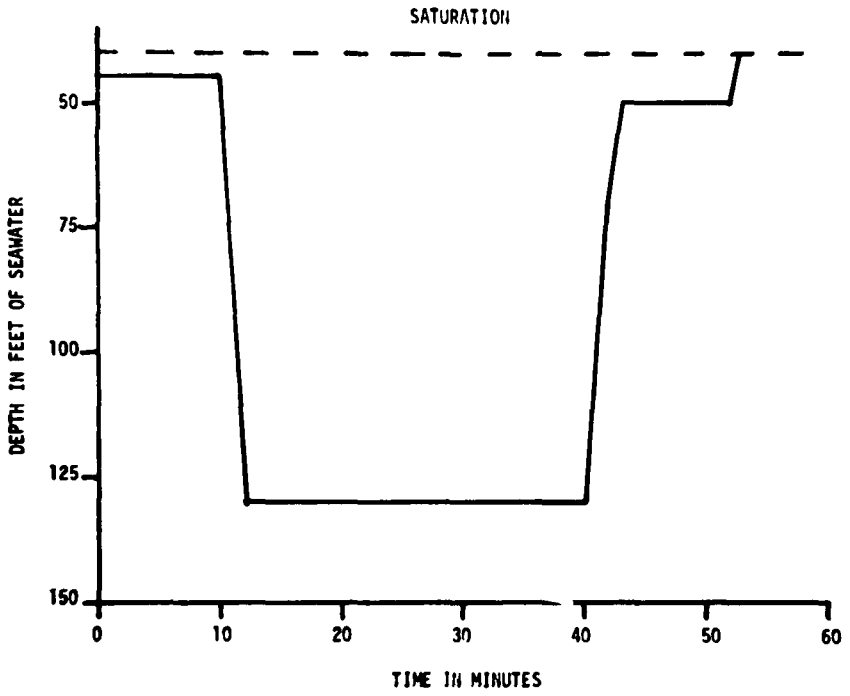

Fig. 2. No-decompression profile used by 25 aquanauts for excursions to 130 fsw from 42-fsw air saturation.

In July 1974 three aquanauts also made a series of excursions to depths of 150 and 200 fsw from a 42-fsw saturation depth. Bottom times at 150 fsw were between 21 and 42 minutes for most of the excursions. As in each deep Hydro-Lab excursion, a swim of about 3,000 feet was required. The seven 200-foot excursions made by the same three aquanauts are shown in Fig. 3. In each of these excursions, the primary mission was to collect biological specimens and to photograph the deep reef wall. All of the 200-fsw excursions were made for either 18 or 19 minutes; no symptoms of bends occurred.

During a different mission in August 1974, a series of 13 man-excursions was conducted by four different divers without incident to depths of 165-175 fsw to carry out geological and oceanographic studies. These excursion profiles are shown in Fig. 4.

In October 1973, two aquanauts (the Hydro-Lab directors) were saturated on air for 6 days at a depth of 42 fsw to conduct studies relating to fish behavior in and around traps. During this period, the two aquanauts made a series of 16 no-decompression excursions to depths ranging from 50-200 fsw. Figure 5 summarizes the depths and times. No symptoms of bends were noted during any of these excursions.

In summary, the Hydro-Lab program has provided a facility for a significant number of excursion dives. To date, no cases of bends have followed any excursion, and in only one case out of 317 involving saturated aquanauts were bends suspected after final decompression.

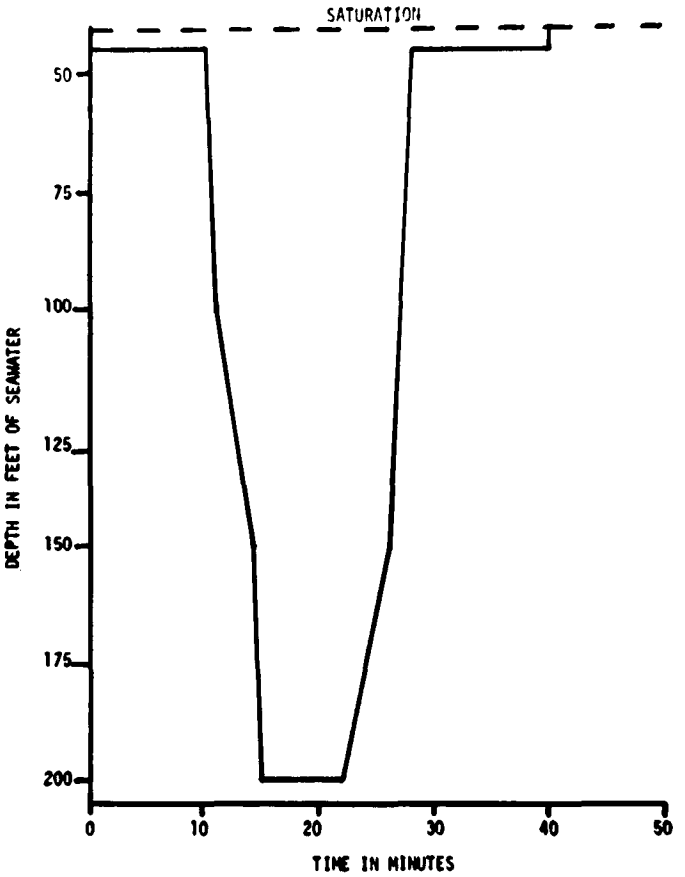

Fig. 3. Typical no-decompression profile used by three aquanauts for 7 excursions to 200 fsw from 42-fsw air saturation.

PRUNE I

Puerto Rico Undersea Nitrogen Excursion (PRUNE I) was an open-sea project conducted in May 1973, which tested, among other things, the upward excursion profiles developed during the NOAA-OPS program. The 14-day, 4-man program was located 10 miles off the southeast coast of Puerto Rico. It utilized the LaChalupa Habitat. The saturation depth was 95 fsw; a normoxic breathing mixture was used as the storage gas and air for all excursions.

The habitat is a 48 × 20 × 10 foot barge-like structure containing living and control chambers, each 8 ft in diameter by 19 ft in length, and a wet laboratory, located in the mid-section.

The living compartment is capable of withstanding an internal pressure of 50 psi and is used as a decompression chamber when the habitat is surfaced at the end of a mission. The control compartment is capable of withstanding an external pressure of 50 psi; in an emergency, it can be used as a decompression chamber on the bottom.

Both compartments have a transfer chamber attached to an upper hatch, which permits transfer of personnel and equipment.

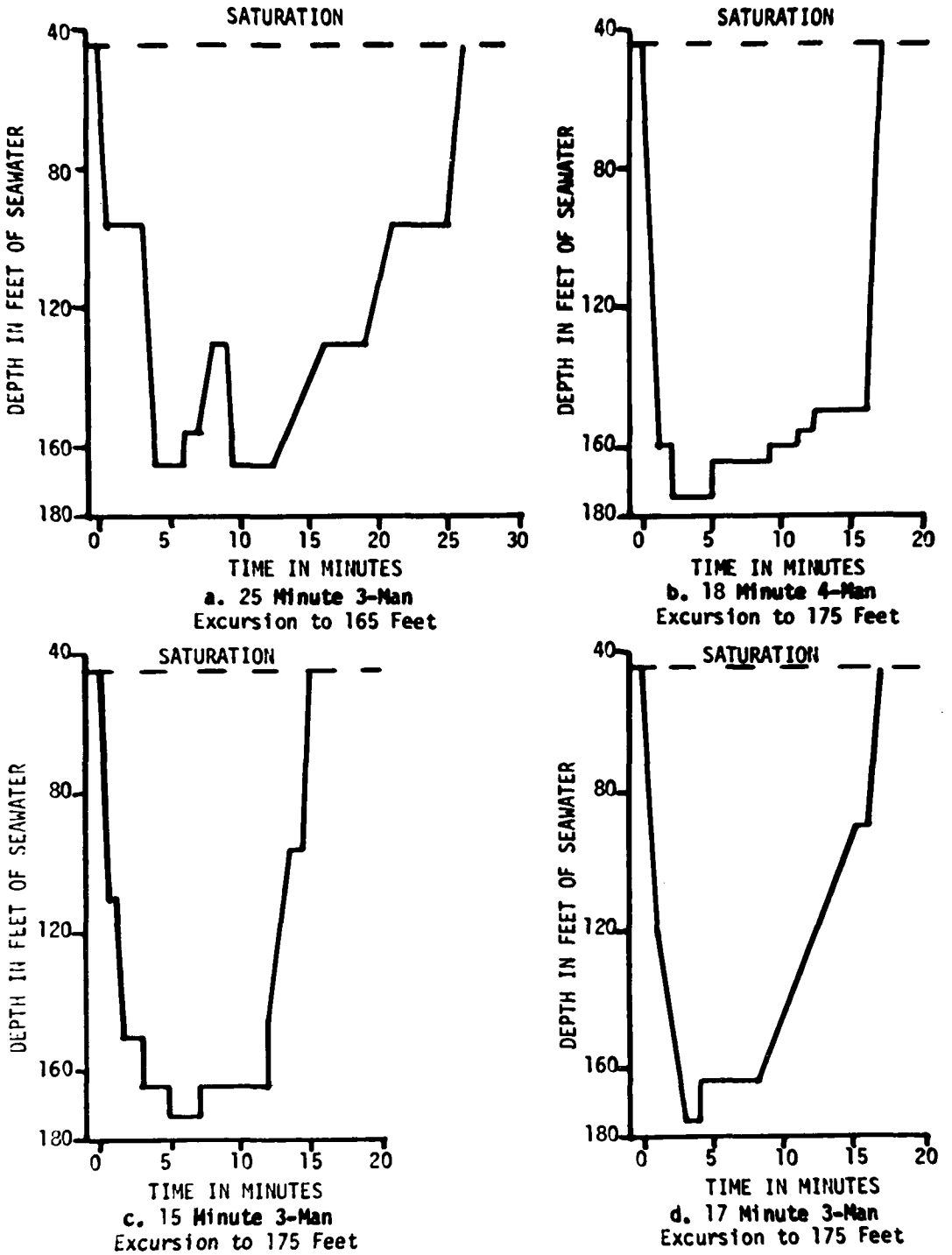

Fig. 4. No-decompression profiles for 13 excursions to depths of 165–175 fsw from 42-fsw air saturation.

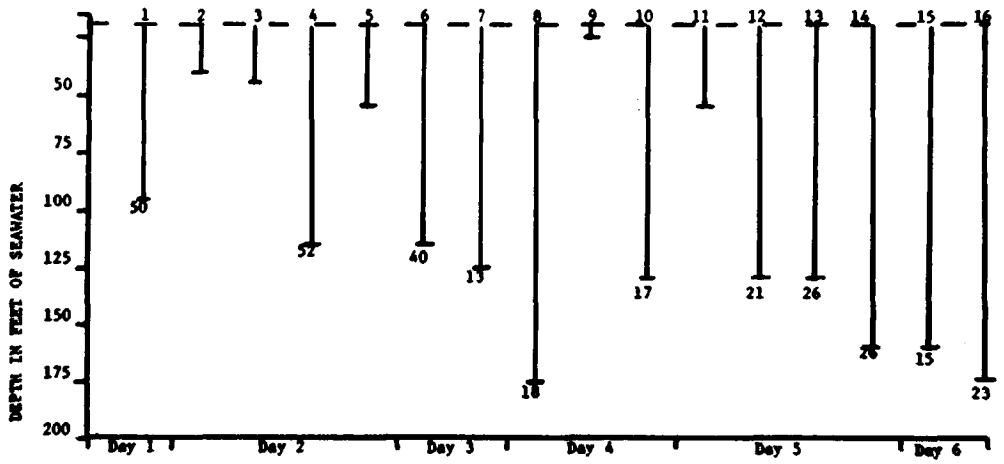

Fig. 5. Representation of 16 no-decompression excursions made by two aquanauts from 42-fsw air saturation; numbers under short horizontal bars represent excursion times in minutes.

The sub-port or wet lab, a captured air space located between the living and control chambers, is open at the bottom. It is used for entrance into the habitat and dry access between living and control compartments.

Once each day an upward excursion was made by two or three divers. On each excursion the divers followed the habitat umbilical to the prescribed depth and maintained physical contact with the umbilical for the entire excursion. The wrist depth gauges used were checked daily with the master gauge in the habitat. The ascent rate was approximately 30 fsw per minute. All excursions were made with the men breathing air from either a standard set of double cylinders or a hookah (breathing hose) attached to the habitat.

The divers checked the time constantly during each excursion and questioned one another for any signs or symptoms of bends. Excursions were made on 10 successive days at times selected for compatability with other planned activities.

Twenty-three man-excursions were successfully completed. Two excursions each were made to depths of 25, 30, 40, 50, and 60 fsw. Table II gives a brief summary of these excursions and their results. No real bends occurred, although one diver felt a few niggles during some excursions. These symptoms are noted in Table II.

PRUNE II

This project was principally designed to test the NOAA-OPS downward excursion profiles to a depth of 300 fsw and to measure any evidence of inert gas narcosis objectively, using tests of time estimation and mental arithmetic. A secondary objective was to conduct a survey of benthic marine organisms.

The program took place during March 1974, in the same general locale as PRUNE I. The saturation depth was 106 fsw, with a nitrogen-oxygen breathing mixture used for storage and air used for all excursions. The LaChalupa Habitat was again employed. The topography of the dive site was dominated by three major features: a broad terrace, an uninterrupted buttress

TABLE II

VERTICAL TEST EXCURSIONS FROM PRINUL HABITAT AT SATURATION DEPTH OF 95 FSW

Excursion Depth, fsw	Duration, min	Habitat Mix, N_2/O_2	Equipment	No. Divers	Symptoms
60	55	96/4	Hookah	2	None
60	55	95/5	Hookah	2	Niggle, rt. knee*
50	30	90/10	Hookah	2	Niggle, lt. elbow
50	31	95/5	SCUBA	3	None
40	18	94/6	SCUBA	2	Slight niggle, rt. knee
40	18	96/4	SCUBA	2	None
30	7	94/6	SCUBA	2	None
30	7	95/5	SCUBA	3	Niggle, rt. knee
25	2	95/5	SCUBA	3	Niggle, lt. elbow
25	2	93/7	SCUBA	2	None

*Same diver each time.

reef on the outer edge of the terrace, and a relatively steep, terraced drop-off into deep water on the outer side of the reef. The buttress reef had a relief of 45 feet and was approximately 400 feet wide. Its location seaward of the habitat required the divers to ascend from the saturation depth of 106 fsw to a depth of 65 fsw on each excursion into the deep water seaward of the buttress reef.

Two divers made each excursion. Air was supplied via a 700-foot hose (hookah) attached to the habitat, and was used for all excursions. Each diver wore a full-face diving helmet (Kirby Morgan Band Mask, KMB-8) connected to the hookah. The helmet also contained hardwire communication to the habitat. In addition to the hookah, each diver wore a set of double 72 cubic foot SCUBA tanks as an emergency backup system. While two divers were making an excursion, the other two were in the habitat manning the communication system, logging times, and recording data.

On each excursion, when time permitted, tests were conducted to detect any manifestation of nitrogen narcosis. Tests were designed to measure the ability to estimate the passage of time and to assess short-term memory. The time-estimation test required the diver to estimate, upon demand from the experimenter in the habitat, the passage of times ranging from 4 to 24 seconds (4, 6, 10, 12, 16, 18, 22, and 24 seconds). Data were collected on the surface prior to the mission, in the habitat, and on most excursions.

The digit span (short-term memory) test was conducted in a similar fashion. The experimenter read sequences of 4-10 digits to the diver, who then repeated them, either in the same sequence or in reverse order.

Excursions were made to depths ranging from 160–265 fsw. The excursion profiles are shown in Table III. Because of the required ascent over the buttress reef, the excursion times originally planned had to be modified. It should be noted that because of the unique topographical features, bottom time began upon reaching the 120-fsw depth on the seaward side of the reef and ended when ascent began from the excursion depth.

TABLE III

PRUNE II EXCURSION PROFILES FROM SATURATION AT 106 FEET

Excursion No.*	Max. Depth, fsw	Time to 120 fsw, min	Time at 120 fsw, min	Time to Max. Depth, min	Time at Max. Depth, min	Time to 120 fsw, min	Time at 120 fsw, min	Time to Habitat, min
1	160	11	2	1	239	3	5	12
2	180	10	2	1	64	3	2	8
3	180	10	2	1	64	4	1	10
4	200	17	2	10	17	4	2	15
5	200	17	2	2	26	6	1	11
6	225	12	1	3	12	3	1	5
7	225	11	2	2	13	5	1	5
8	250	13	13	4	4	5	2	24
9	250	12	8	10	2	6	2	8
10	265	7	8	5	1**	13	4	8

*Two divers each excursion. **Aborted because of nitrogen narcosis in one diver; both divers ascended to 200 fsw and stayed for 8 min.

Table IV shows the depth of each excursion and the times allowed at each depth. These values reflect the time actually spent at depth, which were modified because of the buttress reef, and the times called for by the NOAA-OPS tables.

Although the limits of the NOAA-OPS profiles were not tested because of the required ascent over the buttress reef, the feasibility of making deep excursions using air was demonstrated. These excursions can be classified readily as working dives because of the strenuous physical effort involved in hauling and manipulating the 700-foot hookah hose.

TABLE IV

PRUNE II EXCURSION DEPTHS AND TIMES FROM SATURATION AT 106 FSW

Excursion No.	Excursion Depth, fsw	Time Allowed, No Buttress, min	Time Allowed With Buttress, min	Time Actually Spent at Depth, min
1	160	360	240	239
2	180	360	65	64
3	180	360	65	64
4	200	262	28	17
5	200	262	28	26
6	225	62	163	12
7	225	62	163	13
8	250	35	9.3	4
9	250	35	9.3	2
10*	265	14.7	5.5	1

*One min at 265 fsw where excursion was aborted because of narcosis of one diver; divers stayed for 8 min at 200 fsw.

A total of 20 man-excursions were completed during the mission. There were no respiratory problems or symptoms of bends; one diver, however, experienced nitrogen narcosis at a depth of 265 fsw on an excursion scheduled to go to 275 fsw. This excursion was aborted at 265 fsw. The other diver on this excursion had no apparent symptoms of narcosis.

The results of the digit span memory test were inconclusive, because learning was still occurring throughout the mission. No decrement in short-term memory was noted during the excursions. Though statistically inconclusive, the results indicate that the divers could repeat a series of up to nine digits at depths to 225 fsw.

Results of the time-estimation test show a statistically significant trend in the direction of overestimating elapsed time, that is, estimating times to be longer than they really were. Although the underlying cause for this finding is not clear, the trend observed is consistent with results of other studies where time estimation was used as a human performance measure in stressful situations (1).

SHAD

The Naval Submarine Medical Research Laboratory has for the past three years been conducting a series of air saturation dives (Shallow Habitat Air Dives). Since this program is discussed in detail in the paper in this volume by Adams, Williamson, Harvey, Murray, and Hester, it will only be briefly mentioned here.

Four saturation dives were carried out, principally to study biomedical problems. Air was used both as the storage and excursion gas. The saturation depths for SHAD I, II, and III were 50, 60, and 50 fsw, respectively, and the durations were 29½, 28, and 9 days, respectively. A total of eight ascending excursions and 31 descending excursions were completed to depths ranging from 5–250 fsw.

Significant environmentally related changes were not observed during the residence periods. Three cases of decompression sickness occurred, and signs of oxygen toxicity were also observed during the series. Data analysis has not yet been completed.

SCORE

Prior to the Scientific Cooperative Operational Research Expedition (Project SCORE), air excursions had only been conducted from saturation conditions in a manner which permitted divers to return to the saturation depth without decompression. To increase useful bottom time and to verify earlier studies, excursion profiles were developed which require decompression before returning to storage depth. Evaluation of these new excursion profiles was carried out in the laboratory and in the open sea, along with further tests to determine whether there was any impairment of cognitive, psychomotor, or physiological functions.

The SCORE program had two phases: Phase I took place in the hyperbaric chambers at Duke University; Phase II took place at Grand Bahama Island and utilized the Hydro-Lab habitat and the Johnson-Sea-Link research submersible.

Phase I

The purpose of Phase I was to saturate divers on air at a depth of 60 fsw and to make air excursions to depths of 200, 250, and 300 fsw for periods of one hour, using a diving profile

which included decompression before returning to the 60-fsw storage depth. This five-day saturation program took place during February 1975.

Procedure

Two teams of four men were selected, with a fifth man to act as standby in case of illness. One experienced diver was selected from each group to make scheduled dives in the wet chamber.

Base-line data were collected from all subjects for one week prior to the saturation dive. These data included performance tests consisting of:

(1) Arithmetic test. Two-figure by one-figure multiplication, e.g., 68 × 9;
(2) Ball bearing test; picking up ball bearings with tweezers and placing them in a tube of the same diameter;
(3) Signs and symptoms questionnaire;
(4) Digit span forward. Numbers were read out which the subject had to repeat forward. The numbers increase in length one at a time until the subject makes an error;
(5) Time estimation; subject was required to estimate the passage of times selected at random, ranging from 4–24 seconds.

Venous blood samples were obtained before the dive, throughout the dive, and several days postdive, so that studies could be made of morphology, platelet, or any enzyme changes. Additional data were obtained using a Doppler bubble detector with an over-the-heart sensor during decompressions after excursions.

The subject started the performance tests at the surface and continued regular and frequent practice to eliminate learning characteristics as far as possible within the time available. Control studies were performed at depths of 200, 250, and 300 fsw during which the subjects descended from the surface and remained for 15 minutes while taking performance tests. Further control data were obtained upon reaching the 60-fsw saturation depth.

The excursions were carried out in accordance with the following schedule:

Day 1, 1800—Compress to 60 fsw on air; oxygen in gas was maintained between 20.7 and 20.9%;
Day 2, 0927—Team A compressed to 200 fsw for a 60-min bottom time;
1502—Team B compressed to 200 fsw for a 60-min bottom time;
Day 3, 0912—Team A compressed to 250 fsw for a 60-min bottom time;
1502—Team B compressed to 250 fsw for a 60-min bottom time;
Day 4, 0911—Team A compressed to 300 fsw for a 60-min bottom time;
1500—Team B compressed to 300 fsw for a 57.4-min bottom time;
Day 5, 2000—Begin decompression from 60 fsw to the surface;
Day 6, 1315—Subjects surface.

Decompression profiles for the 200- and 250-fsw excursions are shown in Table V.

Results of Phase I revealed no evidence of decompression sickness on any excursions. During the 250-fsw and 300-fsw excursions, however, symptoms of oxygen toxicity were noted. At 250 fsw, one subject reported numbness of fingers and hands, which was diagnosed as a minor oxygen hit. At 300 fsw, two subjects experienced oxygen toxicity. One reported pain and numbness and the other went into convulsions. Both were treated successfully.

With regard to the performance tests, it was found that the poorest performance was elicited during the presaturation bounce dives. The slight improvement in performance during

TABLE V

AIR DECOMPRESSION TABLES FOR 200- AND 250-FSW EXCURSIONS FROM 60-FOOT STORAGE DEPTH USED IN PROJECT SCORE

Depth, fsw	Rate of Ascent, fsw/min	Elapsed Time, min
	200-fsw Excursion for 1 hour	
200 to 90	30	4
90 stop	—	6
90 to 80	10	1
80 stop	—	4
80 to 70	10	1
70 stop	—	3
70 to 60	10	1
	Total	20
	250-fsw Excursion for 1 hour	
250 to 120	30	4
120 stop	—	1
120 to 110	10	1
110 stop	—	3
110 to 100	10	1
100 stop	—	7
100 to 90	10	1
90 stop	—	6
90 to 80	10	1
80 stop	—	9
80 to 70	10	1
70 stop	—	9
70 to 60	10	1
	Total	45

tests at 60 fsw saturation was minor and may simply have reflected some continued learning. Results obtained during excursions showed considerable decrement during the 300-fsw excursions (and postsaturation bounce dives) compared to data obtained at 200 and 250 fsw. There appeared to be some improvement in performance during the 200- and 250-fsw excursions compared to the initial bounce dives to the same depths. Some learning was still present, however, when the pre- and postsaturation dives to 300 fsw were compared. The improvement in performance during the excursions may thus have been a function of such learning rather than a physiological adaptation to narcosis.

While evidence has been found to suggest an adaptation to nitrogen narcosis in earlier studies, it was concluded that so far as the SCORE study was concerned, there was insufficient evidence to support these earlier findings in compressed air saturation-excursion diving. It cannot be concluded, however, that adaptation to nitrogen narcosis does not occur. The confounding factors of learning and depth in the SCORE studies do not permit any overall final conclusions. When normoxic mixtures were used as storage gas in other studies, there seemed to be clear evidence of an adaptation even though the actual excursions were made on air. More work remains to be done on this aspect of nitrogen-air saturation. Because of the added

risk of oxygen convulsions, however, it is recommended that at the present time no compressed air excursions be made to 300 fsw when the divers are saturated at a storage depth of 60 feet with air as the breathing mixture.

Phase II

Phase II of SCORE was designed to utilize the vertical excursion and decompression profiles tested during Phase I in the open sea. This open-sea project also allowed marine scientists to study and gather samples from a deep vertical reef wall. A further technical objective was the test and evaluation of a newly developed anchoring system which provided stability for the submersible, Johnson-Sea-Link, while it was in the diver lock-out mode suspended next to the vertical wall.

Phase II utilized the lock-out submersible Johnson-Sea-Link, the Hydro-Lab underwater laboratory, the Sub-Igloo diver station, the support ship R/V Johnson, two small hemispherical ocean floor air stations, and miscellaneous support boats.

Four teams of four aquanauts each took part in Phase II. Swimming excursions were made to a depth of 200 fsw for periods up to 60 minutes. The 250-fsw excursions were made by locking out of the Johnson-Sea-Link submersible for periods of up to 45 minutes. The same decompression profiles were used as in Phase I. All teams were saturated on air for five days at a depth of 60 fsw.

A total of 34 200-fsw excursions were made with no evidence of decompression sickness. Figure 6 is typical of a 200-fsw swimming excursion, showing that approximately 53 minutes

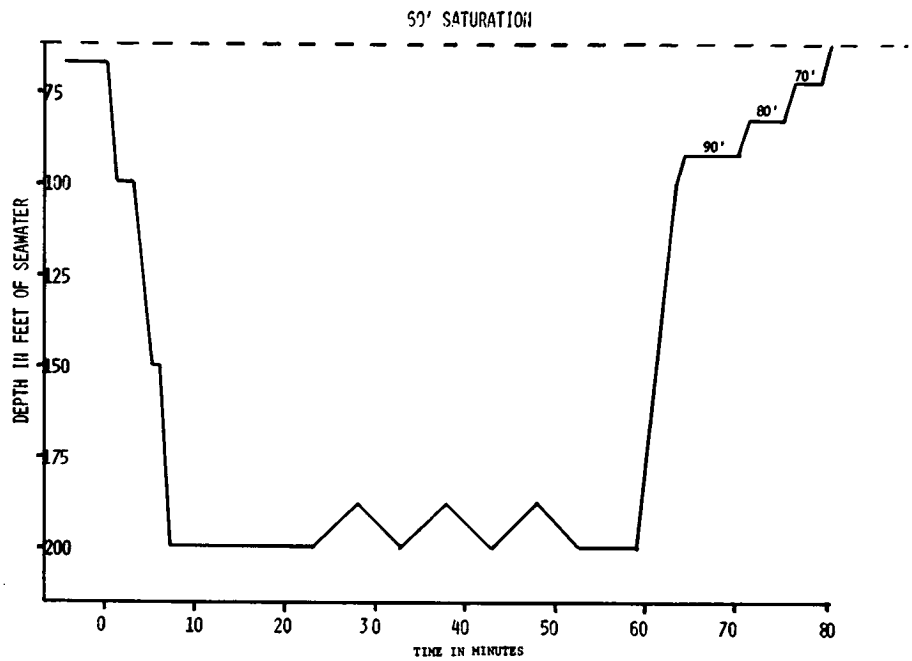

Fig. 6. Swimming Excursion Profile 3, used for eight man-dives.

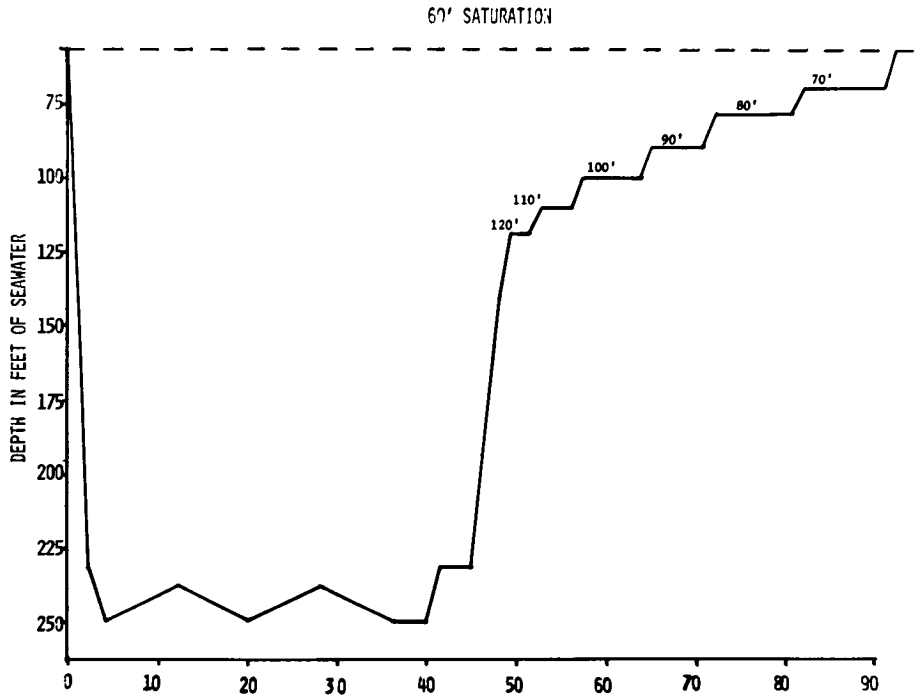

Fig. 7. Excursion profile used for 13 submersible lockout dives.

were spent at about 200 fsw. The excursions were followed by decompression stops at depths of 90, 80, and 70 fsw during the return to the habitat; stops totalled 20 minutes' decompression, as shown in Table V.

Twenty-six man-excursions were made to a depth of 250 fsw with no evidence of decompression sickness. Figure 7 depicts a typical 250-fsw excursion profile. Although an excursion time of 60 minutes had been tested during Phase I, the maximum excursion time in Phase II was about 40 minutes. This permitted an additional safety margin to perform submersible reentry. The 45-minute decompression was carried out in the submersible in accordance with the schedule shown in Table V and outlined in Fig. 7.

Phase II of SCORE demonstrated the feasibility of conducting open-sea, working decompression, excursion dives on air to depths to 250 fsw from an air saturation storage depth of 60 fsw. The open-sea program verified the excursion decompression schedules tested in the Phase I laboratory program. If inert gas narcosis was present, it was not, in the opinion of aquanauts or observers, of sufficient magnitude to impair cognitive or psychomotor performance. The program also demonstrated the viability of combining habitat and submersible operations into a single scientific effort.

Summary

Much remains to be done before it will be possible to exploit fully the potential of air or nitrogen-oxygen saturation diving and excursions. The technical and financial demands of

marine resource development make it imperative that the possibilities of these techniques not be overlooked.

ACKNOWLEDGMENTS

The author appreciates the contributions of Robert Wicklund and Richard Clarke of the Perry Foundation, Inc., and of Peter Bennett of Duke University, who provided some previously unpublished data on the Hydro-Lab and SCORE programs. Appreciation is also expressed to Arthur Bachrach, Micheal Walsh, and Bill Armstrong of the Naval Medical Research Institute, who contributed to the diver performance program of PRUNE II. Acknowledgment is also given to Ian Koblick and the Marine Resources Development Foundation of Puerto Rico for providing support for the PRUNE I and II programs.

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SHALLOW HABITAT AIR DIVING WITH EXCURSIONS BETWEEN 5 AND 250 FSWG: A REVIEW OF FOUR SIMULATED DIVES

G. Adams, R. Williamson, C. Harvey, R. Murray and R. Hester

The Shallow Habitat Air Diving (SHAD) program was initiated at the Naval Submarine Medical Research Laboratory, Groton, Conn. in 1972. The program was designed to assess the biomedical feasibility of man's extended residence in compressed air at relatively shallow depths with periodic brief exposures above and below the residence depth. Compressed air was among the first gases employed in saturation diving (6), and it continues to be employed in shallow and intermediate depth saturation exposures for limited durations (1). The SHAD program was based on earlier saturation experience and on the successful completion of animal experiments at 50 feet of seawater gauge (fswg) for 60 days and at 60 fswg for 36 days in compressed air (16). The SHAD program provided a dry chamber evaluation of pressure profiles applicable in both diving and caisson projects.

Compressed air saturation/excursion exposures inherently have limitations caused by nitrogen narcosis, oxygen toxicity, and gas density elevations. The biomedical evaluations in SHAD were directed toward investigations in these broad areas. The Clark and Lambertsen review on pulmonary oxygen toxicity (8) provided guidance in establishing both the initial residence depth and the SHAD program for pulmonary evaluations. The excursions were designed to incur nitrogen narcosis at the deeper excursion depths (5, 25), and the possibility that repetitive exposures would lead to adaptation (11) was considered in planning the excursion matrix. Hematological alterations caused by elevated oxygen exposures (3, 13) were also considered.

Methods

The SHAD program was conducted in the dry hyperbaric chamber complex at the Naval Submarine Medical Research Laboratory. This complex had been employed previously in both helium-oxygen (4) and nitrogen-oxygen manned exposures. The principal double-lock chamber was nine feet in diameter, with an inner lock 15 feet long and an outer lock 10 feet long. Compressed air was supplied independently to each lock from a common volume tank. Independent life support loops were present in each lock to control carbon dioxide levels, ambient temperature, and ambient humidity. Redundant lighting, visual communication, and audio communication systems were available in each lock. Diver hygiene systems were in the outer lock. A small-item transfer lock was included in the inner lock. Part of the instrumentation required for the various biomedical evaluations was permanently located in the chamber and the remainder was locked into the chamber as required. Table I delineates the investigative program

TABLE I

PRINCIPAL BIOMEDICAL OBSERVATIONS IN SHAD PROGRAM

Pulmonary function (FVC, FEV_1, FEV_2, MEFR, MIFR, MVV)*
Gas exchange (BTPS, BPM, VT, $Paco_2$, RQ)**
Inspired/expired gas analysis
Exercise tolerance
Carbon dioxide tolerance
Mixed venous blood gases
Visual evoked response
EEG
Fundus photography
Night vision sensitivity
Visual fields and acuity
Color vision**
Weight
Rectal temperature
Blood pressure*
ECG (scalar and vector)*
Physical examination*
Longitudinal health study
Long bone radiographs
Adaptive tracking
Mental arithmetic
Short-term memory
Sentence comprehension
Pattern recognition
Audiograms
Ear conduction**
Bone Density†
Precordial Doppler*
Urine (24-hr volume, Ca, Po_4, Na, K, hydroxyproline, creatinine, urea, 17-hydroxysteroids, 17-ketosteroids, osmolarity, protein, ketone, sugar, blood, microscopic cells)
Blood* (RBC, WBC and differential, PCV, H6, MCV, MCH, MCHC, reticulocytes, platelets, Ca (ionic and total), Na, K, Cl, osmolarity, LDH, SCPT, SGOT, CPK, alkaline phosphatase, bilirubin, creatinine, glucose, BUN, protein total and fractions, albumin, haptoglobin, T3, T4, T7; lipoproteins††)
Oral physiology, parotid fluid stimulated
Microbiology (aerobacteriology, skin, oral, nasal, potable water, mechanical environment)

* Evaluated in pre-SHAD I only; **not performed in SHAD I; † not performed in SHAD III; ††performed in SHAD I only.

TABLE II

CHARACTERISTICS OF SHAD DIVERS

Characteristic	Pre-SHAD I	SHAD I	SHAD II	SHAD III
Residence depth, fswg	50	50	60	50
Duration, days	2.5	29.5	28	9
Mean O_2 level, ATA*	0.51	0.51	0.57	0.61
Mean CO_2 level, % SE*	0.111	0.092	0.099	0.172
Mean temperature, °F*	78.0	76.8	73.0	75.8
Mean relative humidity, %*	76.0	62.0	52.0	74.7
No. ascending excursions	0	4	4	0
No. descending excursions	0	14	11	6
UPTD**	101.4	1596.6	7859.0	2578.6

*Residence depth values only, except for SHAD III measurements which are weighted means based on 8-hr per excursion day at 100 fswg; **calculated (excluding decompression) using standard UPTD maximum exposure limit (1425).

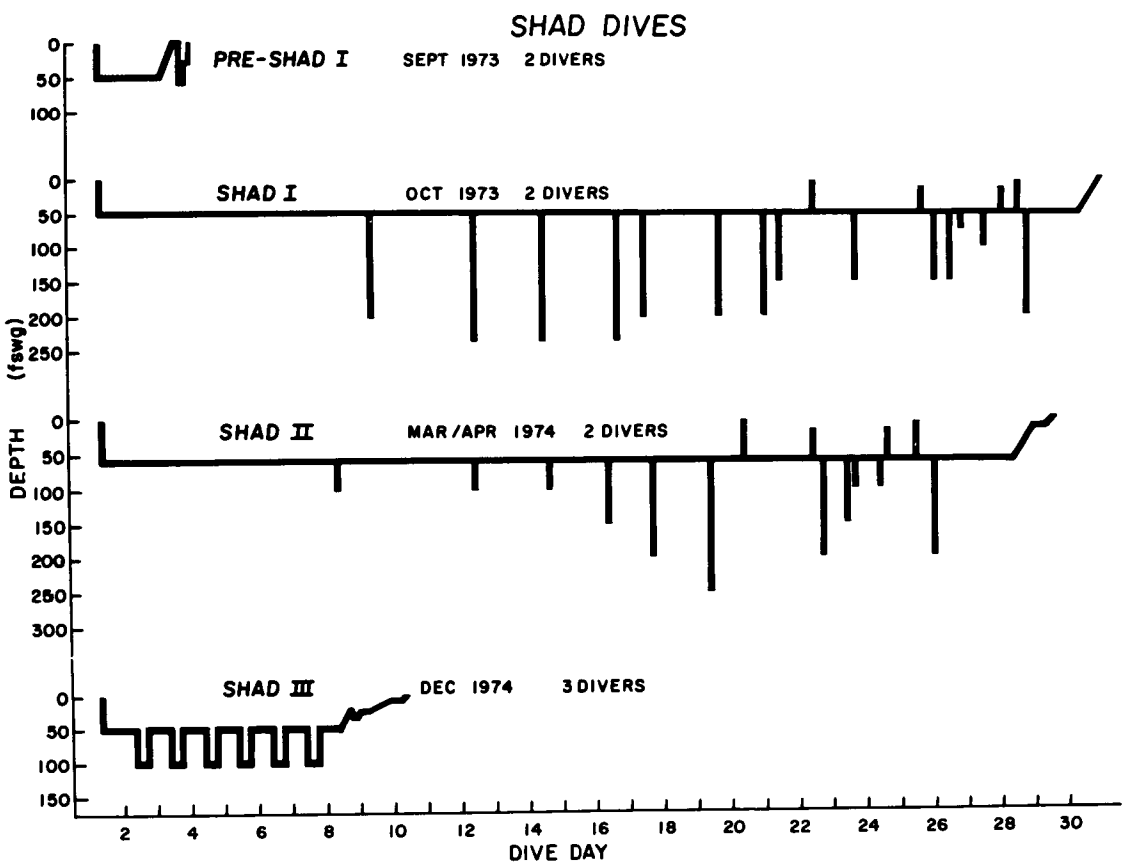

Fig. 1. Pressure profiles of 4 shallow habitat air dives (SHAD).

employed in SHAD. The excursion profiles employed were derived by Dr. R. W. Hamilton, Jr., David J. Kenyon, and Mark Freitag and were predicated, in part, on their experiences in NOAA OPS I and II programs (11).

Results

Four manned dives have been completed in the SHAD program (2). Table II delineates the general characteristics of each SHAD dive. Pre-SHAD I was primarily a habitability verification dive, but it provided significant insight into a potential limitation which will be discussed below. SHAD I, II, and III evaluated residence depths at 50 fswg, 60 fswg, and 50 fswg, respectively, and an excursion matrix between 5 fswg and 250 fswg. The mean residence oxygen partial pressures were slightly below anticipated levels, a result of conservative manual oxygen make-up within defined higher and lower limits. Temperature and humidity levels were maintained at a level comfortable for the divers in the four dives. Figure 1 depicts the pressure profiles of the respective dives.

As depicted in Fig. 1, SHAD I and II were significantly longer dives than pre-SHAD I and SHAD III, and they employed both ascending and decending excursions. Figures 2 and 3 depict the last eight excursions in SHAD I and II, respectively. Figure 3 depicts the last eight excursion dives in SHAD II. Figure 4 depicts the entire residence/excursion profile of SHAD

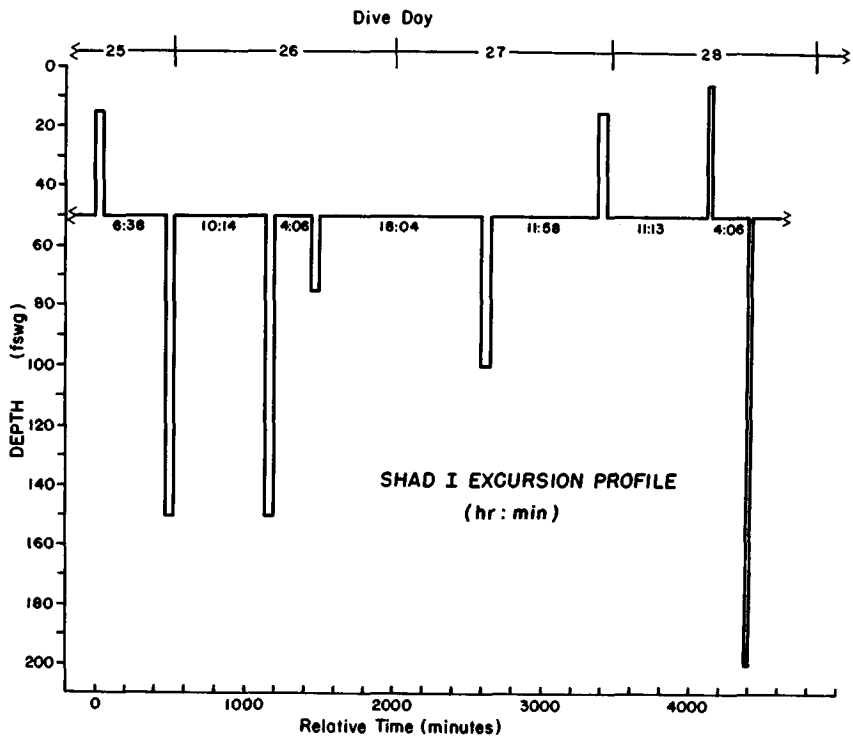

Fig. 2. SHAD I repetitive dive sequence; last 8 excursion dives are represented. Width of bars related to time at depth.

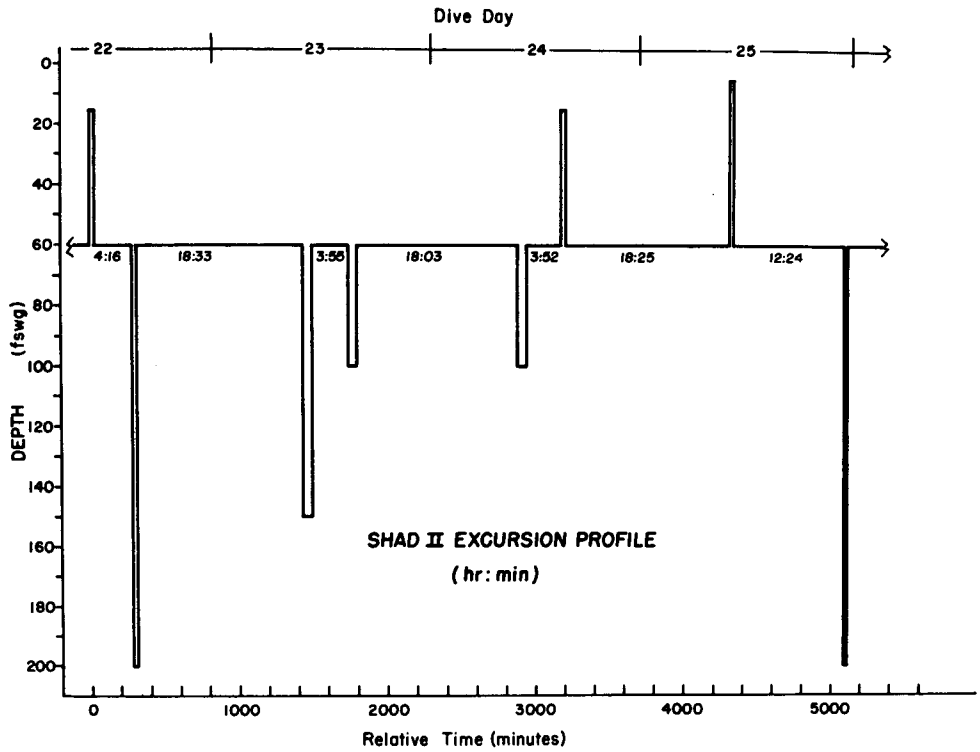

Fig. 3. SHAD II repetitive dive sequence; last 8 excursion dives are represented.

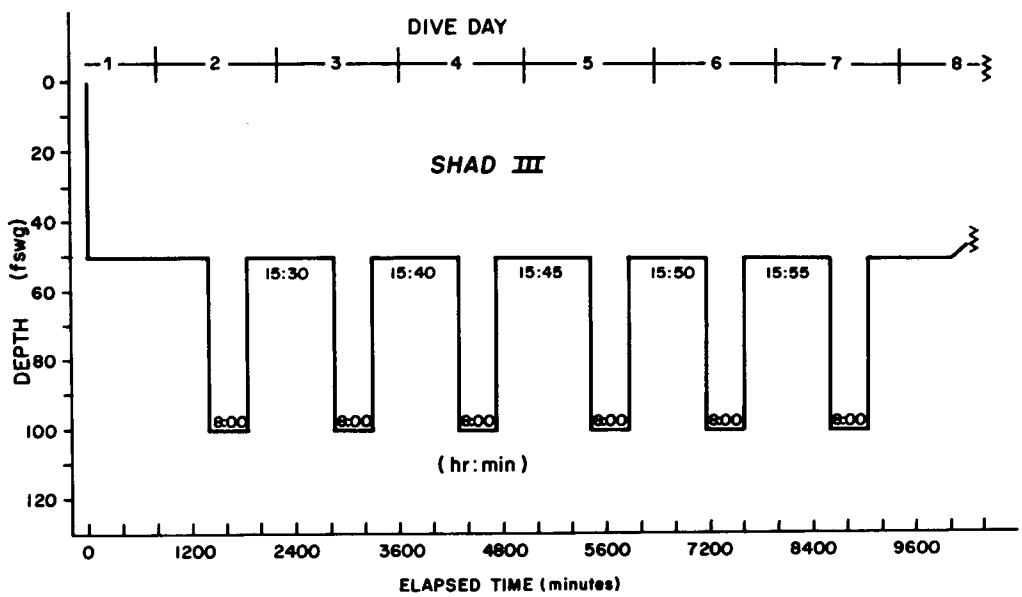

Fig. 4. SHAD III residence depth profile. Times at depth are times at excursion depth; time between excursions indicated by numbers at 50 fswg level.

III; the intervals between each 8-hour excursion to 100 fswg are also indicated. Direct ascent procedures were employed after each excursion, with the first decompression requiring 25 min and each subsequent excursion decompression time decreased by 5 min. Decompression sickness signs and symptoms, with the exception of skin itching (which is commonly observed in dry chamber dives) were not observed during or after the various excursion dives. Pre-cordial Doppler monitoring discerned circulating gas bubbles during the first 5 fswg of each ascending excursion and after the last 200 fswg of each descending excursion in SHAD I. Oxygen toxicity symptoms were not reported or observed during the residence and excursion periods in SHAD I or II. During SHAD III, one diver frequently reported anterior chest discomfort. However, the etiology of this discomfort could not be determined.

In general, the various saturation decompression procedures employed in SHAD were based on the logic developed in NOAA-OPS I and II (11). Figure 5 shows the decompression profiles used. Continuous ascent techniques were utilized; the rate of pressure change decreased at specific depths as the surface was approached. Three profiles were used to effect a 50-fswg air saturation decompression. Upon surfacing, the pre-SHAD I divers reported deep knee pains and were subsequently recompressed and treated on U.S. Navy Treatment Table 6 (22). During the SHAD I decompression, pre-cordial Doppler monitoring discerned circulating emboli continuously in one diver from 40 fswg to the surface; neither the signs nor symptoms of decompression sickness were evident in this diver. During the SHAD III decompression, one diver reported knee pain at 18 fswg after Doppler monitoring detected circulating emboli. A procedure involving 10-fswg recompression followed by four cycles of 10 minutes of breathing 10% oxygen and 5 minutes of air breathing was employed in the therapeutic treatment. Total remission of symptoms was followed by a conservative decompression to the surface. The SHAD II decompression was completed without symptoms or Doppler evidence of circulating emboli.

Biomedical Observations

During the SHAD program, few biomedical changes were observed. Nitrogen narcosis was evident during the deeper excursions (15), but did not significantly inhibit the divers' effectiveness in task completion. Electrocardiographic variations were observed and have been related to the various pressure changes that occurred in the SHAD program (23). Evidence of pulmonary toxicity was only measurable in SHAD III, and has been discussed elsewhere (9). Significant decreases in the red blood cell counts and hemoglobin were observed in SHAD I and II (17) and to a lesser extent in SHAD III. Figure 6 depicts the observed red blood cell decreases. A thrombocytopenia was not evident during the excursion dives of SHAD I and II, but was observed in SHAD III. Thrombocytosis was observed after SHAD I and II. Retinal artery and vein constriction were observed during SHAD I and II (12). Oxygen-inhibited transepithelial transport was suggested by a marked depression of normal stimulated parotid function in the SHAD III divers (14). Although these changes were noted when the dives were evaluated, they were not observed during the daily physical examinations by a diving medical officer each dive day. A significant dyspnea on exertion was noted in the SHAD I and II divers upon surfacing. This condition diminished gradually over the following three-week period.

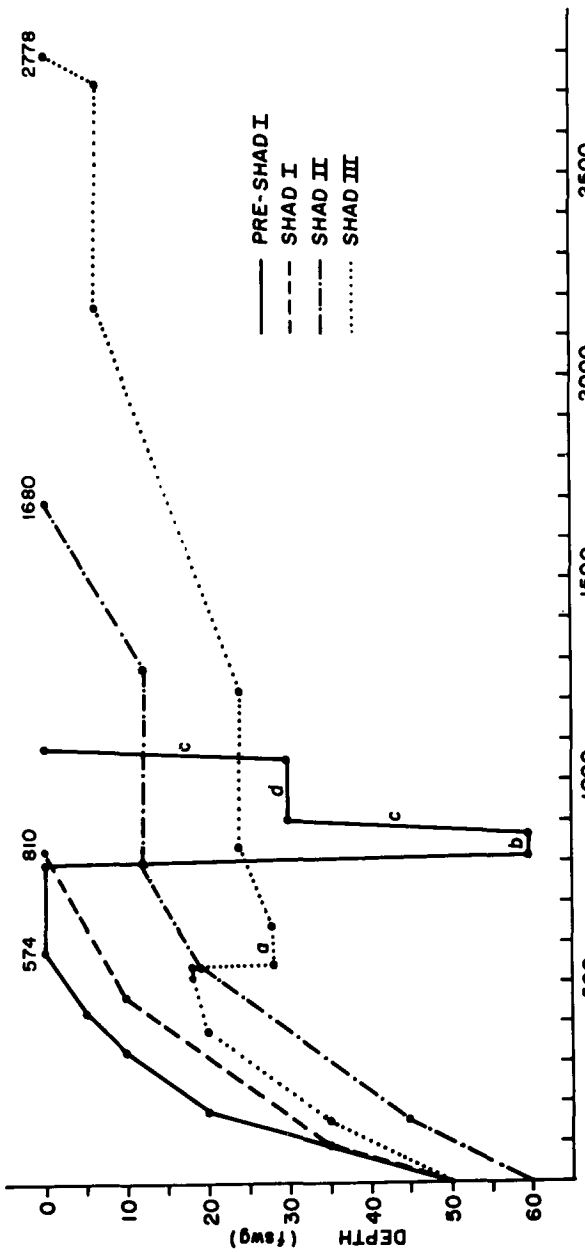

Fig. 5. SHAD dive decompression regimens. Numbers at end of each profile represent total decompression time in minutes; a = intermittent 100% O_2 for 10 min, air for 5 min; b = intermittent 100% O_2 for 20 min, air for 5 min; c = 100% O_2; d = intermittent air for 15 min, 100% O_2 for 60 min.

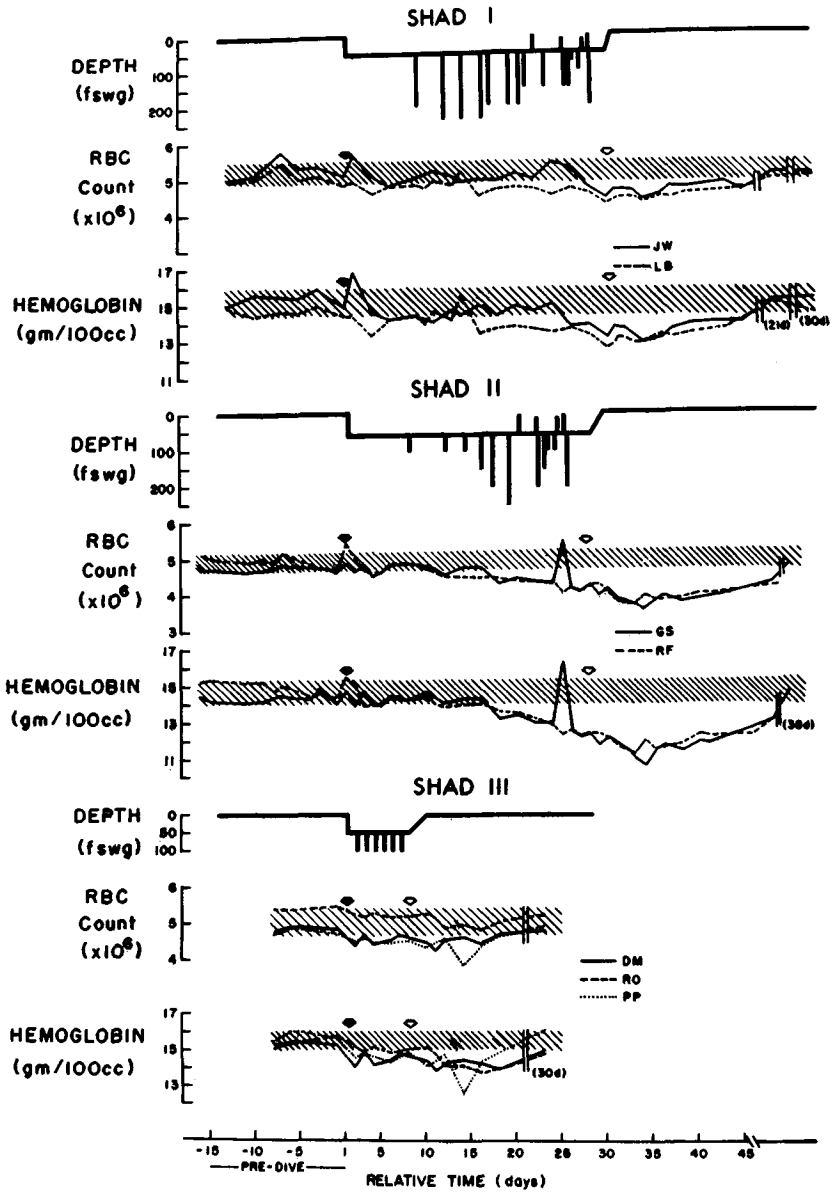

Fig. 6. Hematological variations in SHAD I, II and III. Shaded bands are predive values; compression indicated by solid arrows, decompression by open arrows. Numbers in parentheses indicate number of days postdive.

Discussion

The excursion dive matrix evaluated in the SHAD program was completed without a requirement for decompression procedures on return to residence depth. A significant increase

in bottom time, as compared to that of equivalent surface dives, was obtained in the SHAD program (Table III). The ascending excursion dives evaluated in this program demonstrated the usefulness of these procedures and the practicality of using them in concert with repetitive descending excursions.

Oxygen Toxicity

According to Wright's published procedures (24), the cumulative pulmonary toxicity dose incurred by some of the SHAD divers significantly exceeded safe limits (Table II). These techniques predicted a 6% vital capacity (VC) decrease well in advance of the observed decrease in one SHAD II diver (Fig. 7). Decrements were not seen in the other two SHAD III divers. SHAD II was completed at an average residence oxygen partial pressure of 0.57 ATA, with a decrement in VC. If 0.57 ATA of oxygen is considered a safe pulmonary exposure limit, and Wright's mathematical approach is reevaluated, the predicted time of occurrence and the observed time of occurrence of the vital capacity decrement in the SHAD III diver coincide. Thus, the limiting pulmonary oxygen exposure level in a nitrogen environment may be close to the 0.6-ATA limit suggested in the evaluations of Clark and Lambertsen (7) and Zhironkin (26).

Decompression Sickness

Three cases of decompression sickness were treated in the SHAD series. In each case, 100% oxygen was employed in the treatment regimen, and in each case evidence of oxygen toxicity was observed. The pre-SHAD I divers experienced substernal and deep inhalation discomfort during the latter stages of the treatment, and one of them experienced facial tingling near the end of the last oxygen inhalation period at 30 fswg. The diver treated in SHAD III incurred a post-treatment 24% vital capacity decrement, although he had not earlier had a reduction in vital capacity (9). These observations suggest that pulmonary accommodation to 100% oxygen may have been compromised by the divers' earlier stay at 0.51 ATA oxygen partial pressure for

TABLE III

Bottom Times for Various Total Depths

Total depth, fswg	From surface	From 50 fswg	From 60 fswg
−5	—	33.5 (4)	23 (4)
−15	—	59.0 (4)	33.5 (4)
75	40	60.0 (2)*	—
100	25	480.0 (18)	61.0 (10)*
150	5	45.0 (8)	61.5 (4)
200	0	20.5 (10)	23.5 (6)
235	0	9.5 (6)	—
250	0	—	9.1 (2)

Values are minutes; * = time limits arbitrarily set; numbers in parentheses = number of man-excursion dives evaluated.

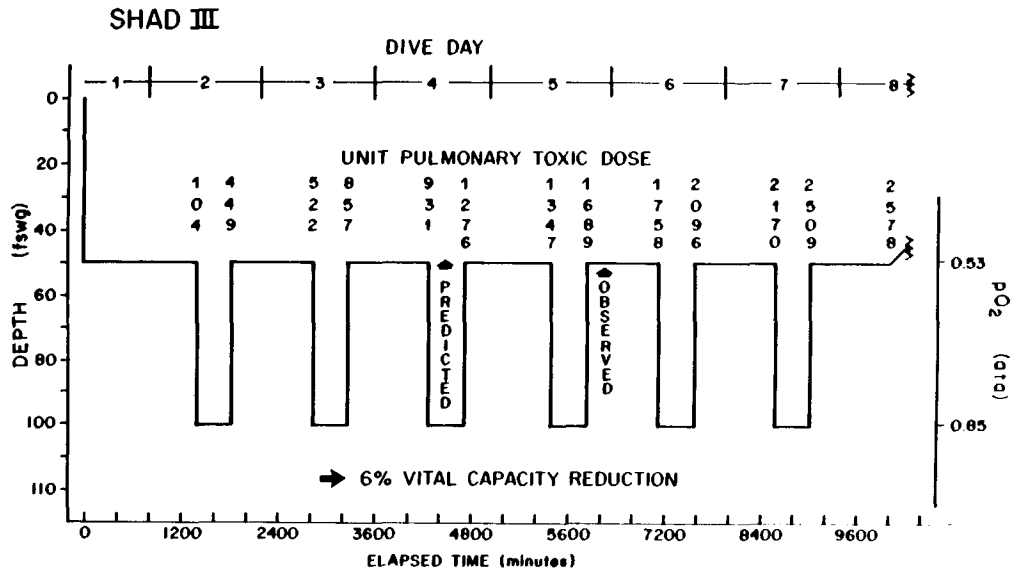

Fig. 7. Cumulative pulmonary oxygen levels in SHAD III. UPTD's shown as dive progressed; for example, 104 UPTD's before and 449 UPTD's after first excursion (derived by Wright's procedures (27)).

a period as short as two days (pre-SHAD I). When a 50% oxygen treatment gas was employed in phase I of the Scientific Cooperative Operational Research Expedition (SCORE), similar symptoms were not observed (unpublished observations).

In phase I of SCORE (a 60-fswg simulated compressed air saturation dive) one diver convulsed 54 minutes into a 300-fswg air excursion dive. Four divers had already completed a 60-minute air excursion at 300 fswg. The onset of this convulsion was consistent with time of onset of central nervous system symptoms for equivalent pure oxygen exposures (21). That the diver had spent time in a hyperoxic environment apparently did not extend the time to onset of symptoms in this case. Exposures to 300 fswg were not included in phase II of SCORE (10), an open-water continuation of the laboratory experiments.

The significant hematological alterations observed during and after SHAD I and II (Fig. 6) may either reflect normal adaptive processes to a hyperoxic environment or deleterious oxygen manifestations (17, 18). A hyperoxic dive appears to produce symptoms inversely related to those of altitude exposure and adaptation. For example, Pace and his group (19) and Pugh (20) have noted variations similar to those observed during SHAD I and II. Less extensive alterations were observed during and after SHAD III (Fig. 6). Both pre-SHAD I and SHAD III were concluded before that point in the dive where red cell count decreases occurred in SHAD I and II. The significant red cell count decreases in SHAD I and II may be related to the exertion-instigated dyspnea observed in each diver on surfacing; this condition did not occur in the SHAD III divers.

ACKNOWLEDGMENTS

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FURTHER STUDIES IN DECOMPRESSION FROM STEADY-STATE EXPOSURE TO 250 METERS

J. Vorosmarti, Jr., R. de G. Hanson and E. E. P. Barnard

Barnard (1) has previously reported a series of dives designed to investigate decompression after steady-state hyperbaric oxygen-helium exposures. To achieve this end, he determined a satisfactory decompression curve to depths of about 100 meters of seawater (msw) using a stepwise progression of 24-hour stops; his curve included large drops to a depth which had been previously determined not to produce decompression sickness. This curve was used to extrapolate, by empirical methods, decompression schedules for deeper depths. This presentation reports the additional dives in this series which were used to establish a safe decompression curve for steady-state exposure to a depth of 250 msw.

Methods

The divers in this series were all from the Royal Navy Saturation Diving Team, Admiralty Experimental Diving Unit. All exposures took place using groups of three divers in the chamber complex of the Deep Trials Unit, Alverstoke, England.

The nitrogen content of the chamber atmosphere was held below 2% of one atmosphere by: (1) emptying the main complex of air before the dive and filling it with the correct oxygen-helium mixture; and (2) having the divers enter the chamber via a lock which was cleared of air by layering in helium while the divers breathed oxygen by mask, followed by addition of the correct amount of oxygen. Since different oxygen partial pressures were used, these will be presented with the specific dives discussed. The carbon dioxide content of the atmosphere was kept below 0.005 bar by using internal scrubbers filled with soda-lime absorbent. A Servomex paramagnetic oxygen analyzer and a Hilger infrared carbon dioxide analyzer were used to monitor these gas levels constantly. The chamber temperature was maintained between 27 and 31 °C, depending on the depth of the dive, and relative humidity was maintained between 60 and 80%. Compression and decompression were both accomplished at the rate of 1 meter/min in all the dives.

The onset of decompression sickness, if it occurred, was always considered the end point of any particular experiment, and its occurrence invalidated the specific decompression schedule being tested, even though, in most cases, this meant a schedule was tested only once. Any persistent pain or central nervous system symptom was regarded as decompression sickness and treated accordingly. In addition, there are several other symptoms which require defini-

tion. "Niggles" refer to mild pain which lasts only for a few minutes and rapidly disappears without residual effects, and which requires no treatment. Another common complaint was mild muscular aching, which was described as similar to the muscular ache one gets after hard exercise if one is not in physical condition. This also generally lasts only for very short periods of time, but it may also disappear completely, only to return within minutes and disappear again. This symptom was not treated in this series. Another symptom which the divers experienced was what they described as "the feeling of something moving through the joints" or "a bubble moving." This symptom lasts only for several seconds and is not painful.

Results

The first dive in this series was to 180 m for 24 hr (dive No. 40). Decompression was calculated using the formula:

$$P_t = (P_o - At) + Bt^C$$

where P_t = depth after time t; P_o = original depth in meters; t = time in hours.

A, *B*, and *c* were constants based on the empirical decompression curve established by Barnard. This schedule is presented in Table I. A possible case of cerebral decompression sickness occurred on decompression to 60 m. The same exposure depth and time were used for dive No. 42. The decompression was similar to that of dive No. 40 in total time and in decompression rates at various depths; however, between 180 and 115 m, decompression was in stages of 1 m rather than 5, since Barnard had found previously that decreasing the distance between stops might prevent decompression sickness. This schedule produced niggles in two divers on the drop to 10 m, and again on the drop to 5 m, and also produced definite decompression sickness in one diver on decompression to the surface. This result led to the use of the same schedule again, and to making all the drops throughout decompression in 1-m increments. Decompression sickness occurred in two divers at 65 m, in one diver at 35 m, and produced niggles in another diver; decompression sickness also occurred at 25 and 2.5 m. The next dive (No. 44) was to a depth of 210 m for 24 hr, with decompression using 5-m drops throughout and lengthening all the stops deeper than 110 m to 4 hr instead of 3 hr (Table I), since it was thought that the deeper decompression, while not fast enough to produce overt decompression sickness, might be causing bubbles which then caused symptoms at a later stage. Decompression sickness occurred in one diver at 25 m. Also on this dive, one diver complained of feeling depressed and irritable during the deeper portions of the dive; these reactions seemed to be relieved when make-up oxygen was jetted into the chamber. The decompression schedule for dive No. 45 (Table I) was modified to slow the midportion of the decompression by extending the 5-hr stops to 130 m instead of 110 m. One diver experienced decompression sickness in both knees and ankles at 15 m with a recurrence at 1.3 m. The lengthening of the decompression in these two dives appeared to have prevented decompression sickness deeper than 25 m but not shallower. It was decided again to try to shorten the drops while keeping the time constant. On dive No. 46, therefore, the drops from 30 m to the surface were in 2.5-m increments for half the time called for with the 5-m drops. Two divers experienced niggles at 12.5 m; one of these divers developed overt decompression sickness which required therapy. It was decided at this point that the relatively small changes that

TABLE I
VARIOUS DECOMPRESSION SCHEDULES USED

Dive No. 40		Dive No. 44	
180-155 m	3 hr/5 m	210-115 m	4 hr/5 m
150-110 m	4 hr/5 m	110-55 m	5 hr/5 m
105-50 m	5 hr/5 m	50-30 m	6 hr/5 m
43-30 m	6 hr/5 m	25-0 m	7 hr/5 m
25-0 m	7 hr/5 m		
Total decompression time: 7 days 2 hr		Total decompression time, 8 days, 9 hr	
Dive No. 45		**Dive No. 47**	
210-135 m	4 hr/5 m	210-135 m	5 hr/5 m
130-55 m	5 hr/5 m	130-55 m	6 hr/5 m
50-30 m	6 hr/5 m	50-30 m	7 hr/5 m
25-0 m	7 hr/5 m	25-0 m	8 hr/5 m
Total decompression time, 8 days, 13 hr		Total decompression time, 10 days, 6 hr	
Dive No. 48		**Dive No. 53**	
210-180 m	4 hr/5 m	210-175 m	3 hr/5 m
175-125 m	5 hr/5 m	170-110 m	4 hr/5 m
120-85 m	6 hr/5 m	105-50 m	5 hr/5 m
80-50 m	7 hr/5 m	45-30 m	6 hr/5 m
45-0 m	8 hr/5 m	25-0 m	7 hr/5 m
Total decompression time, 10 days, 8 hr		Total decompression time, 8 days	

Dive No. 54	
250-175 m	3 hr/5 m
170-110 m	4 hr/5 m
105-50 m	5 hr/5 m
45-30 m	6 hr/5 m
25-0 m	7 hr/5 m
Total decompression time, 9 days	

had been made to the schedule were not enough to prevent decompression sickness. The schedule for dive No. 47 (Table I) was lengthened by one hour at each stop, making the total time of decompression 38 hr longer than dive No. 46. This was also a 210-m, 24-hr bottom time exposure. One case of decompression sickness occurred at 10 m. A review of the previous dives revealed that the decompression from 45 m to the surface was shorter than that determined on the dives using the 24-hr stop, long-drop schedules (1). For dive No. 48, the decompression was shortened for the deeper stops and lengthened for the shallower stops (Table I) to correct for this suspected discrepancy; the overall schedule was kept at approximately the same length as dive No. 47. One diver experienced decompression sickness at 10 m and another developed decompression sickness 12 hr after surfacing. Dive No. 49 was a repeat of dive No. 40. All three divers suffered decompression sickness on this occasion, one at 15 m and the other two at 5 m.

It was obvious at this point that no progress was being made in determining a safe decompression using 0.22 bar oxygen from 180 m. It was decided that since the decompression time of dive No. 40 was very similar to that of the U.S. Navy schedule from the same depth using 0.3 bar oxygen, an attempt to make dive No. 50 using the U.S. Navy schedule would be made. This produced no cases of decompression sickness, but one diver experienced the muscular aches described previously. Another diver surfaced with marked crepitus around both knees and thighs. X-ray examination revealed free gas in both knee joints and in the fascial planes of the thighs (4). Dive No. 51 was a repeat of dives No. 40 and 49, using 0.3 bar oxygen. From 20 m to the surface, all divers complained of "stiffness" in the knees on each drop, but no niggles or decompression sickness occurred. This same schedule, with 0.4 bar of oxygen, was used on dive No. 52. None of the divers reported any symptoms. Dive No. 53 was a 24-hr exposure to 210 m using 0.4 bar oxygen. Decompression followed the schedule shown in Table I and was similar to that used in the previous dive. The only modification was to use the 5-m/3-hr rate up to 175 m instead of 155 m, and to decrease the rate to 5-m/hr between 175 and 155 m. Again, none of the divers had any problems. Extrapolation of the 5-m/3-hr rate provided the decompression for the next dive to 250 m for 24 hr. The only complaint on this dive was from a diver who had injured one knee during exercise and had a niggle in this knee on surfacing. Two more exposures at 250 m, one for three days and one for seven days, were conducted using this decompression schedule. After the 7-day exposure, all three divers stated that they had the feeling of "something moving in the knees" on the drop from 10 to 5 m.

The series of 1-, 3-, and 7-day exposures at 250 m was then repeated with this decompression schedule with a P_{O_2} of 0.22 bar during bottom time and 0.4 bar during decompression. Results of these exposures and of six other 7-day exposures at 250 m are shown in Table II. The only problems on these 36 man-exposures at 250 m were minor: except for two divers who said they had a niggle on surfacing, the other problems were very mild and consisted of the aches or stiffness discussed earlier. In addition, 33 man-exposures to 100 m have been performed using this schedule, beginning with the 95-m stop. Of this group, one diver had a niggle on the the 10 to 5 m drop, and four divers reported fleeting stiffness or "something moving in the knees."

The divers who had been exposed to oxygen partial pressures of both 0.22 bar and 0.40 bar stated that on the dives using the higher partial pressure they felt generally much better, both physically and psychologically. One of the authors (Hanson) participated in the last dive of the series in which the P_{O_2} was 0.22 bar on the first and last days of the bottom time (7 days) and 0.4 bar for the rest of the dive. He reported that when the P_{O_2} was at the lower level he was more irritable and had problems doing the required tasks because his concentration seemed to be decreased. At the higher P_{O_2}, he stated that he felt perfectly normal.

The compression rate of 1 m/min produced minor hyperbaric arthralgia on the 250-m exposures in almost all the divers; symptoms generally disappeared within 24 hr on the bottom. On reaching 250 m, most of the divers also complained of mild dizziness which was annoying but did not interfere with their work. None reported that this problem lasted more than 30 min. Fine hand tremor was experienced by all the divers after compression to 250 m; the tremor was severe enough to postpone drawing venous blood samples until at least 30 min after this depth was reached. The tremor was much reduced by this time but was not completely gone in any of the divers until about 12 h after reaching the bottom.

TABLE II

SUMMARY OF ALL 250-METER EXPOSURES

Dive No.	Bottom Time, Days	P_{O_2}, bar	Symptoms
54	1	0.4	1 diver: niggle on surfacing (knee injury on bottom)
55	3	0.4	None
56	7	0.4	3 divers: "something moving in knee" on surfacing
57	1	0.22 on bottom 0.4 decompression	None
58	3	0.22 on bottom 0.4 decompression	2 divers: mild aches in knees postdive
59	7	0.22 on bottom 0.4 decompression	1 diver: niggle on surfacing
60	7	0.4	None
61	7	0.4	None
64	7	0.4	None
65	7	0.22 on bottom 0.4 decompression	1 diver: transient aching in anterior thighs during drops to 5 m and surface
66	7	0.4	None
67	7	0.4	2 divers: transient knee and thigh stiffness on all drops between 15 m and surface

During three of the later dives (Nos. 60, 66, and 67) excursion dives were done to a depth of 300 m. On all occasions these were done in the wet section of the complex with two of the divers in the water swimming or exercising and the third diver serving as tender; the tender was also exposed to the pressure change. On dive No. 60, three 1-hr excursions per day, separated by 2-h intervals at 250 m, were planned. Compression and decompression were conducted at the rate of 20 m/min. On the last excursion of the second day (excursion 6) one of the divers experienced nausea and vertigo within minutes of reaching saturation depth. These symptoms were relieved by immediate recompression to 300 m, and further excursions on that dive were cancelled. The excursions done on dives No. 66 and 67 were carried out in the same fashion, but only a single 1-hr excursion was done per day, for a total of 21 man-excursions (14 wet, 7 dry). No difficulties were associated with these excursions.

Discussion

This series of dives has accomplished the development of a decompression schedule which can be used for any steady-state exposure to a depth of 250 m. In addition, it has demonstrated for the first time what appears to be a Chouteau effect in man, and has raised again the question of the combined effects of high pressure and oxygen.

First, the safety of the decompression schedule will be discussed. In spite of the small number of dives, it is possible, using standard statistical methods, to hazard a guess about the overall incidence of decompression sickness. Based on the 36 man-exposures to 250 m,

the probabilities of having less than 10, 5, and 2% decompression sickness using this schedule are 0.98, 0.84, and 0.52, respectively. If the 33 successful man-exposures to 100 m using this schedule are added to the total, these probabilities become > 0.99, 0.77, and 0.76, respectively. It remains, of course, for many more decompressions to be done to determine the actual incidence.

In addition to avoiding decompression sickness, a successful decompression schedule should be as short as possible. There was not time to attempt shortening the schedule to determine if this is possible in this series. Because 12 of the 36 divers exposed at 250 m had premonitory signs of decompression inadequacy (niggles, "moving bubbles," muscle aching (Table II)), it is doubtful that the schedule can be significantly shortened. On the other hand, comparing the time of this decompression schedule to that used by the U.S. Navy with 0.3 bar oxygen, this schedule is only 8 hr shorter for a 250-m dive. Since the incidence of decompression sickness with the U.S. Navy schedule is approximately 10%, perhaps a significant decrease in time would be possible.

Chouteau (2) has demonstrated the listlessness and inactivity occurring in animals in normoxic hyperbaric helium environments, which is obviated by increasing the oxygen partial pressure. To the authors' knowledge, this has not previously been reported in man. The subjective evidence expressed by the divers in this series indicates that similar symptoms do occur in men. Though there were no objective measurements of this phenomenon, a difference in well-being was obvious between dives using 0.22 versus 0.4 bars of oxygen. Most divers also stated that fine tremor of the hands decreased when the higher oxygen partial pressure was breathed; again, there was no objective evidence of this.

This is not the only advantage of using an increased oxygen partial pressure. Although the P_{O_2} used during the time on the bottom is important from the point of the Chouteau effect, it does not apparently have any effect on the outcome of the decompression. Dives No. 57, 58, 59, and 65 all used a P_{O_2} of 0.22 bar during the bottom time (Table II) with no obvious difference in decompression. During decompression, however, the P_{O_2} is much more crucial. It is obvious from this dive series that successful decompressions from depths deeper than approximately 150 m were not possible unless the P_{O_2} was increased above normoxic levels. It is generally assumed that decompression can be shortened by increasing the P_{O_2} because this decreases the partial pressure of the inert gas used. This does not appear to be the case in deep saturation diving, however. Increasing the P_{O_2} from 0.22 bar to 0.4 bar makes little difference in the total number of inert gas molecules until the depth becomes very shallow (30 m or less). The effect of the small increase in P_{O_2} makes a greater difference in the decompression than straightforward replacement of inert gas would suggest.

There have been various hypotheses put forward to explain the need for a higher P_{O_2} in the atmosphere. The first, proposed by Chouteau, is that of an alveolar-capillary block; another hypothesis is diffusion dead space in the alveoli (6), and a third is that of stratified inhomogeneity (7). To this list must be added the effect of pressure and/or inert gas on hemoglobin-oxygen dissociation (5). This problem requires a great deal of research, because if the P_{O_2} must be increased as depth increases, a barrier to the depth attainable by man in the ocean may be determined by the maximum P_{O_2} he can breathe for long periods without suffering oxygen toxicity.

Finally, the excursion dives will be discussed. A plot of steady-state exposure depth versus the depth to which one can directly ascend, taken from Barnard (1) and Flynn and Spaur (3),

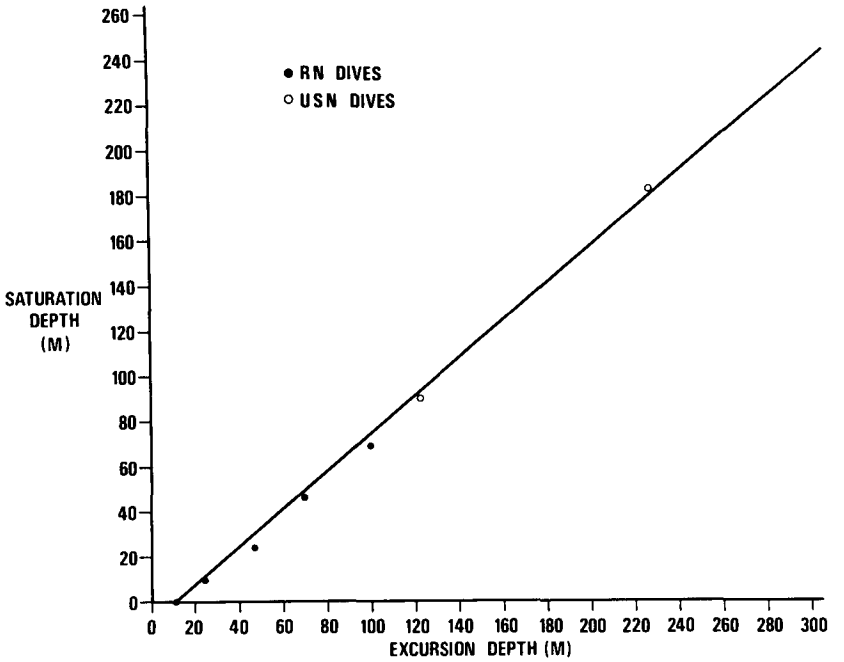

Fig. 1. Relationship of saturation depth and depth to which 24-hr excursions can be made followed by safe no-stop decompression to the saturation depth (1, 3).

produced a straight line which, if extrapolated, indicates that after a steady-state exposure at 300 m one should be able to ascend safely to 240 m (Fig. 1). In the series reported here, this may not have been true in the attempt to do repetitive excursions. There are two possibilities to consider: (1) the relationship of pressure to allowable pressure change may not be linear beyond a certain depth; and (2) if bubbles are formed on any excursion decompression and persist through a surface interval, they will increase in size on the following decompression and cause symptoms.

The entire area of decompression requires a great deal more basic research before the point will be reached when decompression schedules can be scientifically rather than empirically derived.

Acknowledgments

Grateful acknowledgment is made to the Royal Navy Saturation Diving Team, Lt. Cdr. W. Filer, RN, and his staff at the Deep Trials Unit, and R. H. V. Hempleman, Mr. J. Williams, and Petty Officer R. Gray of the RNPL for the long hours and hard work required to make this dive series successful. Any views expressed in this paper are those of the authors and do not necessarily represent those of her Majesty's Government.

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PART VIII. PROGRESS IN SATURATION AND EXCURSION DIVING

DISCUSSION

G. F. Bond, Chairman

Dr. Miller: This is the type of air shelter which was used in this program to provide emergency air on the bottom. This artist's conception (slide shown) gives the general picture of the open-sea portion of SCORE. The submersible picked the divers up, took them out, and anchored at this depth. The divers went out and completed their excursion, locked back in the submersible, and then returned to the habitat for a 45-minute decompression period, which took place in the submersible. The other types of excursions were swimming excursions involving a 700-foot swim out to the depth of 200 feet, for a period of one hour. The aquanauts then swam back to the habitat, stopping on the way at 90, 80, and 70 feet for short decompression stops, totaling 19 minutes. We had one hour of working time at 200 feet from a depth of 60 feet on air, with a 19-minute decompression time prior to returning to 60 feet. This figure shows the decompression stops used (slide shown).

Dr. Youngblood: I would like to add a point in case George Adams' presentation regarding oxygen convulsions at 300 feet at Duke is misunderstood or frightens anyone away from further research in this area. I think there may be a clinical artifact in that convulsion which was not known at the time and is probably not generally known now. The diver involved had, unknown to us, been on disulfiram (Antabuse) for a considerable period prior to the dive and had stopped taking it four days previously. There is a pharmacological hypothesis that disulfiram provides whatever protection it does against oxygen toxicity in mice, and should do the same in humans. However, the fact that the diver stopped it could have made him more susceptible to toxicity. One other point is that we have done eight man-dives using 0.6 ATA oxygen, with the shortest decompression time around 137 hours from 1,000 feet, the most shallow, and 1,525 feet the deepest, with no clinical signs of pulmonary oxygen toxicity. Carbon monoxide diffusion studies were made with the divers from 1,000 feet with no evidence of any pulmonary damage using this oxygen partial pressure for 4-5 days.

Mr. Johnson: If a diver becomes both chilled and fatigued during his work, during a bell excursion decompression or an open ocean decompression, this may have some effect in that the body normally produces a protective mechanism of vasoconstriction. Could the panel comment on whether or not this was taken into consideration in the computation of the excursion decompression tables, and if so, what was the method of doing so?

Unidentified: I don't think it is taken into consideration; we were strictly using dry chamber conditions.

Dr. Miller: With regard to the open sea work, I can't comment on the computation of the tables, but the deep dives that were done from the Puerto Rican habitat were hard working dives. We made these excursions over a rather large rise, pulling behind us a 700-foot hookah hose. Bill Hamilton or Heinz Schreiner could perhaps comment about the computation of those tables. They were tested under what I would consider hard working conditions, and for fairly long excursions, up to four hours.

Dr. Hamilton: Jim, what you did was part of the test. We didn't do anything different in computing the tables used at sea and the ones used in our laboratory or at Duke.

Dr. Miller: That is what I suspected.

Dr. Vorosmarti: All of our excursions were done wet with the divers actually in the water swimming and working hard. During the hour the excursion took, they spent at least 50 minutes working hard. However, the water was not cold; it was somewhere around 24°C.

Dr. Spencer: I don't know whether it is relevant to that question, but we compared direct decompression dives that we performed in the laboratory with open sea dives. The two profiles that were compared were for 165 feet for

10 minutes, and we used the same divers in the chamber and in the open ocean. The open ocean was the North Pacific, which is cold, and they were very hard dives on an open sea mountain 350 feet from the shore. We found an increased incidence of bubbles, precordial bubbles in the open sea. We found increased incidence of bends and we found that the people that tended to be prone to bubbles in the laboratory went on to bends in the open sea dives. In other words, there was just a general tendency, remembering that there were only six divers, for each of them to move towards more bubbles and a higher incidence of bends in the working dives.

Mr. Adams: If I could make a comment with respect to the Chouteau effect. We completed a 200-foot saturation exposure on nitrogen and oxygen not too long ago in which we initially compressed the divers on 0.24 atmospheres of oxygen and the balance nitrogen. We believed we very well documented the Chouteau effect. After about 12 hours of very listless behavior in two of the three divers, we raised the oxygen to around 0.33 ATA in preparation to abort the dive, only to have all three of the divers begin to feel very good and recover essentially thoroughly. We then continued the dive for an additional seven days.

Dr. Vorosmarti: I would like to make a comment about that because I think that it is very important to establish some limits for oxygen right now, because if the oxygen content of the breathing gas has to be raised for deeper dives this may very well limit the depth to which divers can go, in addition to the obvious problems of the High Pressure Nervous Syndrome and the respiratory problems.

Dr. Bennett: Just one word of caution: we seem to be using the Chouteau effect for an effect I don't believe Chouteau described. You are using it in the decompression sense, when he was only interested in the depth sense.

Dr. Vorosmarti: No, I am using it for botton time; I'm not talking about decompression.

Mr. Le Pechon: On the Chouteau effect. I was an assistant of Professor Chouteau in Marseille for four years, and he never discovered this Chouteau effect on men because he never tried it on men. But he has in his records many letters from people who wrote to him that they had observed it in men. He probably would be very glad to have an official confirmation on that. Dr. Vorosmarti, what is your interest in making rapid decompressions and long stops for saturation dives, which probably produce longer times than if you do continuous decompression, which probably produces less physical stress?

Dr. Vorosmarti: The whole program was started originally to develop a schedule that could be entered at any depth; to do this, Peter Barnard would not have let himself be biased by any mathematical theory or anything else. He decided to go back to basics, starting at 10 meters for 24 hours and using a Po_2 of 0.22 bar, establishing a curve which could then be extrapolated for deeper depths. I am not sure that using a linear rate can shorten decompression, because we attempted that on three different occasions and it didn't work; we ended up going back to the 5-meter increments.

Mr. Le Pechon: I do not want to speak of linear decompression but of continuous decompression.

Dr. Vorosmarti: Yes, continuous.

Mr. Le Pechon: For a given total decompression time, linear decompression is of course too long at the beginning and too short at the end.

Dr. Vorosmarti: Yes.

Mr. Le Pechon: But with continuous decompression, you can always, from any depth, get back to a standard decompression. Just the very first hours are changed. If you work with supersaturation gradients to install the necessary supersaturation gradient, you can then follow the same table and then the same decompression schedule from any depth. We have, for example, just decompressed two people from 1,000 feet in 7 days and 2 hours with a continuous decompression with 0.45 atmospheres of oxygen. I think this is less stressful than rapid drops and long stops.

Dr. Akers: A comment and a caution on the Chouteau effect. I have been diving animals and chick embryos in all sorts of gadgets this way for many years. We observed the Chouteau effect in chick embryo, which is a beautiful tissue to study oxygen levels in, and we backed up Hyatt and Weiss and their studies. As you increase the pressure you have to increase the oxygen to avoid anoxic effects on developing tissue. This is a beautiful example of the Chouteau effect. However, once you pass a half bar or get into the 400 millimeter level, you have to be cautious with long-term exposure. If you run the study for 12 days, you begin to get very subtle changes in the lung structure in guinea pigs, for example, which have lung nets as close to those of humans as you can find in an animal model. I would suggest that if you are going to employ added oxygen in your dives for long-term saturation, this be kept in mind so as not to run the oxygen up and give the divers an atelectasis and hyaline membrane disease from the oxygen.

Dr. Bühlmann: We use stops lasting 120 minutes for decompression after saturation dives. According to our method, total decompression time after saturation at 250 meters is 3 days and 5 hours.

Unidentified: What Po_2?

Dr. Bühlmann: Po_2 around 0.5, 0.6, 0.4.

Dr. Vorosmarti: Well, it may work. We tried it on three dives and it didn't. The problem is we have done 47 dives in three years, each of which takes about 16 days, and we just haven't had time to try to find ways of shortening the schedule. We were attempting to find a basic schedule which worked.

Dr. Bennett: Jim, you have been consistently saying that it doesn't work. Dr. Youngblood explained very definitely that our work with 0.6 ATA oxygen shows that it is safe and very effective in shortening decompression without bends.

Dr. Vorosmarti: I said that it didn't work on three of the dives that we tried it on. I believe the rest of you, I have no argument about that. It may work wonderfully for the rest of the world, but we just had bad luck and haven't had a chance to explore it further.

Dr. Bennett: Maybe you should try it at 0.6 atmospheres of oxygen.

Part IX. DETECTION, GROWTH, AND RESOLUTION OF BUBBLES

ROLE OF BUBBLE GROWTH KINETICS IN DECOMPRESSION

E. N. Lightfoot, A. Baz, E. H. Lanphier, E. P. Kindwall and A. Seireg

The purpose of this paper is to suggest a fundamental reexamination of gas elimination theories to use as a basis for more effective and economical decompression procedures.

The shortcomings of Haldanian theory (5) are recognized by diving physiologists; the effects of such shortcomings have been mitigated by empirical modification. The result has been an informal evolutionary optimization, which has been very effective in the long run in finding locally optimum conditions. However, after so many decades of trial-and-error experimentation, it does not seem reasonable to expect major advances through further refinements of an old model.

Instead, it seems preferable to seek an entirely new approach for devising improved decompression schedules. Since it is much too dangerous to try radical changes blindly, it is important to note whether there are any indications of promising alternative procedures in the available data.

The key to at least one promising alternative to the Haldanian approach is presented by the realization that gas bubbles are present even during normal decompressions. Further, it is believed that intravascular bubbles can aid gas elimination substantially, and may therefore prove beneficial under favorable conditions. If this hypothesis is correct, faster and safer decompression schedules should be possible.

Speculations on Gas Transport and its Relation to Decompression Sickness

All the physical bases of the Haldanian theory are questioned in this paper: the need to avoid all gas nucleation, the existence of a critical pressure ratio for nucleation, and the assumption of homogeneous perfusion-limited transport. Attention should be directed to discrepancies between predictions of the present model and those of three important bodies of information: accumulated diving experience, the physical chemistry of gas-liquid systems, and mammalian physiology.

Kindwall's recent confirmation (9) of the anomalous effect of ambient pressure on nitrogen elimination, first noted by Willmon and Behnke in 1941 (18), focused the authors' attention on this problem. Kindwall's procedures are diagrammed in Fig. 1. Normal subjects were compressed in air to an equivalent of 100 fsw for 40 minutes. After these exposures to compressed air, they were switched to 80% He/20% O_2 and maintained at equivalent depths of either 10, 50, or 100 fsw for an additional 90 minutes. Nitrogen elimination was measured during these 90-min periods, and the averages of individual results are plotted in Fig. 2.

This figure shows the times required to eliminate given quantities of nitrogen as a function of equivalent decompression depth; these were always lowest for the 50-fsw decompression. More specifically, times were 1.2 to 1.3 times larger at 10 fsw, and 1.5 to 1.9 times larger at 100 fsw. Furthermore, these ratios increased with time or volume of nitrogen removed.

These differences were unexpected, since the nitrogen partial pressure driving forces for elimination are initially the same in all three situations. Furthermore, as discussed by Kindwall et al. (10), the results do not appear to be artifacts of the experimental procedures. An explanation must therefore be sought outside the present theory, either on the basis of a changed

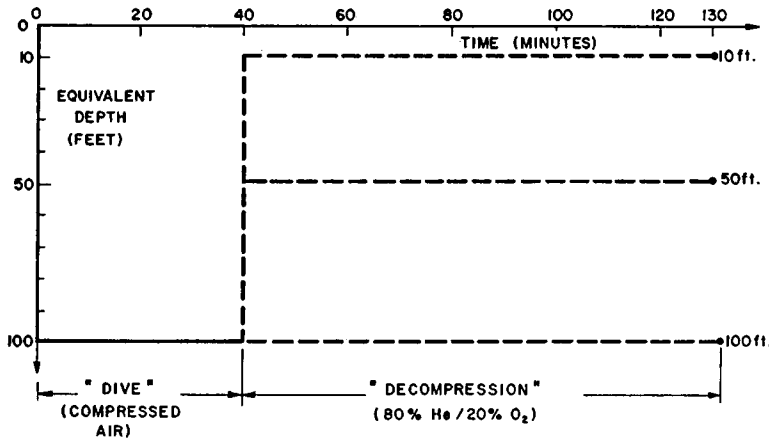

Fig. 1. Diagram of Kindwall et al. experiments (10).

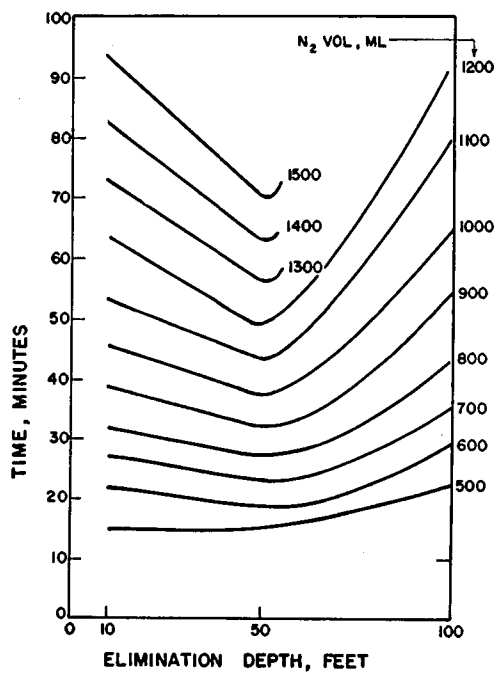

Fig. 2. Effect of depth on gas elimination time.

physiological state of the diver or the changed physical state of the nitrogen. Pending confirmatory tests, the first possibility cannot be ruled out completely, but the second seems much more likely.

The 100-fsw elimination rates represent the transport of dissolved nitrogen according to Haldanian theory. At 50 fsw, gas bubbles form in the systemic capillaries, thereby increasing both transport capacity and the driving force for diffusion from tissue to blood. This process is indicated diagrammatically in Fig. 3. Since the molar concentration of nitrogen in bubbles is roughly 50 times that in adjacent blood, only 2% of bubbles by volume are sufficient to double nitrogen transport capacity.

At first sight, convective transport should be even more effective at 10 fsw than at 50, but this is clearly not the case. Two possible explanations are suggested and illustrated in Fig. 4. One possibility is the formation of extravascular bubbles which, because of their very high nitrogen content, lower tissue nitrogen pressures markedly. This reduction would in turn lower the amount of nitrogen available to the perfusing blood in direct proportion to the lowering of tissue nitrogen tension. The other possibility is that gas bubbles grow so quickly at 10 fsw that they occlude systemic capillaries and decrease perfusion rate as a result. Occlusion of blood vessels, in this case veins, by gas bubbles was observed over 100 years ago by Hoppe-Seyler (8).

There are numerous indications that bubbles exist during decompressions conducted according to presently accepted schedules. They have been found in many investigations that used various ultrasonic techniques. Among these are the studies of Smith and Jacobson (15), and Rubissow and MacKay (14), who reviewed much of the earlier literature. There are also numerous reports of hematological changes which are apparently related to bubble formation during asymptomatic decompressions (1, 12, 13). Stegall and Smith (16) also found bubbles during asymptomatic decompression and tied bubble-detection findings and system pathophysiology together. D'Aoust, Newman, and White (6) have perhaps come closest to verifying the contention that extravascular bubbles can reduce gas elimination. They reported a negative correlation between nitrogen elimination and the existence of bubbles, and they concluded that their results were "consistent with the presence of extravascular separated gas."

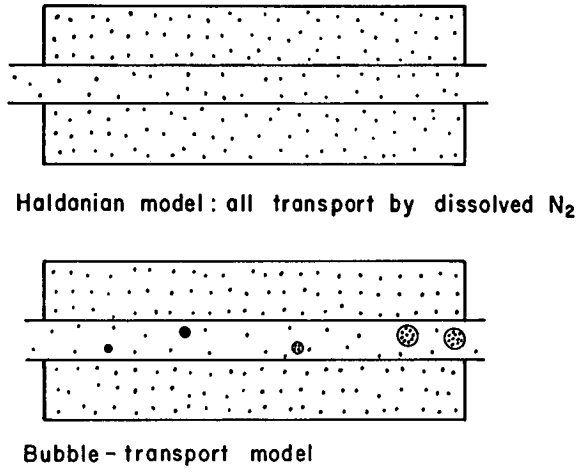

Fig. 3. Effect of small intravascular bubbles on gas transport.

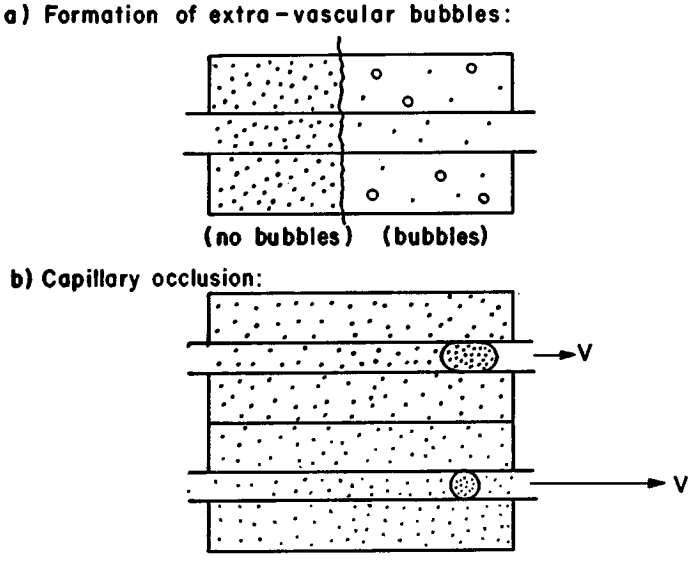

Fig. 4. Proposed bubble-induced retardation of gas elimination.

There seems then to be no real doubt that bubbles are present during normal decompression, but their effect on gas elimination rate remains to be established. This will ultimately have to be done through experimentation, but it is also important to compare our hypothesis and existing models with available data mathematically.

Gas-Transport Models and Diving Experience

Diving experience, as embodied in U.S. Navy decompression tables, has been compared in this paper with models of gas transport and elimination. For example, supersaturations have been calculated for no-decompression dives of maximum duration (Fig. 5).

These familiar data contain several features not adequately explained by current gas-transport models. First, supersaturations for the fast 5- and 10-min tissues increase rapidly with depth and become substantially larger than those tolerated for shorter dives. Second, the 30-fsw saturation dives seem anomalous because calculated supersaturation pressures are substantially higher than those in all but the 5- and 10-min tissues for the longer dives.

Observations of high apparent supersaturations in fast tissues on surfacing from deeper no-decompression dives are not new, but they have never been explained. It seems probable that the 5- and 10-min tissues eliminate gas more effectively than expected as a result of intravascular nucleation, in accordance with our hypothesis. However, the possibility of very high supersaturations cannot be ruled out. Calculations focused on the first decompression stop of longer dives yield results comparable to those above.

Haldane-type decompression calculations deal with supersaturation in terms of postulated critical pressure ratios, usually with substantial empirical modifications. There is, however, no basis for postulating a critical pressure ratio in nucleation theory, which is quite well developed and has been established for many years (see, for example, Frankel (7)). Critical

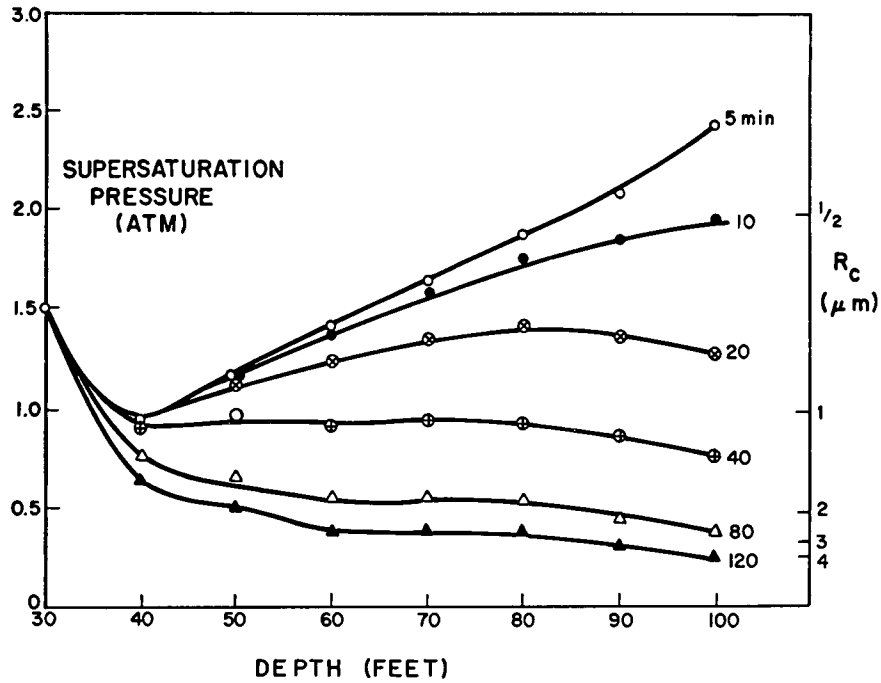

Fig. 5. Calculated supersaturation pressures and critical nucleation radii for maximum no-decompression dives.

pressure difference depends only on the chemical and physical condition of the system, and is not influenced to any appreciable degree by total pressure in the range used in current diving tables.

The experimental decompressions of Willmon and Behnke (18) and Kindwall (9) can be examined by estimating maximum supersaturations for each tissue in the three sets of experiments. Unfortunately, this presents a major problem: there is no valid way to calculate combined helium and nitrogen pressures in a given tissue. Two sets of estimates were therefore prepared: (1) one based on nitrogen alone; and (2) another which added tissue partial pressure of helium, assuming helium half times to be two-thirds those of nitrogen, which probably underestimates the helium effect. Once again, it would be highly desirable to have more solid anatomic and physiologic bases for such calculations. Nevertheless, a few possibilities present themselves.

Negative supersaturation and therefore the absence of bubble formation was predicted for the 100-fsw decompressions. Even if helium had a larger effect than assumed, supersaturations would be modest and bubble formation would be rather unlikely.

Decompressions at 50 fsw show rather modest supersaturations also, especially if the influx of helium is neglected. There is thus reason to suspect that helium may have triggered nucleation in the slower tissues and increased the elimination rate above that which would have been obtained otherwise. This is a possibility worth checking, especially since there is strong evidence in the Kindwall work (10) of enhanced nitrogen removal from the perhaps critical slower tissues.

Supersaturations are very large in the 10-fsw decompressions, especially for the slower tissues if the effect of helium has really been underestimated. These high pressures would indeed suggest vascular occlusion or extravascular nucleation.

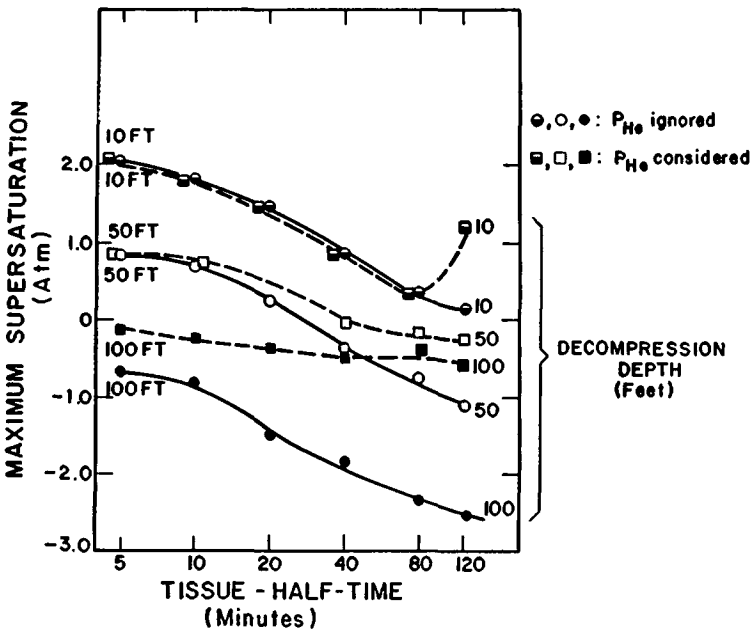

Fig. 6. Calculated maximum supersaturations for the Kindwall et al. experiment (10).

On balance, a preliminary quantitative assessment of the Kindwall data (10) is not in disagreement with our hypothesis, although it raises additional questions.

An Estimate of Bubble Growth Rates

Our bubble transport hypothesis, in conjunction with an examination of available data, leads to the belief that nucleation occurs at supersaturation pressures well below those supported painlessly by divers. The growth behavior of bubbles under representative conditions remains to be determined to see if these rates are compatible with the elements of this discussion.

To do this, a simplified bubble growth estimate was used:

$$\frac{dR}{dt} = \alpha_i D_{im}\left(\frac{T\ 1\ atm}{273°K}\right)\left[\frac{P_i - \left(P_a + \frac{2\gamma}{R} - \Sigma P\right)}{R\left(P_a + \frac{2\gamma}{R} - \Sigma P\right)}\right] \tag{1}$$

where α_i = solubility of inert gas (ml STPD per ml of solution at P_i of 1 ATA); D_{im} = effective liquid-phase diffusivity of inert gas; P_i = partial pressure of i in liquid phase; P_a = ambient pressure; ΣP = combined partial pressures of oxygen, carbon dioxide, and water vapor; and γ = effective surface tension. The origin of Eq. 1 is described in the Appendix.

For discussion purposes, nitrogen at 2 ATA with an ambient pressure of 1 ATA and temperature of 310°K was used. α_{N_2} was assumed to be 0.0122, D_{N_2M} to be 10^{-5} cm^2/sec, and

ΣP to be 0.18 atm. It remains to estimate γ and original bubble size; for simplicity, two limiting cases were considered:

(1) α = 50 dynes/cm, for which R_o, the initial bubble radius, should be about 1.25 μm (see Appendix). This is reasonably consistent with laboratory measurements.
(2) $\gamma = 0$ for which R_o is also 0. This is roughly consistent with Hills' suggestion (4).

In general, these conditions correspond to a typical 5-min tissue at the end of a representative dive to about 100 fsw.

Calculated bubble radii are given as a function of time for these two limiting calculations (Fig. 7). The 50-dynes/cm calculation predicts bubbles will reach the approximate capillary radius of 5 μm in the reasonable capillary transit time of 1 sec. The zero surface tension calculation, which should overestimate growth rate, predicts that capillary radius is reached in half a second. On balance, both these calculations suggest that bubble growth rates accord quite well with the hypothesis.

Conclusion

The bubble transport hypothesis is in reasonable agreement with both available data and transport theory, and it suggests several possibilities for increasing gas elimination: proper, presumably deeper, choice of decompression stages, and, most promising for slower tissues, triggering with an insoluble gas like helium. This suggestion appears consistent with the faster decompression schedules discussed elsewhere in this volume.

Further tests of this hypothesis are indicated, and these should take at least three forms:

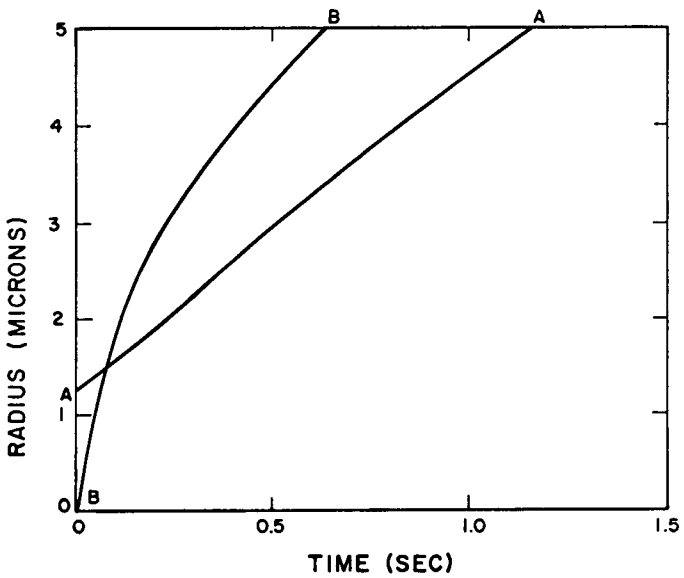

Fig. 7. Calculated bubble growth rates; P_{N_2} = 2 ATA; P_a = 1 ATA; T = 310°K, α = 0.0122 ATA^{-1}; $D_{N_2B} = 10^{-5}$ cm^2/sec. A = γ = 50 dynes/cm, R_o = 1.25 μm; B = γ = 0, R_o = 0.

(1) direct measurement of bubbles by ultrasonic devices; (2) additional simulated dives, under water, in which effects of oxygen, helium, and bottom time and pressure are systematically determined; and (3) careful comparison of absorption and elimination for various fractional saturations. More generally, a serious attempt should be made to connect diving theories with available physiological data, for example by careful localized perfusion studies.

Appendix

Derivation of Eq. 1

The rate of input of mass M_i of inert gas i to a growing bubble is:

$$\frac{dM_i}{dt} = (4\pi R^2) D_{im} \frac{\partial C_i}{\partial r}\bigg|_R$$

where r = radial distance; R = bubble radius; and C_i = molar concentration of i in the surrounding liquid. The rate of volume increase, V, of the bubble is then:

$$\frac{dV}{dt} = 4\pi R^2 \frac{dR}{dt} = 4\pi R^2 D_{im} \alpha_i \left(\frac{T}{273°K}\right) \frac{1 \text{ atm}}{\left(P_a + 2\frac{\gamma}{R} - \Sigma P\right)} \frac{\partial P_i}{\partial r}\bigg|_R$$

where P_i is liquid (or gas) partial pressure of i.

For small bubbles (see Lightfoot (11) or van Lieuw and Hlastala (17)):

$$\frac{\partial P_i}{\partial r} = \frac{P_i - \left(P_a + \frac{2\gamma}{R} - \Sigma P\right)}{R}$$

so that:

$$\frac{dR}{dt} = \alpha_i D_{im} \left(\frac{T\ 1 \text{ atm}}{273°K}\right) \frac{P_i - \left(P_a + \frac{2\gamma}{R} - \Sigma P\right)}{R\left(P_a + \frac{2\gamma}{r} + \Sigma P\right)}$$

It then follows on integration that:

$$\alpha_i D_{im} \left(\frac{T\ 1 \text{ atm}}{273°K}\right) t$$

$$= \left(\frac{P_a - \Sigma P}{[P_i - (P_a - \Sigma P)]^3}\right)\left[\frac{A^2}{2} + 4\gamma A + 4\gamma^2 \ln A\right]_{A(o)}^{A(t)}$$

$$+ \frac{2\gamma}{[P_i - (P_a - \Sigma P)]^2}\left[A + 2 \ln A\right]_{A(o)}^{A(t)}$$

where $A = [P_i - (P_a - \Sigma P)] R - 2\gamma$.

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GAS NUCLEATION IN GELATIN

T. D. Kunkle and D. E. Yount

The observation of gas bubbles forming in supersaturated aqueous media is as commonplace as beer or Coca Cola. That gas phases also develop within decompressed animals should surprise no one: living systems consist mainly of water, and the production of bubbles is a physical process that occurs in water whether it is a component of blood, tissue, beer, or gelatin. What is very surprising, in view of homogeneous nucleation theory (3, 5, 6, 9, 13), is that bubbles form so readily, frequently at supersaturation pressures below 1 atm. In the absence of nuclei or nucleation processes other than random molecular motion, the threshold for bubble formation is expected to be of the order of 1440 atm (24).

Clearly, it is of great interest to those working in the field of decompression sickness to understand why bubble formation is so precocious. Transparent gelatin (14, 22, 23, 26) has proved to be an excellent model for such a study, not only because it closely approximates interstitial tissue, but more practically because it yields bubbles that are stationary and can be counted and measured. The technique is also simple, humane, and reproducible.

In this report, the findings to date concerning the nucleation problem are summarized. The apparatus and some of the results have been described elsewhere (22, 23, 26), and such topics will only be discussed briefly for the sake of completeness and continuity.

Apparatus

The primary apparatus, shown in Fig. 1, consists of a small pressure vessel limited to 300 psig, a gas supply, a microsope with a magnification of seven times, and four counting chambers suspended in a water bath to buffer the temperature. The counting chambers are 27 mm wide and 6 mm along the line of sight, and they are usually filled to a depth of 4 mm.

The gelatin is normally prepared in large batches and stored in individual 10-ml aliquots by freezing. A standard batch consists of 127 g of unflavored Knox® gelatin crystals dissolved in 5 liters of distilled water. The transition from gel to sol occurs at about 25 °C for this mixture, and samples are maintained near 21 °C during tests. Compression is usually carried out with two samples in the gel state and two samples in the sol state, all samples being in the gel state by the time decompression occurs. The close agreement between gel and sol results indicates that nucleation is not related to any trapping action of the gelatin infrastructure and suggests that elasticity is negligible in comparison with surface tension, as would be expected for so lean a mixture.

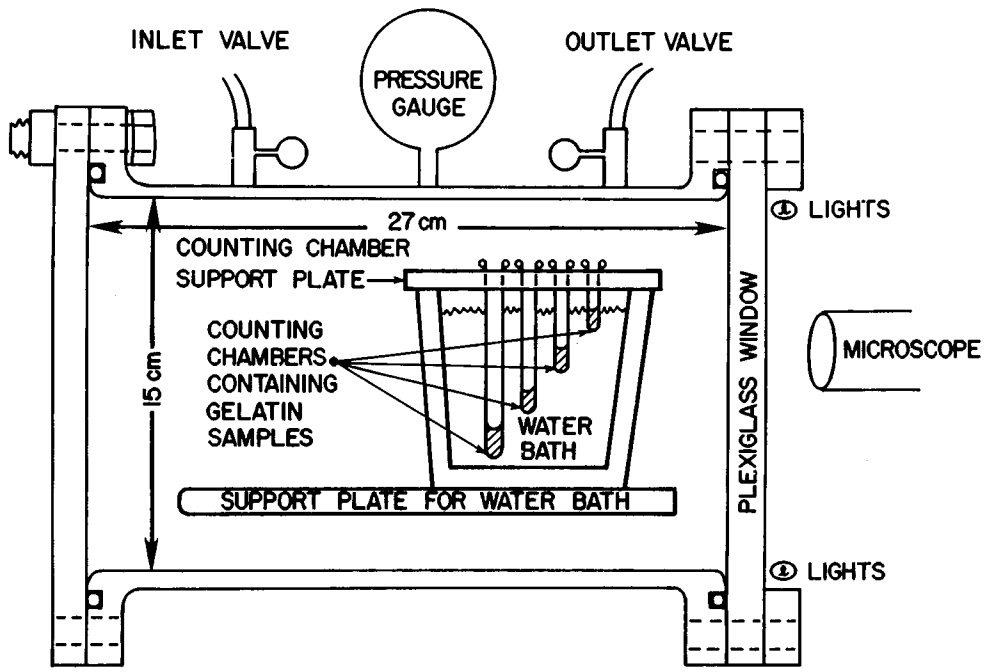

Fig. 1. Pressure vessel and counting chambers used to study decompression of gelatin.

Smaller batches are prepared with the same proportions, and test substances are occasionally added both to standard and to special batches. Frozen samples from a given batch are stable and give reproducible bubble counts for periods exceeding one year. Samples taken from different batches generally give different bubble counts for a given pressure schedule. As will become clear shortly, this statement is synonymous with the statement that different batches have different distributions of gas nuclei, which in no way affects the major conclusions of this work, verifiable with any similar gelatin mixture.

Figure 2 is a photograph of a counting chamber and gelatin sample taken a few minutes after decompression. Ordinarily, bubbles become visible within seconds after a rapid decompression and grow for some minutes before stabilizing. If no further changes in pressure occur, the sample will remain stable for several days. This photograph is typical in that bubbles are: (1) approximately spherical; (2) generally well separated from one another and from the chamber walls; and (3) much larger than the observational limit of about 10 μ in radius. Bubbles in the lower 3 mm are also of fairly uniform radius and randomly distributed in space. Those in the upper 1 mm and meniscus are noticably smaller and appear to be more numerous than elsewhere. For this reason, only bubbles in the lower 3 mm, defined as the fiducial volume, are counted. The tendency of bubbles to form with fairly large and uniform radii reflects the fact that bubble size is determined mainly by how much gas diffuses into each nucleus rather than by the initial characteristics of individual nuclei.

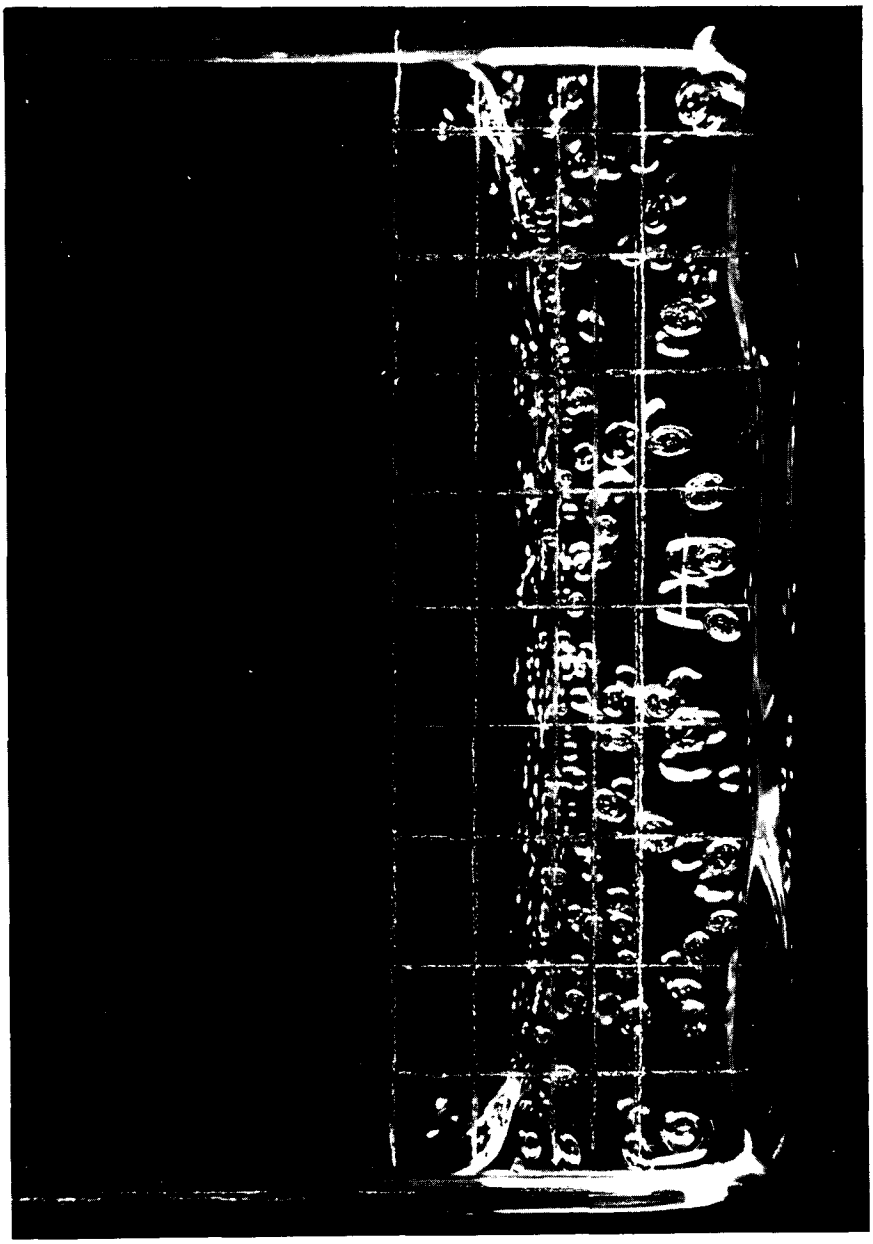

Fig. 2. Photograph of a counting chamber and gelatin sample taken a few minutes after decompression; bubbles in the upper 1 mm were noticeably smaller and more numerous than elsewhere; only those in the lower 3 mm were counted (this corresponds to a fiducial volume of about 3 mm × 6 mm × 27 mm).

Denucleation by Centrifuging

Various methods of denucleation were compared (26) by subjecting gelatin samples to the following pressure schedule:

$$\text{initial pressure} \equiv p_o = 0 \text{ psig;} \tag{1a}$$
$$\text{saturation pressure} \equiv p_s = 300 \text{ psig; and} \tag{1b}$$
$$\text{final pressure} \equiv p_f = 0 \text{ psig.} \tag{1c}$$

Compressions and decompressions were carried out at about 3 psi/sec, and the time at saturation pressure, p_s, was

$$\text{saturation time} \equiv t_s = 5.25 \text{ hours.} \tag{1d}$$

This is much longer than the range of time constants calculated for the 0-3 mm fiducial volume and 4 mm depth

$$\sigma(0 \text{ mm}/4 \text{ mm}) = 74 \text{ min (bottom of fiducial volume); and} \tag{2a}$$
$$\sigma(3 \text{ mm}/4 \text{ mm}) = 19 \text{ min (top of fiducial volume)} \tag{2b}$$

Thus the gas tension τ throughout the sample was closely equal to the saturation pressure immediately prior to decompression

$$\tau \simeq p_s \text{ (for saturation at } p_s), \tag{3}$$

and the supersaturation pressure, p_{ss}, was approximately

$$p_{ss} \equiv (\tau - p_{amb}) \simeq (p_s - p_f) \tag{4}$$

where p_{amb} is the ambient pressure. For the test schedule of Eqs. 1a,b,c, and d, this gives

$$p_{ss} \simeq 300 \text{ psi} \tag{5}$$

When samples from Batch A were subjected to the test schedule just described, they yielded about 400 bubbles per fiducial volume, whether compression took place in the gel or in the sol state. The schedule was then applied to samples from the same batch that had been centrifuged for 15 minutes at 20,000 rpm, corresponding to about 17,000 *g* near the top of the centrifuged mass and about 36,000 *g* near the bottom. Three samples centrifuged in the sol state yielded 1.0 ± 0.6 bubbles per sample, while 15 samples centrifuged in the gel state averaged 0.33 ± 0.15 bubbles per sample. The combined average for gel and sol states is 0.44 ± 0.16 bubbles per sample, corresponding to 0.11 ± 0.04% of the number obtained without centrifuging. The fact that centrifuging is effective in the gel state as well as in the sol state suggests that centrifuging eliminates nuclei by compression or crushing rather than by precipitation.

Hydrostatic crushing was tested directly by subjecting samples to a pressure spike at the beginning of a pressure schedule, and it was found that compression to a maximum of $p_m = 300$ psig for 10 minutes would eliminate 97% of the bubbles normally formed in a test schedule similar to that described above, but with Eq. 1b replaced by $p_s = 150$ psig. Crushing is a specific test for gas nuclei: thus gas nuclei account for at least 99.9% of the bubbles formed in gelatin.

To determine whether gas nuclei are associated with powdered gelatin, with the mixing and preparation of samples, or with water per se, a special batch of gelatin was prepared using dis-

tilled water that had been centrifuged at 20,000 rpm for 15 minutes. The standard proportions of powdered gelatin to water were used. When subjected to the test schedule described by Eqs. 1a,b,c, and d, this batch yielded 28 ± 3 bubbles per sample averaged over three samples. This test indicates that approximately 93% of the bubbles produced in gelatin are associated with pre-existing gas nuclei in the water and are not introduced by the powdered gelatin or by our method of preparing samples.

Seeding Experiments

Seeding with Powdered Gelatin

To determine the source of the remaining nuclei, i.e., those not associated with water in the previous test, gelatin powder was placed at the bottom of individual counting chambers in variable quantities. Denucleated sol was then added and the sample allowed to stand until the powder dissolved. The pressure schedule of Eqs. 1a,b,c, and d yielded no bubbles for a sample into which no powder had been introduced and 7.0 ± 2.6 bubbles for a sample seeded with 4 mg of gelatin powder. A third sample seeded with 8 mg of powder yielded 15 ± 4 bubbles. It was concluded that some nuclei are associated with the gelatin powder and that the number of such nuclei is roughly proportional to the amount of powder used. An estimate of the fraction of the nuclei introduced into Batch A via the original gelatin powder can be made from these data and is consistent with the 7% not associated with water. Furthermore, no evidence was found that any of the procedures used in preparing gelatin, e.g., stirring, heating, cooling, freezing, melting, etc., introduces nuclei. It was concluded that about 93% of the nuclei observed in gelatin are associated with water and the remaining 7% with the gelatin powder.

Seeding with Hydrophobic Spheres

Plesset (16) has suggested that the difference in the threshold for bubble formation predicted by homogeneous nucleation theory and that observed experimentally may be caused by the presence of solid impurities. To explore this idea further, Plesset carried out specific calculations for hydrophobic spheres and found that the probability of forming a concentric gas or vapor phase approaches unity as the cavity radius approaches that of the enclosed sphere. From this point of view, a hydrophobic sphere in water may be thought of as being surrounded at all times by an arbitrarily thin shell of vapor or gas.

Experimental attempts to seed liquids with solid impurities have met with very little success. For example, Bateman and Lang (2) tried charcoal, blood corpuscles, dialyzed colloidal ferric hydroxide, ivory black, and sodium bicarbonate. Only the last two had any effect, and the authors considered these results to be inconclusive. Richardson (17), on the other hand, found that salt grains can carry nuclei into solution, but since the salt dissolves completely, these must be gaseous rather than solid. Furthermore, Richardson's technique, absorption of ultrasonics, is sensitive specifically to gas phases.

It is clear from the denucleation tests discussed above that gas nuclei also account for virtually all of the bubbles observed in gelatin. Plesset's model is interesting, nevertheless, and it could be important in other circumstances; for example, it might provide a means of putting

nuclei of known size into a sample for calibration purposes. With this in mind, we have seeded denucleated gelatin with polystyrene spheres (7), noting that polystyrene (contact angle $\simeq 98° - 132°$) is rather hydrophobic (limiting contact angle of 180°).

The smallest radius detectable with our apparatus is given by (26)

$$r_c^{min} = 2\gamma/p_{ss} \tag{6}$$

where p_{ss} = 300 psi from Eq. 5, and where

$$\gamma = (51 \pm 5) \text{ dynes/cm} \tag{7}$$

is the measured surface tension in gelatin (26). The result is

$$r_c^{min} = (0.049 \pm 0.005)\,\mu \tag{8a}$$

The largest nuclei actually observed in gelatin are characterized by radii of (26)

$$r_c^{max} = (1.24 \pm 0.13)\,\mu \tag{8b}$$

In separate tests, we have used polystyrene spheres of two different radii, both well within the range defined by Eqs. 8a and b. These are, respectively,

$$r_1 = (0.117 \pm 0.0013)\,\mu; \text{ and} \tag{9a}$$
$$r_2 = (0.397 \pm 0.0022)\,\mu \tag{9b}$$

The spheres were suspended in 10% aqueous solutions (7), which were mixed with sol in proportions of about 1/750 to give 0.013% final concentrations. This corresponds to about 2×10^8 spheres per sample of the smaller size and about 0.9×10^8 spheres per sample of the larger. Gas nuclei were removed in various ways, the simplest being to centrifuge the seeded samples in the gel state. Centrifuging crushes any gas phases that may be present, while the gel state prevents precipitation and thus holds the solid spheres in suspension. This was easily checked by noting that samples were equally cloudy before and after centrifuging. The results of all such tests were negative. Specifically, it was observed that there were 0.60 ± 0.25 bubbles per seeded sample averaged over 10 assorted samples.

The inability to generate bubbles on hydrophobic spheres caused a re-examination of Plesset's model in some detail (25). Using his formulas, it was shown (25) that the probability for cavitation is low if: (1) the spheres have radii larger than 10 A; and (2) the thicknesses of the vapor or gas shells surrounding them are required to be at least one average intermolecular distance, about 3 Å in water. Since homogeneous nucleation is expected to be effective in producing vapor phases with radii up to about 10 Å, the presence of smooth spheres of any size, whether hydrophobic or not, can reduce the threshold from that predicted for homogeneous bubble formation (1440 atm) by at most 30%.

Seeding with Salt

At this point in the investigation it was evident: (1) that gas nuclei account for virtually all of the bubbles observed in gelatin; (2) that such nuclei are abundantly present in distilled water; and (3) that the gas phases present in water are stable for periods of hours or longer. The two major questions were: (1) How are gas nuclei produced? (2) How are gas nuclei stabilized? The occurrence of nuclei in distilled water seemed to rule out the Harvey model (11, 12) in which gas phases develop spontaneously and are stabilized in hydrophobic cracks, although

this mechanism could operate in the interstitial spaces between cell walls (1). Similarly, the organic skin theory (8) was untenable because (16): (1) Gas phases grow quite readily by rectified diffusion in oscillating pressure fields so that there is no evidence for inhibition of diffusion at bubble boundaries; and (2) Chemical reagents which in small amounts strongly affect organic materials, including proteins, do not have a noticeable effect on cavitation thresholds. The most promising explanation at this stage seemed to be: (1) production by ionizing radiation, principally cosmic rays and their secondaries; and (2) stabilization by electrostatic forces such as might exist in the vicinity of Na^+ and Cl^- ions.

The ionic model is supported by Richardson's observation that salt grains can carry gas phases into solution (17). To pursue this further, centrifuged-gelatin samples were seeded with pure reagent salt, with Springfield table salt, and with various saline solutions prepared with centrifuged water. These tests were all negative, yielding 0.57 ± 0.20 bubbles per seeded sample averaged over 14 assorted samples. It was concluded that any gas phases carried into solution by dissolving salt grains are relatively unstable and that the presence of large numbers of Na^+ and Cl^- ions does not significantly enhance the bubble yield in decompressed gelatin. It is important to add that these conclusions are entirely consistent with Richardson's data in which the gas phases introduced by dissolving salt grains were studied only for short periods after the salt was released. After several hundred seconds, virtually all of Richardson's bubbles had either dissolved or risen to the surface. The two experiments together indicate that gas phases can be introduced by dissolving salt grains, but this is not a source of stable gas nuclei.

Nucleation by Ionizing Radiation

It has been known since the invention of the bubble chamber (10) that ionizing radiation can cause bubble formation in a superheated liquid. In the case of liquid hydrogen, the critical radius is about 40 Å (18) and the threshold energy about 500 eV (18). These levels can be reached by some of the δ rays (atomic electrons) that recoil from soft collisions of an incident charged particle, such as a cosmic ray muon. Ionizing radiation is also capable of producing gas or vapor phases in supersaturated water (4, 19, 20) and some other liquids (15), but in aqueous media the threshold energy is about 5 MeV for a 1-μ critical radius (4).

The ultrasonic-cavitation experiments of Sette and Wanderlingh (19) indicate that oxygen nuclei, recoiling from collisions of cosmic-ray neutrons with water molecules, can produce gas or vapor phases in water. The oxygen nuclei eject thousands of δ rays in the last few microns of a stopping trajectory, and these δ rays are so densely packed that their ionization channels coalesce to form cylindrical cavities that may be 10 μ long and may initially have radii of about 20 Å. The temperature within the cavity is about 600°K, and it contains oxygen and hydrogen gas from radiolysis, as well as water vapor, excited atoms, ions, and free electrons (4). The cavity is thus both supersaturated and superheated, and this permits it to grow and reorganize itself in a more stable configuration. That this actually happens is evidenced by the experimental observation (19) that gas nuclei formed in this way are stable for periods on the order of hours.

The marginal nature of the process just described is suggested by the experimental observation (19) that recoil hydrogen nuclei (protons) are not able to generate stable gas phases in water, although the δ-ray density is only a factor of 10 lower than for recoil oxygen nuclei. On

the other hand, recoil carbon nuclei are able to produce cavities in pentane and acetone by this mechanism (15).

Clearly, it is of great interest to determine whether ionizing radiation is capable of generating stable gas or vapor phases in gelatin. Not only could this explain precocious bubble formation and the presence of gas nuclei in aqueous media generally, but in addition, the gelatin model would have many advantages as a technique for pursuing the topic further. In particular, the number of nuclei versus nuclear radius could be measured (26), whereas ultrasonic cavitation in water measures only the formation threshold, i.e., the radius r_c^{max} of the largest nucleus present.

In testing the radiation sensitivity of gelatin, a plutonium-beryllium source (21), $^{239}Pu^{13}Be$, rated at 1.6×10^6 neutrons per second, was used. The neutron energy spectrum extends from roughly zero to about 10.6 MeV, with an average neutron energy of about 4.2 MeV. The half life of ^{239}Pu is 24,3000 years. The source consists of four cylindrical slugs mounted along the axis and near the center of a large cylindrical tank filled with water. Fast neutrons are obtained by removing wax plugs positioned radially with respect to the source, permitting gelatin and water samples to be placed in the immediate neighborhood of the slugs. This source is about one order of magnitude stronger than the $^{226}Ra^{13}Be$ source used by Sette and Wanderlingh (19), which has a maximum energy of about 13 MeV and an average energy of about 4 MeV. The $^{210}Po^{13}Be$ source used by Lieberman (15) generated about 4×10^4 neutrons per second in the range from 1 to 10.8 MeV, with an average neutron energy of about 4.5 MeV.

In the first series of experiments, denucleated gelatin was placed adjacent to the source slugs immediately after decompression from 300 psig, the last step of the usual test schedule described by Eqs. 1a,b,c, and d. Since these samples were supersaturated at $p_{ss} \simeq 300$ psi at the time of irradiation, they were expected to yield bubbles whether or not the neutron-induced nuclei were stable. Results of these tests, with variable exposures from a few minutes to several hours, were negative.

A possible explanation for this result is that the gel structure somehow inhibits nucleation. This was tested by irradiating denucleated sol samples before compression and denucleated water samples before mixing. These test results were also negative.

Next, the hypothesis was advanced that ionizing radiation works by enlarging pre-existing nuclei rather than by initiating new ones. This was tested by irradiating samples that had not been centrifuged, either before or after the samples were subjected to various pressure schedules. The yields in this case were consistent within statistical errors with the bubble counts ordinarily found.

In the most recent attempt, uncentrifuged samples were decompressed from the 0-psig mixing pressure to determine whether the threshold for bubble formation, normally about -12 psig, was in any way influenced by fast neutron bombardment. Again, the results were negative.

At this time the negative results of the neutron survey are not fully understood. It is worth noting, however, that the density of gas nuclei at cavitation threshold in the experiments of Sette and Wanderlingh (19) was about 2.5×10^{-2} per cm^3 with incident cosmic rays. This is in good agreement with their calculated rate of 2.17×10^{-2} gas nuclei per cm^3, which was obtained by assuming: (1) that the rate of incident cosmic-ray neutrons above 10 MeV is 2×10^{-4} per cm^2 per second; (2) that one gas nucleus is formed for every incident neutron above 10 MeV; and (3) that the half-life for gas nuclei is 3×10^3 sec (determined by their measurements). Sette and Wanderlingh (19) also found that the density of gas nuclei was not

significantly higher when the $^{226}Ra^{13}Be$ source was used, even though the source intensity was many orders of magnitude higher than the rate for cosmic-ray neutrons. Sette and Wanderlingh suggest (19) "that either only a few neutrons are emitted from the source with sufficient energy for the formation of nuclei, or that the equilibrium density of nuclei in the liquid is controlled by elements other than radiation, such as impurities present in the liquid." In any case, the densities of gas nuclei obtainable with cosmic-ray neutrons or with radioactive neutron sources would be less than 0.01 per gelatin sample, well below our experimental sensitivity.

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NATURAL URANIUM AND DECOMPRESSION SICKNESS

A. Evans and D. N. Walder

Although there is still speculation about the way decompression sickness symptoms are caused, there is widespread agreement that bubbles of gas within the body must be an important factor. If this were not so, why would recompression treatment alleviate the symptoms so effectively? What else could reflect or attenuate the ultrasonic beams directed into the bodies of animals and men to monitor the results of decompression?

During the early 1940's, Harvey and his group (4) demonstrated that some gas nucleus had to be present in a supersaturated fluid before any visible bubble could arise. Harvey's work is still widely quoted in the physical sciences, where the absence of gas micronuclei is essential to superheat a pure fluid above its normal boiling point, achieve a stable supersaturated gaseous solution, or place a liquid in a state of tension (or negative pressure) either continuously or transiently, as occurs in ultrasonic cavitation studies. Denucleated water can be heated at atmospheric pressure to above 200 °C, or withstand a tension of some tens of atmospheres, and stable solutions of water containing gases at over 100 atmospheres have been described.

Since gas bubbles arise in the body at the modest gaseous supersaturations permitted under properly controlled decompression procedures, a mechanism must exist in the bodies of normal men for the production of readily expandable gas nuclei. To create a new gas phase in an otherwise continuous liquid requires the injection of a large amount of energy into a region whose dimensions are at most of the order of a micron (6), and one possible source of this energy is the particles associated with the decay of radioactive elements. The critical factor is the linear energy transfer (LET), which is a measure of the amount of energy lost by the particle to its host fluid per unit path length. The LET of β and γ rays is not sufficient to nucleate water containing a modest supersaturation of air, though the authors found that bubbles did arise in solutions containing uranium 238 (7). The obvious explanation for this was that the LET of the α particles, which are liberated abundantly by ^{238}U, was sufficient for nucleation. However, in further experiments, solutions containing thorium, which liberates similar α particles, remained as stable as controls. The authors were thus forced to the conclusion that it was the much less frequent spontaneous nuclear fission of the uranium 238 which caused the bubbles. The fission products are highly charged and energetic, and so have a very high LET.

Since uranium 238 is the major constituent of natural uranium, it has recently been suggested (7) that spontaneous nuclear fission within the body burden of natural uranium may

have a place in the nucleation of bubbles associated with decompression sickness. At first sight this may seem unlikely, for the spontaneous fission half-life of ^{238}U is about 10^{16} years (2), and the average body burden is only about 1/10 mg (3, 8). However, even this small amount represents a great many atoms, and calculation shows that a fission event can be expected for the average man about once every three weeks.

This tells us nothing about the outcome of any one decompression for a single man, but it does allow prediction of the bends rate which might be experienced by a group of men undergoing many regular decompressions; how many decompressions is dictated by the statistics of counting. For a bends rate of 2%, 1000 man-decompressions are needed to achieve 20 bends. The standard deviation of a number is its square root, so 20 is really 20 ± 4.5, an uncertainty of over 20%. To have any confidence in a computed bends rate, therefore, it must refer to at least 1000 decompressions, and preferably to many more.

Numbers of this magnitude are not available for divers. However, the Medical Research Council Registry at Newcastle upon Tyne has for many years been collecting complete data from British compressed air contracts, and some of these data are now stored on the computer for rapid analysis. To determine the best way to present these data in the present context, the possible consequences of a uranium fission in a man's body must be considered.

If uranium fission occurs at atmospheric pressure, the fission event will pass unnoticed. Similarly, if it occurs during the free air rest period of a man working regularly in compressed air, it may be of no consequence because when he is next compressed the friable skin on a newly created micronucleus may be crushed so that the gas goes back into solution. However, a nucleus created either during the working period at high pressure or during the subsequent decompression when the tissues are supersaturated is liable to persist, grow, and multiply with the decompression to lead to an attack of decompression sickness. Thus a risk time can be identified which consists of the working period plus the time spent on decompression; the predicted bends rate therefore increases linearly with the risk time over a certain range of exposures.

We have previously predicted the bends rate for one exposure period at the Tyne Road Tunnel (7). The remarkable agreement with the observed rate can be no more than coincidental, and it was merely intended to establish that the orders of magnitude were correct. Figure 1 shows more detail of the men's performance at the Tyne Road Tunnel (the last major contract to use the 1958 tables), and also some bends rates from the Dungeness B power station contract, on which Hempleman's Blackpool Tables were used for the first time. The solid line shows the linear increase of predicted bends rate with risk time based on Hamilton's (3) estimate of uranium body burden for a normal man with no occupational exposure. The points show the observed bends rates; the error bars indicate one standard deviation each way from counting statistics. To make up sufficient numbers it was necessary to consider all exposures to pressure above 14 psig, where timed decompressions start, in 1-hr exposure increments. The mean risk time was computed for each group with the aid of the appropriate decompression table, so that each rate could be properly compared with the predicted rate (Fig. 1).

The points for 7-8 and 8-9 hr exposures at the Tyne Tunnel fall on the solid line in Fig. 1, as do the means for all exposures and the point for the 3-4 hr group. The regulation of shift length was not within our control, so there were some time intervals where the number of exposures was too small to yield meaningful bends rate data.

The spread of exposure times was greater at Dungeness though the majority of exposures

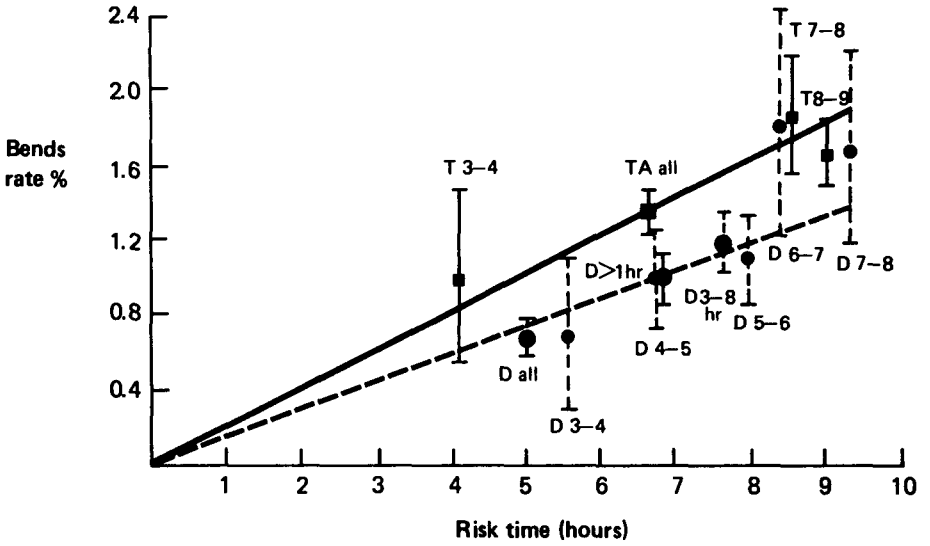

Fig. 1. Variation of observed bends rate with calculated risk time (grouped data for various working periods) for men at 2 British compressed air contracts. T and upper line: Tyne Road Tunnel, 1962-6, using 1958 Tables; D and lower line: Dungeness B Power Station, 1966-8, using Blackpool Tables. Numbers beside each point indicate period of work in hr.

were for 5-6 and 6-7 hr. The longer decompression times of the Blackpool Tables, however, made these men's risk times comparable with the Tyne Road Tunnel 7-9 hr group, and the bends rates are much the same. However, the Dungeness observations based on the shorter shift length fall below the predicted bends rate line, though a straight line, of lower slope, is compatible with them all (broken line, Fig. 1). It is not relevant to discuss here why this should be so; but it should be pointed out that experience based on two separate decompression tables appears to be consistent with a linear dependence of bends rate on risk time.

Although the response of a group of many men yielded data which seem consistent with the uranium fission hypothesis, considerable individual variation may be expected among that group. At the Tyne, for instance, one man underwent more than 1300 decompressions without a bend, whereas one of his workmates had six incidents of bends in 25 exposures. Several factors probably combine to produce these extremes, and one of them might be differences in the body burden of uranium. The figure of 1/10 mg is an estimated average for individuals with no occupational exposure. Clearly, a man living in an area where the water supply and food are comparatively rich in uranium should accumulate a much larger retained body burden than a man from a low uranium district. Geographical variations do occur in the uranium content of water, and the total α activity of ashed human tissue specimens also covers a considerable range, though this is not all caused by uranium.

If body burdens could be estimated, it might be possible to prove or refute the fission hypothesis by studying men who are known to be sensitive or resistant to decompression sickness. Unfortunately, whole body or selective counting techniques are of no use at such low levels, so it is necessary to resort to destructive analysis of body fluids. The most obvious of these are blood and urine, in which the normal uranium contents are both about 1 μg per

liter. It is much more convenient to take a liter of urine for analysis, especially if the analysis is to be repeated several times.

Two methods are available for the detection of uranium in urine. The first is a fluorimetric technique (1) which is widely used for monitoring workers in uranium laboratories to detect accidental ingestion. This technique does not have to be very sensitive; the lower limit for measurement with the standard procedure is about 5 μg/liter. However, concentration of the urine by a factor of 10 should allow measurement down to the natural levels for nonexposed men.

A preliminary trial on 24-hr urine samples from compressed air workers has now been carried out; samples were collected at the Second Dartford Tunnel project, which was in progress at the time. Concentrated aliquots from these samples were analyzed by fluorimetry at the UKAEA Harwell laboratories, and the estimated daily excretion for each man was compared with the men's susceptibility to decompression sickness. There was no immediately apparent correlation. One reason for this may be the insensitivity of the method, even with concentration, so that for many men only upper limits to the daily excretion could be established. Another problem, however, concerns quenching. Constituents of the urine which are present in variable amounts quench the fluorescence (1) to an unknown extent, so that many of the determinations may be underestimates.

A fundamentally different technique which does not present this problem is neutron activation analysis (5). In this technique, the sample is introduced into the core of a nuclear reactor, where a flux of about 10^{13} thermal neutrons/cm^2/sec causes fission of the uranium 235. The sample is then removed, the nitrogen 17 is allowed to decay, and the delayed neutrons from the fission products are counted for a standard time. This count is directly proportional to the ^{235}U content of the sample, and the technique is sufficiently sensitive to measure less than 0.1 μg of natural uranium to $\pm$ 10%. The only assumption necessary in assaying ^{235}U to estimate ^{238}U is that the sample must display the natural isotopic ratio; for divers or compressed air workers with no occupational exposure, this is no problem.

Neutron activation analysis of urine is clearly the technique of choice; a program to determine the natural uranium excretion of individuals of known susceptibility is presently underway. This may help to confirm or refute the suggestion that uranium may have an influence in the etiology of decompression sickness; until then, it would probably be prudent to suggest that uranium miners refrain from diving.

ACKNOWLEDGMENT

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USE OF EXOTIC GASES FOR THE STUDY OF DECOMPRESSION SICKNESS

S. Daniels, W. D. M. Paton and E. B. Smith

This study is primarily concerned with the effects that may be observed after decompression from atmospheres containing sulphur hexafluoride, although comparative experiments were conducted with carbon tetrafluoride (CF_4), nitrous oxide (N_2O), hexafluoroethane (C_2F_6) and nitrogen. The use of these exotic gases is important for the eventual understanding of the mechanism of decompression sickness because, though their use in diving is not recommended, they do produce unusual symptoms, and they possess a range of physical properties greater than those of the conventional diving gases, nitrogen and helium.

Sulphur hexafluoride was chosen primarily because of two unusual observations made some years ago (3). First, on decompression from an exposure to SF_6, bubble formation was scanty even when death occurred. Second, a white or pink foam was observed in the mouths of some animals after such decompression. This foam seemed to be the direct cause of death in some instances; the animals had apparently become anoxic.

Two factors have therefore been studied: low overall bubble formation and anoxic death due to the foam.

Lethality and Microscopic Examination of Decompression Sickness after Exposure to Raised Pressures of Sulphur Hexafluoride

A standard LD_{50} determination was carried out with SF_6 using groups of five male albino CD1 mice. A 90-min exposure to various pressures of SF_6 was given, followed by a rapid (15 sec) decompression and a 30-min interval in 1 ATA pure oxygen. During the postdecompression period in oxygen, the mice were under close observation. Compression was achieved in 1 min, a process which had previously been shown to produce no detectable ill effects (3). Four groups of 5 mice at each pressure were deemed sufficient for statistical accuracy. A comparative LD_{50} determination was performed using nitrogen. Probit analysis on the Oxford University I.C.L. 1906A computer gave an LD_{50} for SF_6 of 71.5 $\pm$ 3.5 psi, and an LD_{50} for N_2 of 203 $\pm$ 3.5 psi ($\pm$ Finney's SD).

The mice were examined after decompression using an Olympus dissecting microscope with a maximum magnification of 80. The bubble formation found after SF_6 dives differed significantly from that found after dives with N_2. Table I gives the analysis.

The overall incidence of bubble formation after SF_6 decompression was very low. If only those animals that died as a result of decompression are considered, the difference between

TABLE I

ANALYSIS OF BUBBLE FORMATION AFTER SF_6 AND N_2 EXPOSURES

	SF_6 Dives (LD_{50} = 71 psi)			N_2 Dives (LD_{50} = 203 psi)			
Pressure, % of LD	Extravascular Bubbles, %	Intravascular Bubbles, %	Incidence of Death, %	Pressure, % LD	Extravascular Bubbles, %	Intravascular Bubbles	Incidence of Death, %
—	—	—	—	74	100	100	0
63	0	0	0	79	100	100	6
70	5	0	0	89	100	100	7
84	15	10	15	98	100	100	38
98	28	15	45	108	100	100	53
—	—			113	100	100	58

nitrogen decompression and SF_6 decompression becomes more clearly marked. Table II gives the analysis for the mice that died as a result of decompression with SF_6.

Of the 12 mice that died as a result of decompression with SF_6, 7 showed a complete absence of detectable intravascular bubbles and 3 mice showed no visible bubble formation. This compares with the N_2 dives, where in every case of mice dying from decompression sickness, both extra- and intravascular bubble formation was extensive.

Comparable experiments performed with CF_4 and N_2O showed that bubble formation in CF_4 dives was similar in every respect to that in N_2 dives; with N_2O dives, bubble formation was extensive, and has been described as "fulminating decompression sickness (3)."

This pattern of very low incidence of intravascular bubble formation was altered when the mice were exposed to 70 psi SF_6 for various periods (10, 20, 40, and 60 min), rapidly decompressed, and killed immediately after decompression without further exposure to O_2. All the mice were observed to have very small (20-300 μ diameter) discrete bubbles in the inferior vena cava, renal vein, pulmonary vein, and right auricle. No extravascular or peripheral intravascular bubble formation was seen. These bubbles were not observed to grow or to coalesce with other bubbles over a period of 30 min, even when the vessel containing them was manipulated.

TABLE II

ANALYSIS OF BUBBLE FORMATION IN MICE DYING AS A RESULT OF DECOMPRESSION WITH SF_6

Pressure, psi	Extravascular Bubbles	Intravascular Bubbles
45	no deaths	—
50	no deaths	—
60	3/3 (100%)	2/3 (66.6%)
70	6/7 (85%)	3/9 (33.3%)
Total incidence in total cases	9/12 (75%)	5/12 (41.7%)

Values are number of animals showing bubble formation and total number of animals in group.

It is possible that the small size and stability of these bubbles could be due to a surfactant skin and that they originate from the lungs through a rupture and are released directly into the pulmonary vein and then into the circulation.

Pulmonary Edema and Anoxic Death

After SF_6 and N_2 exposures, the lungs of the mice were removed, weighed wet, freeze-dried, and weighed dry. The freeze-drying was done by immersing the lungs in an acetone/solid CO_2 bath (-75 °C) and then desiccating in vacuo for 24 hours with KOH and 24 hours with P_2O_5. The dry/wet ratio of lung weight was used as a measure of pulmonary edema. A group of 21 mice that had not been exposed to pressure was used as the control.

Statistical analysis of the results showed that the dry/wet ratios for N_2 exposure did not differ significantly from that of the controls. The dry/wet ratios after SF_6 dives, however, were significantly different from the controls (0.1% significance level) and from the N_2 values (0.1% significance level). Further, the edema that developed was found to be dependent on the exposure pressure (Figs. 1*A* and *B*).

The incidence of foam observed in the trachea and lungs correlates with the dry/wet lung weight ratios. At 70 psi, 80% of the mice had visible foam in the lungs and trachea, and at 45 psi, 10% of the mice had visible foam. Foam was never observed in the lungs or trachea of mice after N_2 dives. The failure of the lungs to collapse after exposure to SF_6 indicated that the compliance of the lungs had been destroyed.

The mean weight of fluid appearing in affected lungs (calculated from the change in dry/wet ratios and lung weight) was 0.28 ± 0.02 g. This is approximately 1% of the body weight and, in a 75-kg man, would correspond to 1½ pints of fluid in the lungs.

Comparable experiments with CF_4 and N_2O were performed and the results are shown in Fig. 1, *C* and *D*.

For CF_4, the number of cases showing visible foam is shown in Table III.

With N_2O, foam was only observed on three occasions. An exploratory experiment with C_2F_6 showed that at 70 psi the lung ratio was significantly lower than the control value, indicating a high degree of pulmonary edema. Furthermore, all the animals died after decompression and no intravascular bubbles were observed. Foam was observed in the lungs and trachea of all the animals.

The question of how long an exposure to SF_6 had to be to produce these effects was studied in two different experiments. The first involved a series of exposures to SF_6 at 70 psi for 10, 20, 40, and 60 min, followed by rapid decompression and a 30-min interval of pure O_2 at 1 ATA. The second involved the same exposures, but the mice were killed immediately after decompression to examine the time course of the edema. The dry/wet lung weight ratios for the two experiments are shown in Fig. 2.

In both these experiments the incidence of visible foam was the same. This indicates that the foam itself requires very small amounts of fluid to form, and that it is damage to the structure of the lung, removal of the surfactant lining layer, which leads to gross edema formation. The accumulation of fluid in the lungs takes time to develop, whereas the formation of foam follows directly on the decompression. The fact that even after a 10-min exposure there was a measurable degree of pulmonary edema and also a positive incidence of foam indicates that

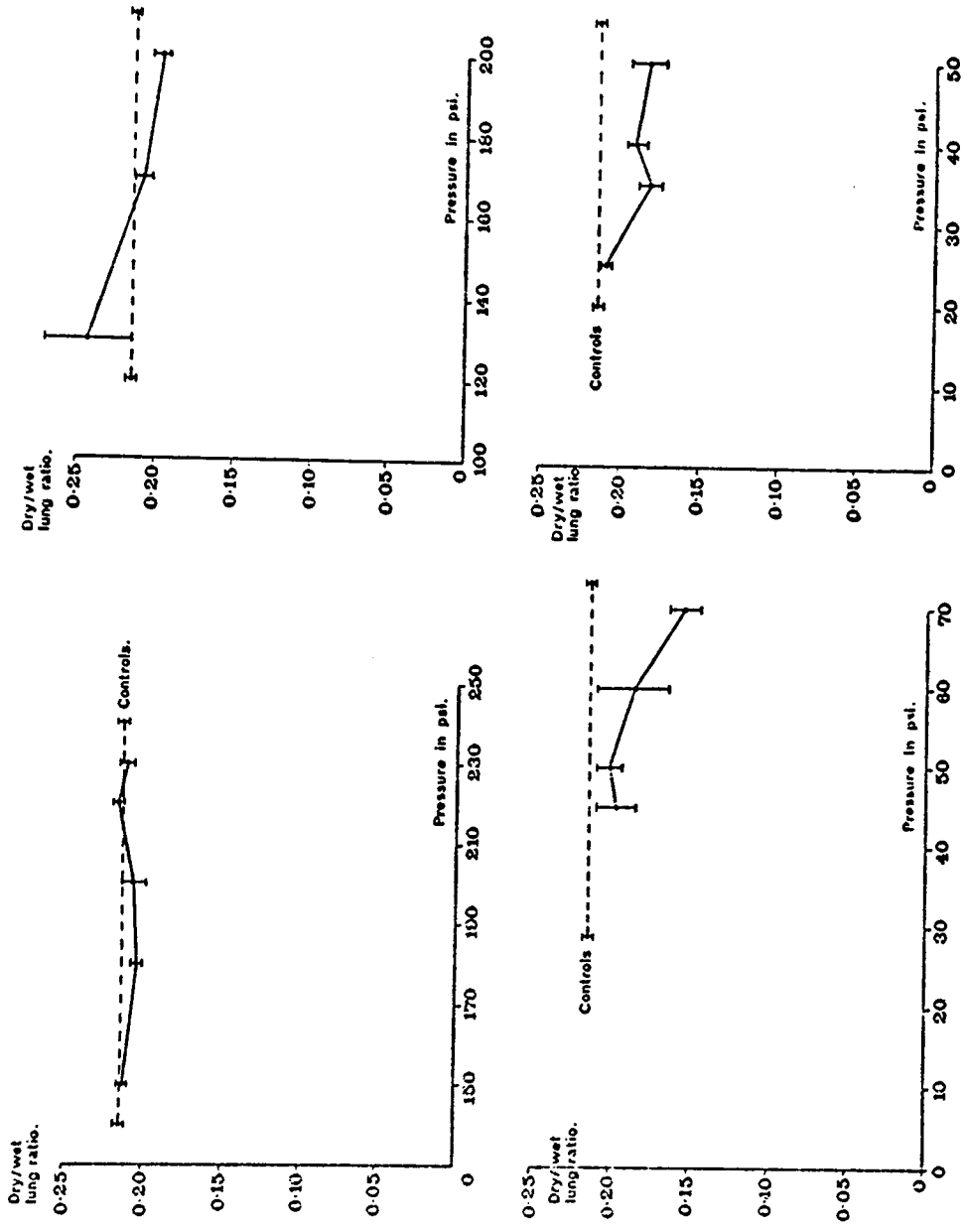

Fig. 1. Dry/wet lung ratios after decompression from raised pressures of nitrogen (*upper left panel*), sulphur hexafluoride (*lower left panel*), carbon tetrafluoride (*upper right panel*), and nitrous oxide (*lower right panel*).

TABLE III

NUMBER OF CF_4 CASES SHOWING VISIBLE FOAM

Pressure, psi	Visible Foam, % of Cases
130	10
170	10
200	20

the mechanism by which SF_6 removes surfactant from the lining layer of the lungs is relatively rapid.

Figure 3 shows the combined incidence of visible foam, death, and intravascular bubbles as a function of time of exposure to 70 psi SF_6. The interesting feature in this figure is the discontinuity in the plot of the incidence of death. At first the incidence of death increases sharply with increasing time of exposure, as does the incidence of visible foam. However, the incidence of visible foam quickly reaches a maximum value, while the incidence of death remains con-

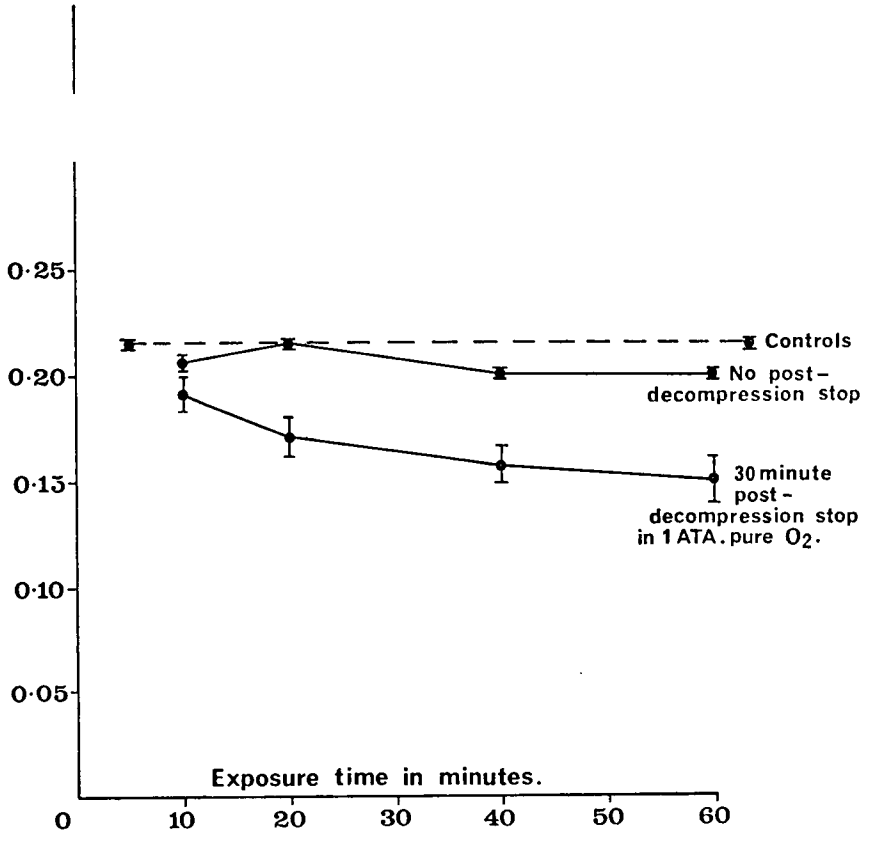

Fig. 2. Dry/wet lung ratios after decompression from sulphur hexafluoride at 70 psi.

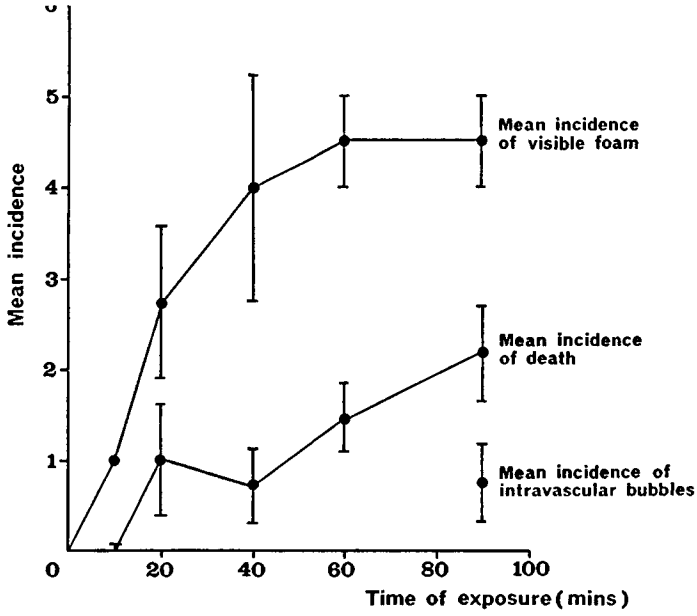

Fig. 3. Incidence of foam, death, and intravascular bubbles after exposure to sulphur hexafluoride at 70 psi followed by 30 min in 100% O_2 at 1 ATA.

stant against increasing time of exposure before beginning to increase again (Fig. 3). The second increase in the incidence of death is much slower than the first (Fig. 3). This would seem to indicate a dual mechanism for death; initially, death is linked to the production of foam in the lungs, and is caused by anoxia. This happens after a short exposure, but the incidence rapidly reaches a plateau. The second mechansim of death may be classical decompression sickness, which requires a longer exposure and reaches a maximum incidence with saturation. This fits with the fact that the rate of uptake of SF_6 is very slow, approximately 25 times slower than the rate of uptake of nitrogen.

The possibility that the lung damage is caused or contributed to by the 30-min period in 1 ATA pure O_2 was investigated in two control experiments. In the first, 10 mice were kept in 1 ATA pure O_2 for 1½ hours, killed, and their dry/wet lung weight ratios were then determined. The second experiment involved keeping 8 mice in 1 ATA pure O_2 for 5 hr and 20 min, and then killing them and measuring their dry/wet lung weight ratios. These experimental values are shown in Table IV. There was no difference between the controls and the mice kept in 1 ATA pure O_2.

It was thought possible that while 1 ATA of pure O_2 did not cause any observable lung damage, the situation might be different after decompression. A series of experiments was therefore performed in which groups of 5 mice were exposed to 70 psi SF_6 (P_{O_2} = 15 psi) for 40 min, rapidly decompressed, and then allowed 30 min in air at atmospheric pressure instead of the usual 30 min in O_2 at atmospheric pressure. Comparing these results with those of the 40-min exposure to 70 psi SF_6 group, in which the mice were decompressed into O_2, showed that the incidence of death was much higher, 60% compared to 15%; the incidence of visible foam was similar, 75% compared to 80%; and the extent of pulmonary edema as measured by dry/wet

TABLE IV

DRY/WET LUNG WEIGHT RATIOS AFTER EXPOSURE TO 100% O_2 AT 1 ATA

	Control Group	1.5-hr Exposure Group	5.3-hr Exposure Group
Ratio	0.2147	0.2192	0.2138
SD	±0.003	±0.002	±0.002
n	21	10	8

lung weight ratio was lower, 0.1988 compared to 0.1588. However, the mice that died did so within 2 min after decompression, and they exhibited the symptoms of anoxic death: convulsions, gasping for breath, and a visible blue tinge to the ears. In all the mice that died, foam was observed. Therefore, far from having a damaging effect, the 30 min in O_2 provided protection from anoxic death, and the measured decrease in the extent of pulmonary edema merely reflected the fact that 60% of the animals died before a significant quantity of fluid accumulated in their lungs.

In light of other known causes of pulmonary edema, the possibilities accounting for the foam were listed: (1) neurogenic (vagal) actions (1); (2) vasodilation; or (3) an inflammatory response. These possibilities were evaluated by testing the effect of pretreatment with atropine, ephedrine or hydrocortisone. The drugs were all administered intraperitoneally 15 min prior to compression. Incidence of death and dry/wet lung weight ratios were recorded after decompression. No difference was found in the dry/wet lung weight ratios. The only difference observed was that the incidence of death among the atropine- and ephedrine-treated mice was higher.

The action of SF_6, therefore, appears to depend on some direct physical interaction. Whether some cardiovascular mechanism was involved that required an intact circulation was explored. This was done by performing a series of in vitro experiments using solutions of volatile anesthetics injected into the excised lungs of guinea pigs. The volatile anesthetics were chosen because they possess high fat but low water solubility, one of the physical characteristics of SF_6. Saturated solutions of halothane, chloroform, and ether in saline all reproduced the loss of compliance and the production of a large amount of foam that characterized decompression with SF_6. The ability of anesthetic solutions to reproduce this effect in vitro decreases the likelihood that a primary circulatory mechanism is responsible for the production of foam by SF_6.

The next series of experiments was designed to elucidate the part played by decompression. In these experiments, SF_6 exposure and decompression were separated in time. In the first, mice were compressed to 70 psi with SF_6 and maintained for 40 min. This would cause foam and edema if followed by rapid decompression. Instead, the SF_6 was exchanged for air at 70 psi for one hour more. This was followed by rapid decompression, and the dry/wet lung weight ratios were determined, along with the incidence of death and visible foam. Two experiments were performed: in the first, there was a 30-min period in 1 ATA pure O_2 after decompression; in the second, the mice were killed immediately after decompression. Another experiment designed to explore the relative roles of exposure and decompression was also performed. In this case the mice were given a 40-min exposure to 70 psi SF_6 (P_{O_2} 15 psi); after 40 min the pres-

sure was increased to 230 psi with He and maintained for 10 min. Pressure was then quickly reduced to 70 psi and maintained for a further 30 min, followed by final decompression. The mice were then immediately killed. Figure 4 shows the results of these experiments.

The dry/wet lung weight ratios for the two groups receiving a recovery period in air prior to decompression (Fig. 4*C* and *D*) are not significantly different from each other, nor from those of the group in which mice were given a 40-min exposure to SF_6 at 70 psi, decompressed and killed (Fig. 4*B*), or the control group's value. However, the incidence of visible foam in those groups which received a recovery period (Fig. 4*C, D*) is significantly lower than in the group that was decompressed from 70 psi of SF_6 and killed immediately (Fig. 4*B*). This would indicate that when SF_6 is present, decompression is an important factor in the production of foam. This is also supported by the finding that if He exposure is superimposed on the SF_6 exposure, significant pulmonary edema occurs (Fig. 4*E*).

Further evidence supporting the hypothesis that decompression is important in the occurrence of lung damage is provided by decompressing the mice slowly. Groups of 5 mice were given a 40-min exposure to 70 psi SF_6 and then decompressed at 10 psi per minute to atmospheric pressure. They were then left in O_2 at atmospheric pressure for 30 min. Comparing this to results from groups exposed similarly but decompressed rapidly provides clear evidence of the importance of decompression. When the mice were decompressed slowly, the incidence of death was 0 compared to 15%; the incidence of visible foam was 10% compared to 80%, and the lung weight ratios were 0.2072 compared to 0.1588. By decompressing slowly, the extent of pulmonary damage was greatly reduced.

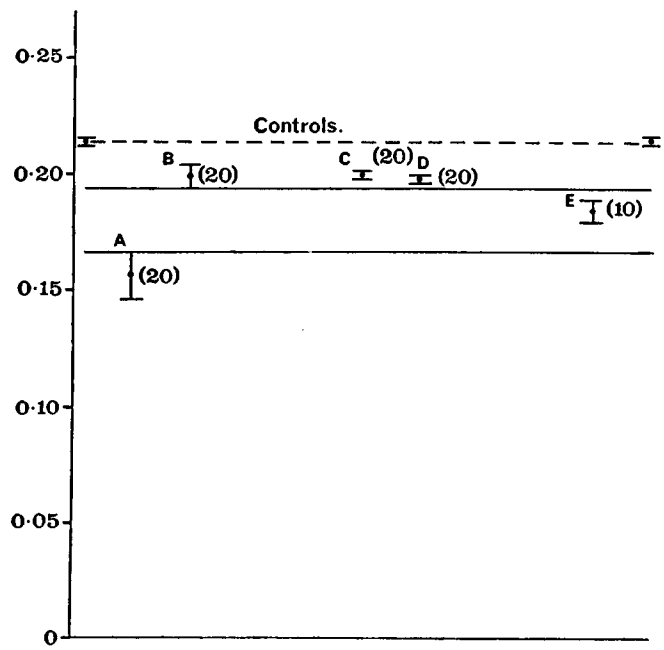

Fig. 4. Results of decompression experiments. A = 40 min at 70 psi SF_6 → rapid decompression → 30 min 1 ATA O_2; B = 40 min at 70 psi SF_6 → rapid decompression; C = 40 min at 70 psi SF_6 → 60 min at 70 psi air → rapid decompression; D = 40 min at 70 psi SF_6 → 60 min at 70 psi air → rapid decompression → 30 min 1 ATA O_2; E = 40 min at 70 psi SF_6 → He to 230 psi for 10 min → decompression to 70 psi for 30 min → rapid decompression.

Conclusions

The degree of pulmonary edema produced by SF_6 depends on the exposure pressure; the time of exposure necessary is very much less than that required for saturation, as estimated from the incidence of death plotted against time extrapolated to give a constant incidence of death. Edema and production of foam, while related, are actually distinct from each other in occurrence. Thirty minutes in O_2 at atmospheric pressure is required to allow time for edema fluid to build up in the lungs; the foam, however, is produced upon decompression and requires very little fluid for formation.

The exact composition of this foam is unknown, but it is comprised largely of surfactant material, e.g., di-palmitoyl lecithin (2, 7). This was deduced from observations of the high stability of the foam for long periods of time, its resistance to powerful antifoams (4, 5) (except in the presence of proteolytic enzymes) (6) and the similarity of the infrared spectrum of lung foam to that of synthetic di-palmitoyl lecithin.

The experiments performed suggest that SF_6 causes some dissociation of surfactant from the alveolar surface, that this mechanism is physical, and that decompression is itself important in the final effects of exposure to SF_6. A common factor between SF_6 and the volatile anesthetics halothane, ether, and chloroform, which have been shown to produce the same effect in vitro, is a high fat solubility and a lower water solubility. This, coupled with large molecular size, may be sufficient to produce a degree of local mechanical stress at the membrane level which releases surfactant. The act of decompression would aggravate an already stressed situation, either by causing a large flux of gas molecules out of the alveoli, or by causing transient distension (due to gas expansion) of the alveoli.

Although such gross effects have never been observed in dives with nitrogen or helium, mechanisms of this sort may contribute to the lesser respiratory pathology recorded after these dives. Also, the effects of decompression on the surfactant system might be important if the ability of anesthetics to antagonize the effects of pressure were exploited by using anesthetics more potent than nitrogen which have a larger molecular size and higher fat solubility.

Acknowledgment

Thanks are due to D. H. Elliott for suggesting the use of dry/wet lung weight ratios as a quantitative measure of pulmonary edema.

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DECOMPRESSION SICKNESS IN RATS AND MICE RAPIDLY DECOMPRESSED AFTER BREATHING VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

T. E. Berghage, L. J. Keating and J. M. Woolley

It is well documented that the breathing of increased percentages of carbon dioxide can greatly affect the respiratory cycle and alter the time course of gas exchange. It therefore seems reasonable to expect that such changes would also alter decompression requirements. Behnke's (1) now-famous chapter in the book *Decompression Sickness* states that "With respect to work in compressed air, empirical evidence points to a higher incidence of bends in association with a rise in the carbon dioxide level." This position, taken by Behnke in 1951, is still widely held today.

The 1970 version of the *U.S. Navy Diving Manual* (19) states that "Excess carbon dioxide breathed during a dive is...believed to increase the likelihood of decompression sickness, but the reasons for the increase are unclear." The 1973 version of the *Manual* (20) has softened this position somewhat. It states that "Breathing an excess of carbon dioxide may increase the possibility of decompression sickness...." If one does not accept these statements at face value and goes into the research literature to find confirmation, the problem worsens.

Physical models of bubble formation and growth have all shown that carbon dioxide (CO_2) infuses readily into existing bubbles because of its high solubility. A classic demonstration of this has been reported by Harvey (10). A physical model was constructed which consisted of layers of water alternately saturated with air and CO_2, each at a pressure of 1 ATA. Small bubbles moving from the air-saturated stratum rapidly increased in size as they reached the CO_2-saturated stratum and decreased in size as rapidly as they passed into a new region of air-saturated water.

Dean (5), reporting on several physical studies of bubble growth, concluded that "since the diffusion coefficients of most gases are so nearly equal, the rate at which gas enters a bubble will be controlled largely by the amount of gas close to the bubble, that is, by the absolute solubility of the gas."

The solubilities of several of the diving gases in 100 parts of water at 0°C are:

Carbon dioxide	179.70
Helium	1.49
Hydrogen	2.15
Nitrogen	2.35
Neon	1.14
Oxygen	4.89

From these figures it is easy to see why researchers have readily accepted the idea that increased CO_2 levels increase the incidence of decompression sickness. When, however, one moves from a simple physical concept to a physiological model, the results are not nearly as clean and precise.

Experimental results of studies using animals and humans can be interpreted to support almost any position on the effects of CO_2. The earliest reference these authors were able to find on the relationship between CO_2 and the outcome of decompression occurred in a report by Snell (18) in 1896. Working as a physician during the excavating for the Blackwall Tunnel in England, Snell kept careful records of the daily environmental fluctuations and related these to the incidence of decompression sickness. During the time the workers were at 2.36 ATA, Snell observed that the incidence of decompression sickness was related to the ventilation rate of the tunnel (Table I). He suggested that the CO_2 accumulation in the tunnel was responsible for the increased incidence.

In 1942, Margaria et al. (14) found that the administration of 12% CO_2 in air actually facilitated decompression in ducks. Breathing CO_2 in air allowed the ducks to almost double their resistance to decompression sickness after a 4-hr hyperbaric exposure. Decompressing ducks from 3.5 to 1 ATA in air produced the same incidence of decompression sickness as decompressing them from 5 to 1 ATA in a mixture of 12% CO_2 in air. These investigators concluded that the stimulation to respiration and circulation caused by the increased percentages of CO_2 actually facilitated the decompression.

In 1944, Harris et al. (9) reported placing bullfrogs in an atmosphere of 60-70% CO_2 (balance air) for 2-3 hr, which they tolerated very well. After this very high CO_2 exposure, the frogs were decompressed to 60,000 ft. Bubble formation occurred in a majority of the CO_2-treated animals, while only 3 out of 18 frogs in the control group showed bubbles. The researchers reported that "high concentrations of CO_2 are required to produce this effect"; experiments using only 25% CO_2 showed only slight, nonstatistically significant effects (Table II). Harris et al. (9) also reported an interaction between muscular activity and CO_2 in producing decompression sickness. They suggested that local high concentrations of CO_2 in active muscle interact to produce bubble nuclei.

TABLE I

RELATIONSHIP BETWEEN INCIDENCE OF DECOMPRESSION SICKNESS AND VENTILATION RATE DURING BUILDING OF THE BLACKWALL TUNNEL (ENGLAND)

Ventilation Rate, $ft^3/min/hr$	Incidence of Decompression Sickness, cases/100 days
Below 4000	28.5
4000 to 8000	19.1
8000 to 12,000	11.2
Above 12,000	0

From Snell, E. H. Compressed Air Illness. London: H. K. Lewis (18). Courtesy of H. K. Lewis.

TABLE II

BUBBLE FORMATION IN DECOMPRESSED FROGS PREVIOUSLY TREATED WITH CO_2 COMPARED WITH UNTREATED CONTROLS DECOMPRESSED SIMILARLY

Pretreatment, % CO_2	Altitude, ft	Activity	Treated Animals		Controls Decompressed Similarly Without CO_2 Pretreatment	
			Bubbles Present	No Bubbles	Bubbles Present	No Bubbles
60-70	60,000	None (urethanized)	6	3	3	15
60-70	50,000	None (2 urethanized)	0	3	0	2
60-70	50,000	Slight (spontaneous)	6	0	0	7
60-70	15,000	Violent (stimulated)	2	3	0	5
25	50,000	Slight (spontaneous)	1	3	0	5
25	50,000	Moderate	2	0	7	0

Experimental animals placed in CO_2 mixtures for 1.5 to 3.5 hr before decompression; duration of decompression 2 to 10 min.

From Harris, M., W. E. Berg, D. M. Whitaker, V. C. Twitty and L. R. Blinks. Carbon dioxide as a facilitating agent in the initiation and growth of bubbles in animals decompressed to simulated altitudes. *J. Gen. Physiol.* **28:** 225-240, 1945 (9). Courtesy *J. Gen. Physiol.*

In 1945 two apparently well-controlled studies of the relationship between CO_2 and decompression sickness were conducted. Both studies were done in altitude chambers using aviation students. The first study, conducted by Gray et al. (8), involved 408 male subjects. The chamber flight consisted of a 4000 ft/min ascent to 38,000 ft, followed by a 2-hr exposure to the latter altitude. The subjects used demand oxygen (O_2) equipment with pure oxygen for the initial decompression. Immediately upon reaching 38,000 ft, one-half of the subjects on each flight changed from pure oxygen to a CO_2-in-O_2 mixture. The percentage of CO_2 varied in the different flights; the average concentration was 12%, and in the majority of cases, it was between 10 and 15%. The average CO_2 tension in the inspired mixture was 19 mmHg, which is the sea-level equivalent of 2.5%. The incidence of decompression sickness for this study is shown in Table III; there are no significant differences between the two groups.

TABLE III

EFFECTS OF ADDING 12% CO_2 TO O_2 AT ALTITUDE

	≈ 12% CO_2		100% O_2	
	n	%	*n*	%
Total number of subjects	204	100	204	100
Total symptoms of decompression sickness	90	44.1	96	47.0
Total descents	43	21.0	44	21.6

Adapted from Gray et al. (8).

The second study was done by Hodes and Larrabee (11, 12). As with the Gray et al. (8) study, the experimental subjects were taken to 38,000 ft in an altitude chamber. Although not directly stated, it appears that 11 subjects were used in the study; each subject made more than one ascent. Ascents were made on pure oxygen at the rate of 3000 ft/min. Five minutes after reaching 38,000 ft and every 10 min thereafter, the subjects did three deep knee-bends and three arm exercises. The subjects remained at altitude for 2 hr, or until forced to descend due to decompression sickness. Just prior to ascent, each subject sat quietly for at least 15 min. At the end of this rest period, one or two end-tidal alveolar gas samples were taken. The relationship between the alveolar CO_2 levels and the incidence of decompression sickness is shown in Fig. 1. The results are quite impressive ($r = 0.96$) and certainly cannot be ignored, but measures of end-tidal CO_2 are notoriously unstable and can be altered almost at will. Any degree of anxiety or hyperventilation will tend to reduce the end-tidal CO_2.

Smedal and Graybiel (17) also attempted to correlate alveolar CO_2 tension with decompression sickness susceptibility. From a total subject population of 35 aviators who had undergone repeated altitude exposure, 3 subjects were selected who had few symptoms and 3 who had displayed many symptoms. The mean alveolar CO_2 tension for the group with many symptoms was 39.9 mmHg. The group with few symptoms had a mean of 36.4 mmHg. The mean difference of 3.51, divided by the SE, gave a critical ratio of 0.865, which is nonsignificant ($P < 0.44$).

Catchpole and Gersh (4) decompressed rabbits to an altitude of 45,000 ft in 6 min to study the effects of various factors on bubble formation. One of the factors they studied was the effect

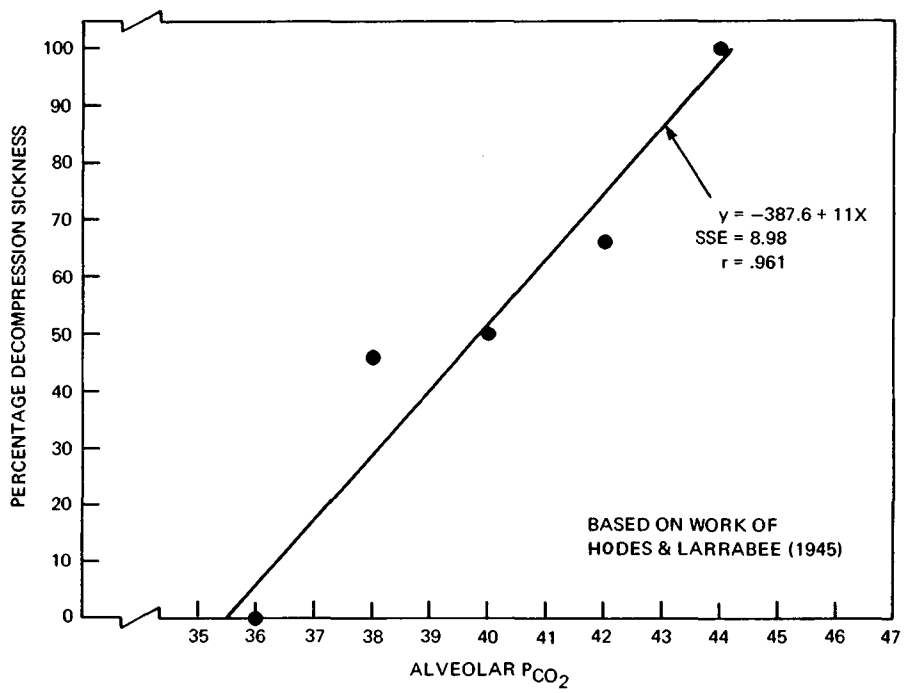

Fig. 1. Relationship between pre-altitude exposure, end-tidal CO_2 measures, and incidence of decompression sickness.

of CO_2 concentration. They used three conditions: pure oxygen, 5% CO_2 in oxygen, and 10% CO_2 in oxygen. The results they obtained are shown in Table IV. These researchers acknowledged that there were no differences in the mortality of rabbits exposed to CO_2, but they indicated that in 3 of the 12 animals breathing CO_2, there were greater bubble intensities than in the control group. This may be a valid observation, but the results are not statistically significant ($P < 0.29$). In other words, their bubble intensity results could very well be due to chance alone. All indications in this study are that the CO_2 produced no changes in decompression outcome.

The final studies in our literature review were done by Philp (16) and Gowdey and Philp (7). These investigators exposed rats to 5.42 ATA for 2 hr, then decompressed them to the surface. After spending either 2-5 min or 5-15 min at 1 ATA, the rats were further decompressed to an altitude of 10,000 ft, where they were observed and evaluated for signs of decompression sickness. Results of these exposures are shown in Table V. The increase in incidence associated with the level of CO_2 is statistically significant ($P < 0.05$ and $P < 0.002$) for both surface intervals.

What conclusions can be drawn from the results of these many studies? As can be seen from Table VI, six of the eight studies reviewed have results based on observations made at pressures less than one atmosphere. There is nothing wrong with gathering information on the effects of CO_2 in the hypobaric environment as long as one does not try to generalize it beyond this realm. Researchers have long recognized that decompression sickness occurring at altitude is qualitatively different from that occurring during high-pressure exposure (6). As

TABLE IV

INCIDENCE OF DEATH AND BUBBLE INTENSITY AMONG RABBITS DECOMPRESSED TO AN ALTITUDE OF 45,000 FT

Conditions	Decompression Outcome		Bubble Intensity		
	Dead	Survived	Heavy	Light	Absent
Pure O_2	5	1	0	4	2
5% CO_2 in O_2	5	1	1	4	1
10% CO_2 in O_2	4	2	2	1	3

Values are number of animals. Adapted from Catchpole and Gersh (4).

TABLE V

INCIDENCE OF DECOMPRESSION SICKNESS ASSOCIATED WITH TWO LEVELS OF CO_2

CO_2 Exposure Level	Effects of Exposure at 1 ATA			
	2-5 min		5-15 min	
	No Signs	DS	No Signs	DS
< 0.8% CO_2	8	0	8	4
≈ 4.5% CO_2	14	13	1	17

DS = decompression sickness. Adapted from Gowdey and Philp (7).

TABLE VI

SUMMARY OF EFFECTS OF CO_2 ON DECOMPRESSION OUTCOME REPORTED IN EIGHT STUDIES

Study	Year	Subjects	Atmospheric Conditions		Results
			Altitude	Surface	
Snell (18)	1896	human		X	adverse
Margaria et al. (14)	1942	ducks		X	facilitate
Gray et al. (8)	1945	human	X		no difference
Harris et al. (9)	1945	frogs	X		adverse
Hodes and Larabee (11)	1945	human	X		adverse
Catchpole and Gersh (4)	1946	rabbits	X		no difference
Smedal and Graybiel (17)	1947	human	X		no difference
Philp (16)	1964	rats	X		adverse

shown in Fig. 2, the alveolar partial pressures at altitude are greatly altered; CO_2 makes up a greater proportion of the total alveolar gas present. The mole concentration of CO_2 may well be important when one talks of narcosis, but in terms of decompression sickness, the proportion of the various gases takes on greater significance.

If the review of CO_2 studies is restricted only to those studies involving hyperbaric exposures with observations at pressures of 1 ATA or more, only two studies remain, and these have completely opposite results. In summary, for hyperbaric exposures with a return to normobaric pressure, we have little or no knowledge of the effects of non-narcotic levels of CO_2. If one considers the observations made at altitude, the picture becomes a little better, but it is still far from clear.

With this literature review as background, the experiments reported in this paper were designed to define the relationship between inspired CO_2 level and decompression outcome.

Methods and Procedure

EXPERIMENTAL DESIGN

Initially, a single study using mice was planned with an augmented 3 × 5 factorial experimental design. Three pressure levels (12, 14, and 16 ATA) were combined with 5 CO_2 levels (3.0, 9.1, 15.2, 30.4, and 45.6 mmHg) to form the 15 cells for the basic experimental design. This basic design was then augmented with two additional experimental conditions to allow the investigators to assess the effects of no exercise. The two no-exercise conditions were run at 12 ATA with 15.2 and 45.6 mmHg CO_2, respectively.

After this initial study using mice, it was decided that the results needed to be replicated using a larger animal. A 2 × 4 factorial design was selected in which rats were exposed to two different inert gases (nitrogen-oxygen (N_2) and helium-oxygen (He)) and 4 levels of CO_2 (3.8, 7.6, 15.2, and 30.4 mmHg). For the nitrogen exposures, one additional level of CO_2 (22.8 mmHg) was added after the fact to double-check the results obtained during the running of the basic design.

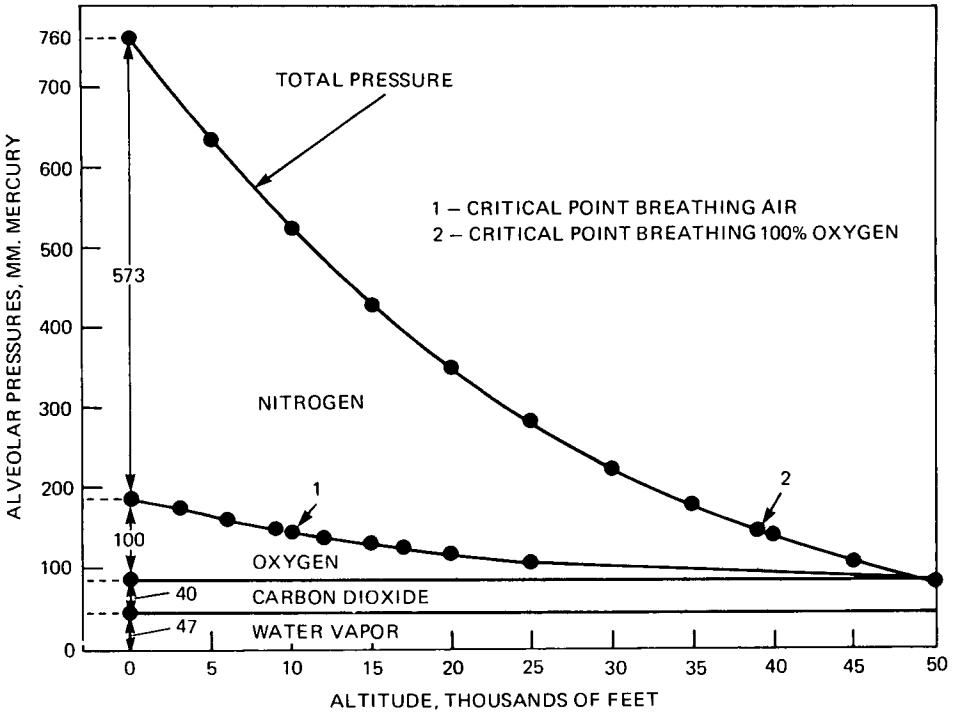

Fig. 2. Relation of altitude above sea level to barometric pressure and partial pressures of gases in alveoli. From Ferris, E. B., Jr., and G. L. Engel. The clinical nature of high altitude decompression sickness. *Decompression Sickness. Caisson Sickness, Diver's and Flier's Bends and Related Syndromes.* Fulton, J. F., (ed.) Philadelphia: W. B. Saunders Co., 1951 (6).

All pressure exposures in these two studies were made in a Bethlehem Model 1836 10-HP chamber, which has a volume of approximately 170 liters. The chamber atmosphere was monitored with a Beckman Model F-3 paramagnetic oxygen analyzer and the oxygen partial pressure was maintained at 0.51 ATA at the exposure depth. Carbon dioxide levels in the chamber were monitored with a Beckman IR 315 analyzer and kept within ±2 mmHg of the desired level. Chamber pressure was monitored with a 0- to 2000-fsw Heise gauge and maintained within ±2 fsw of the specified pressure. The temperature in the chamber during the experiment was kept at 30 ± 2°C.

Mouse Study

Eighty-five chamber dives were made in Phase 1 of the study. During each pressure exposure, eight mice were exercised at a rate of 5 rpm (3.19 m/min) in an 8-inch diameter rotating cage. In all, 680 mice were used in the study. The mice were a healthy, homogeneous strain from the Naval Medical Research Institute animal colony. They weighed between 18 and 22 g.

The experimental procedure involved compressing to 1.3 ATA with pure oxygen to establish the oxygen partial pressure at 0.51 ATA (388 mmHg). Carbon dioxide was then added to

achieve the desired dose level of 3.0, 9.1, 15.2, 30.4, or 45.6 mmHg (0.4, 1.2, 2, 4, or 6% surface equivalent). Subsequent compression to the exposure pressure (12, 14, or 16 ATA) was with pure nitrogen. The mice were kept at the exposure pressure for 15 min. The chamber was then ventilated for less than 30 sec with oxygen to raise the oxygen percentage above 15% for surfacing. As soon as the ventilation was secured, the chamber was immediately decompressed to the surface at the rate of 0.68 ± 0.1 atm/sec. After the decompression, each animal was observed for 5 min. At the end of that period, each animal's behavior was scaled as follows: 1, normal walk; 2, paralysis or convulsions; and 3, tumbling in the cage.

Rat Study

Forty-four chamber dives were made during Phase 2 of the study. During each pressure exposure, five rats were exercised at a rate of 5 rpm (3.33 m/min) in an 8¾-inch diameter rotating cage. A total of 220 rats were used in the study. They were healthy, male, Sprague-Dawley-derived albino rats from the Naval Medical Research Institute animal colony. They weighed between 200 and 300 g.

All of the pressure exposures were made to a depth equivalent to 180 fsw (6.45 ATA). Because two different inert gases were used in this phase of the study, two different experimental procedures had to be used.

Procedure for use with nitrogen

1. Compress to 10 fsw (1.3 ATA) with O_2.
2. Add CO_2 to give the proper surface equivalent percentage.
3. Compress to 6.45 ATA with N_2.
4. Remain at the maximum pressure for 30 min.
5. Ventilate the chamber with O_2 in the last 60 sec of the "bottom time." The O_2 content was increased to between 15 and 20%.
6. Decompress to the surface in 16 sec and observe the rats for 15 min. The manifest symptoms were scaled just as they were for the mice in Phase 1.

Procedure for use with helium

1. Ventilate the chamber for 5 min with O_2 to remove the 0.79 ATA of N_2.
2. Compress to 3 ATA with helium.
3. Ventilate the chamber with helium until the O_2 is 17% of the volume.
4. Add CO_2 to give the proper surface equivalent percentage.
5. Compress to 6.45 ATA with helium.
6. Remain at the maximum pressure for 10 min.
7. Ventilate the chamber with O_2 in the last 60 sec of the "bottom time." The oxygen content was increased to between 15 and 20%.
8. Decompress to the surface in 16 sec and observe the rats for 15 min. The manifest symptoms were scaled just as they were for the mice in Phase 1.

Results

The results of Phase 1 of this study (Fig. 3) demonstrate that the incidence of decompression sickness can be shifted depending on the concentration of CO_2 in the breathing media. Results indicate that for the mouse, the maximum effect of CO_2 is reached at a dose of about 15.2 mmHg (2% surface equivalent). From that point on, no additional increase in the incidence of decompression sickness is noted. Previous studies of mice by Berghage et al. (2, 3) have shown that a 15-min exposure to 13.8 ATA with a subsequent rapid decompression to the surface will produce decompression sickness in 50% of the animals. Present results show that this ED_{50} (effective dose sufficient to produce symptoms in 50% of the animals) point is subject to change, depending on the concentration of CO_2. Negative results were obtained in the two additional experimental conditions run at 12 ATA to determine if exercise was an additional factor to be considered. There was no difference in the incidence of decompression sickness between those mice that exercised and those that remained relatively inactive.

The results of Phase 2 of this study are shown in Fig. 4A and B. The effect of CO_2 on the incidence of decompression sickness in rats is not nearly as clean and precise as that for mice. The results, however, do show a statistically significant rise in decompression sickness when the animals breathed elevated levels of CO_2 in either $He-O_2$ or N_2-O_2 mixtures.

Discussion

Clearly, the results of the two experiments with mice and rats are not going to answer the question about the effects of CO_2 on the incidence of decompression sickness. These results,

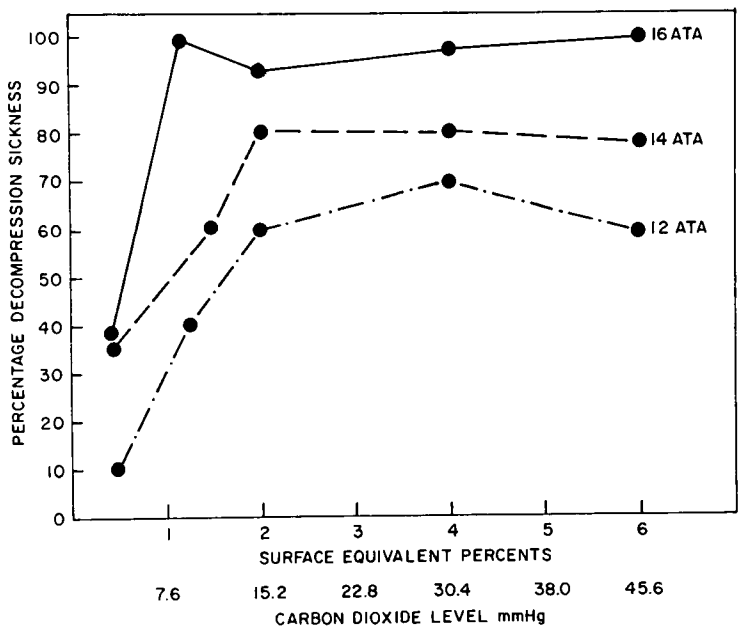

Fig. 3. Incidence of decompression sickness in mice associated with exposure to various levels of CO_2.

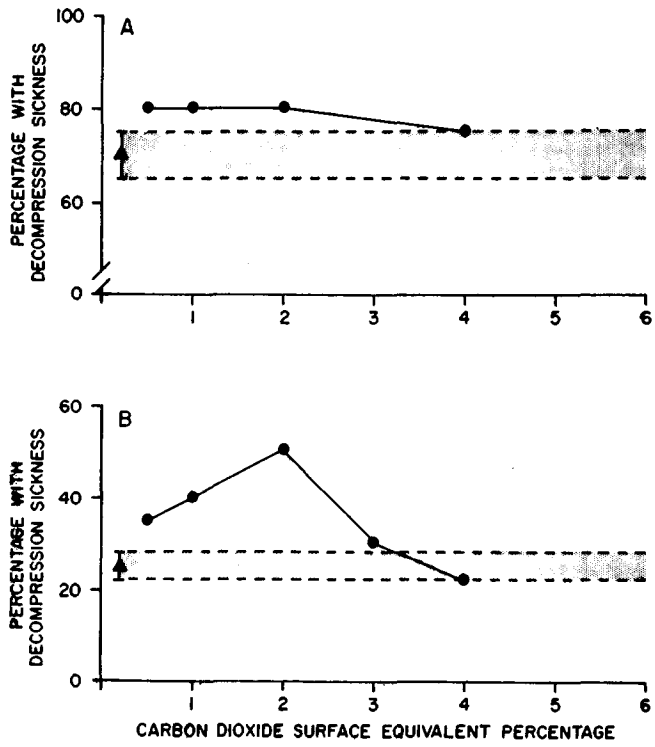

Fig. 4. Incidence of decompression sickness in rats breathing various levels of CO_2 in either a helium-oxygen (A) or nitrogen-oxygen (B) mixture. Incidence of decompression sickness of control rats breathing CO_2-free gas mixtures is shown by shaded areas.

however, do provide some additional evidence that can be evaluated along with the existing information on the topic. To aid in this discussion, the results are summarized in Table VII, along with existing information.

The weight of present-day evidence still clearly supports the position taken by Behnke in 1951. The presence of elevated levels of CO_2 in the breathing media has had, as shown in the majority of the studies, an adverse effect on subsequent decompression. One should not ignore, however, those studies that have yielded contrary results. The four contrary studies serve the important function of alerting the reader to the fact that the effects of CO_2 may not

TABLE VII

SUMMARY OF PRESENT INFORMATION ON EFFECTS OF CO_2 ON DECOMPRESSION OUTCOME

Site of Observation	Study Results		
	Adverse Effects	No Effect	Facilitative Effect
Surface, 1 ATA	3	0	1
Altitude, < 1 ATA	3	3	0
Total	6	3	1

be as important as other possible variables. Since it is possible for Kenrick et al. (13) to saturate water with O_2, N_2, or CO_2 at 100 atm and then to reduce the pressure to 1 ATA without producing bubbles, the high solubility of CO_2 may not be as important as originally thought.

McElroy et al. (15) attempted to evaluate the importance of CO_2 concentration compared to that of variables such as tissue damage and exercise. They concluded that in both muscle contraction (exercise) and leg crushing (tissue damage), the primary factor in bubble formation is reduced hydrostatic pressure due to mechanical tension. Carbon dioxide appeared to play a relatively minor role compared to the other two factors. The authors stated that "under extreme experimental conditions it should be possible to produce bubbles by increasing CO_2 concentration but it is doubtful whether these conditions would be realized ..." [in an actual diving situation]. In other words, it is possible to demonstrate an adverse effect due to CO_2, but the conditions necessary for such a demonstration are artificial and are rarely found in the real world.

It is also possible that part of the demonstrated CO_2 effect is due to the species of animal involved. There appears to be a general relationship between animal size and results. Small animals appear to be more sensitive to elevated levels of CO_2. This observation remains to be confirmed.

Summary

Experimental data presently available indicate that elevated levels of CO_2 in the breathing media can increase the incidence of decompression sickness. Despite the statistical significance and the logical physical rationale for these findings, the experimental results have not been consistent in all cases. The inconsistency is an indication of the importance or impact of this variable in the overall decompression picture. Of the hundreds of possible variables that could be incorporated in a decompression model, CO_2 level appears to be one of lesser priority.

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The animals used in this study were handled in accordance with the provisions of Public Law 89-44 as amended by Public Law 91-579, the "Animal Welfare Act of 1970," and the principles outlined in the "Guide for Care and Use of Laboratory Animals," U.S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

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RELATIONSHIP BETWEEN PRECORDIAL DOPPLER ULTRASOUND RECORDS AND DECOMPRESSION SICKNESS

Ichiro Nashimoto and Yoshiyuki Gotoh

Since the appearance of Bert's paper (1), it has been thought that gas bubbles formed in the body play an important role in the occurrence of decompression sickness. Use of the Doppler ultrasound technique developed by Spencer and Campbell (4) in 1968 enables easy detection of vascular bubbles. Initial studies showed, however, that bubble signals occurred in subjects who were not experiencing decompression sickness (2, 3, 5). This study was undertaken to observe the relationship between bubble signals and decompression sickness.

Methods

Sixty-eight caisson workers were investigated in this study. Their working time was from two to four hours at 2.3 to 3.5 kg/cm^2 (3.2 to 4.4 ATA).

The Doppler ultrasound bubble detector used was of the type designed by Spencer and Clarke (6). Subjects were monitored after locking out. The probe was applied along the left midsternal border and held in the examiner's hand. At the same time, signs and/or symptoms of decompression sickness were recorded.

Precordial Doppler ultrasounds were recorded on magnetic tape so that they could be analyzed later. They were classified on a zero to four scale, according to M. P. Spencer's classification (7). Changes in the character and intensity of all sounds were determined aurally. Some were analyzed using a Rion 07 sound spectrograph. Signs or symptoms of decompression sickness were classified as none, itches (skin involvement), bends (muscular or articular involvement), chokes (pulmonary and circulatory involvement), and paralysis (central nervous system involvement).

Results

Results of the 150 recordings are summarized in Table I. The data were divided into several groups according to the grade of bubble signals and the type of decompression sickness, so that they could be analyzed statistically.

The following results were obtained:

(1) Bubble signals and decompression sickness. As indicated in Table II, bubble signals were heard in 48 (48.5%) of 99 men without decompression sickness. On the other hand,

TABLE I

APPEARANCE OF BUBBLE SIGNALS RELATED TO SIGNS OR SYMPTOMS OF DECOMPRESSION SICKNESS

Bubble Signals	Signs or Symptoms				
	None	Itches	Bends	Chokes	Paralysis
Grade 0	51	8	0	0	0
Grade 1	28	16	2	0	0
Grade 2	15	11	4	0	0
Grade 3	5	4	4	1	0
Grade 4	0	0	0	1	0

Values are number of cases.

TABLE II

OCCURRENCE OF BUBBLE SIGNALS IN MEN WITH AND WITHOUT DECOMPRESSION SICKNESS

Bubble Signals	None	Decompression Sickness	Total
With bubble signals	48 (48.5%)	43 (84.3%)	91
Without bubble signals	51	8	59
Total	99	51	150

$\chi^2 = 15.25$, 1 df; $P < 0.001$.

there were bubble signals in 43 (84.3%) of 52 men with decompression sickness. The difference is highly significant ($\chi^2 = 15.25$, $P < 0.001$).

(2) Bubble signals and itches. Whereas low-grade (Grade 1 and 2) bubble signals were heard in 43 (45.7%) of 94 men without decompression sickness, they were heard in 27 (77.1%) of 35 men with skin itches. This difference is highly significant ($\chi^2 = 8.90$, $P < 0.005$). High-grade (Grade 3 and 4) bubble signals were heard in 5 (9.8%) of 56 men without decompression sickness, while they were heard in 4 (33.3%) of 12 men with skin itches. The difference is not statistically significant ($\chi^2 = 3.22$, $P > 0.05$).

(3) Bubble signals and bends. Low grade bubble signals were heard in 43 (45.7%) of 94 men who did not suffer from decompression sickness, while they were heard in 6 (100%) of 6 men who suffered from bends. This difference is statistically significant ($P < 0.02$). High-grade bubble signals were heard in 5 (9.8%) of 56 men who did not suffer from decompression sickness, while they were heard in 4 (100%) of 4 men who suffered from bends. The difference is highly significant ($P < 0.001$).

(4) Bubble signals and chokes. Low-grade bubble signals were not heard in any men with chokes. The two men (100%) who had chokes had high-grade bubble signals. High-grade bubble signals were heard in 5 (9.8%) of 56 men who did not suffer from decompression sickness. The difference is statistically significant ($P < 0.02$, Table III).

A shower of bubbles was heard in a caisson worker who suffered from chokes after the second work period of the day (Fig 1). He did not complain of symptoms after the first work period, although high-grade bubble signals were heard at that time.

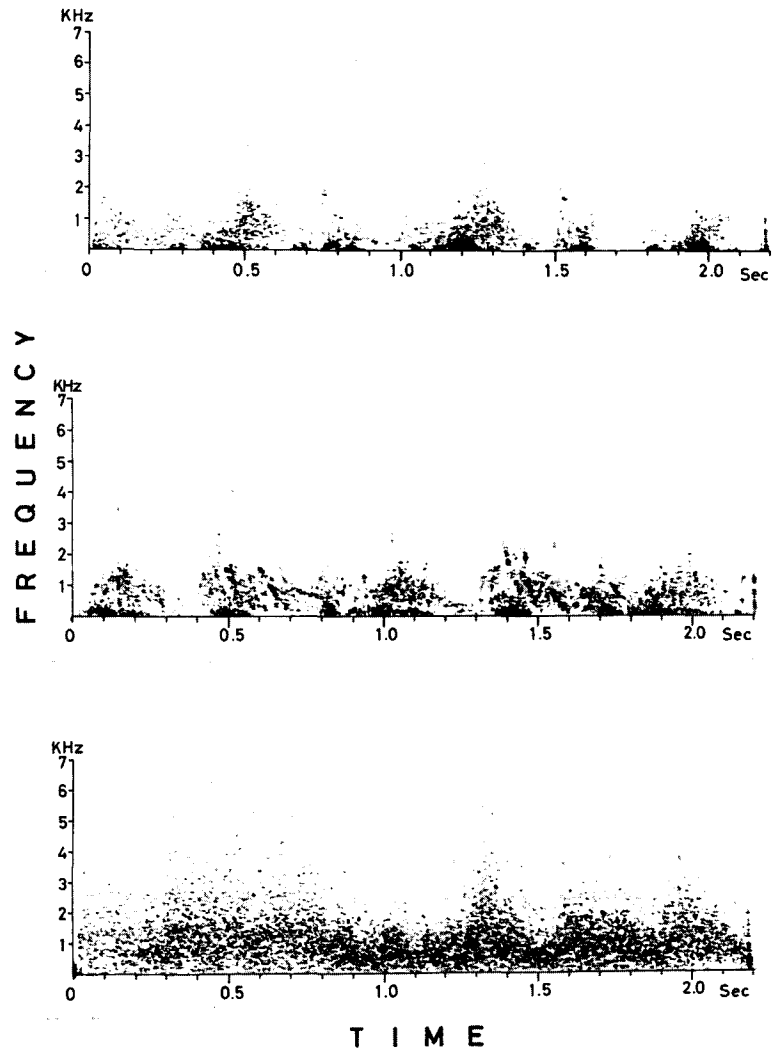

Fig. 1. Changes in Doppler sound spectrogram in a caisson worker who suffered from chokes after second locking out. His precordial Doppler ultrasounds were grade 0 before locking in (*upper panel*), grade 3 after first locking out (*middle panel*), and grade 4 after second locking out (*bottom panel*).

Discussion

Bubble signals were heard in 48 (48.5%) of 99 men without decompression sickness. Such asymptomatic bubbles have been demonstrated in previous studies (2, 3, 5). There was, however, a greater prevalence of bubble signals in men with decompression sickness than those without decompression sickness ($P < 0.001$, Table II). There was a statistically significant association between the presence of skin itches and low-grade bubble signals ($P < 0.005$).

TABLE III
RELATIONSHIP BETWEEN HIGH AND ZERO GRADE BUBBLE SIGNALS IN MEN WITH CHOKES AND WITHOUT DECOMPRESSION SICKNESS

Bubble Signals	None	Chokes	Total
High grade	5 (9.8%)	2 (100%)	7
Zero	51	0	51
Total	56	2	58

$P < 0.02$.

Bubble signals were heard in all cases of bends or chokes. Four out of twelve men, however, were asymptomatic when they were monitored after surfacing. Furthermore, a greater prevalence of low- or high-grade bubble signals was found in men with bends than in those without decompression sickness ($P < 0.02$ and $P < 0.001$). There was also a greater prevalence of high-grade bubble signals in men with chokes than in those without decompression sickness ($P < 0.02$, Table III). Therefore, it is logical to believe that more intravascular bubbles appear in severe cases of decompression sickness.

There were four cases in which bubble signals were heard prior to the occurrence of bends or chokes. Bends or chokes occurred in eight cases before precordial Doppler ultrasounds were monitored. Because precordial Doppler ultrasounds were not monitored during the entire decompression period, it was not clear when these bubble signals appeared. A previous study (7) suggests, however, that bubble signals are detected ultrasonically before symptoms of decompression sickness develop.

One man with asymptomatic bubble signals (Grade 3) suffered from chokes after a second work period (Fig 1). His precordial Doppler ultrasounds then became grade 4. From these data, it is reasonable to assume that silent bubbles remain and grow after repetitive dives, and may thus trigger chokes. This worker might not have suffered from chokes had he not engaged in repetitive work. Further studies of this problem are needed.

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ULTRASOUND MONITORING AND DECOMPRESSION SICKNESS

Michael R. Powell and David C. Johanson

The role of gas phase separation in the etiology of decompression sickness is poorly understood even after almost a century of scientific investigation. In experiments performed several years ago, through-transmission ultrasound was employed to study the time course of gas phase formation in muscle tissues of rats after decompression from pressure (4). The degree of gas separation, as indicated by the degree of ultrasound attenuation, paralleled the severity of the dive profile. Also, the time course for the appearance and disappearance of the gas phase paralleled the time course for the observance of decompression sickness in rats. Bubbles in the venous system of rats were of a more transitory nature, and the time course for their presence did not parallel the observance of signs of limb-bend decompression sickness (5). It has been postulated (5) that the gas phase responsible for limb-bend decompression sickness is formed in situ, with a locus in the microcirculatory system, and this "thrombus-like" gas phase was termed "Class I bubbles" (Fig. 1). This gas could later be released into larger venous channels where it would be essentially harmless unless it was present in sufficient quantity to cause pulmonary vascular obstruction. Class II bubbles were initially postulated in this model to be of little consequence in normal dive situations, i.e., situations other than explosive decompressions. However, depending on the degree to which blood-bubble interactions play a role in decompression sickness, this "essentially harmless" position needs modification (3, 7).

Doppler Sounds and Decompresion Sickness in Swine

The fact that a large number of venous bubbles in the body does not necessarily lead to decompression sickness was demonstrated earlier in a series of experiments using miniature swine diving on nitrogen, helium, or neon mixtures (6, 8). In this series of experiments, a group of miniature pigs was titrated on profiles in which the final decompression stop was successively truncated until the point at which decompression sickness appeared was reached; further truncation produced decompression sickness signs which increased in severity. At the same time, the animals were monitored precordially for degree of bubbles.

In the series of pig dives using air, inability to distinguish between a "bending" and "non-bending" outcome using the precordial system was noted, because all pigs produced Grade IV bubbles independent of whether they displayed signs of decompression sickness. However, when helium or neon mixtures were used as the compression gas, a better gradation was seen:

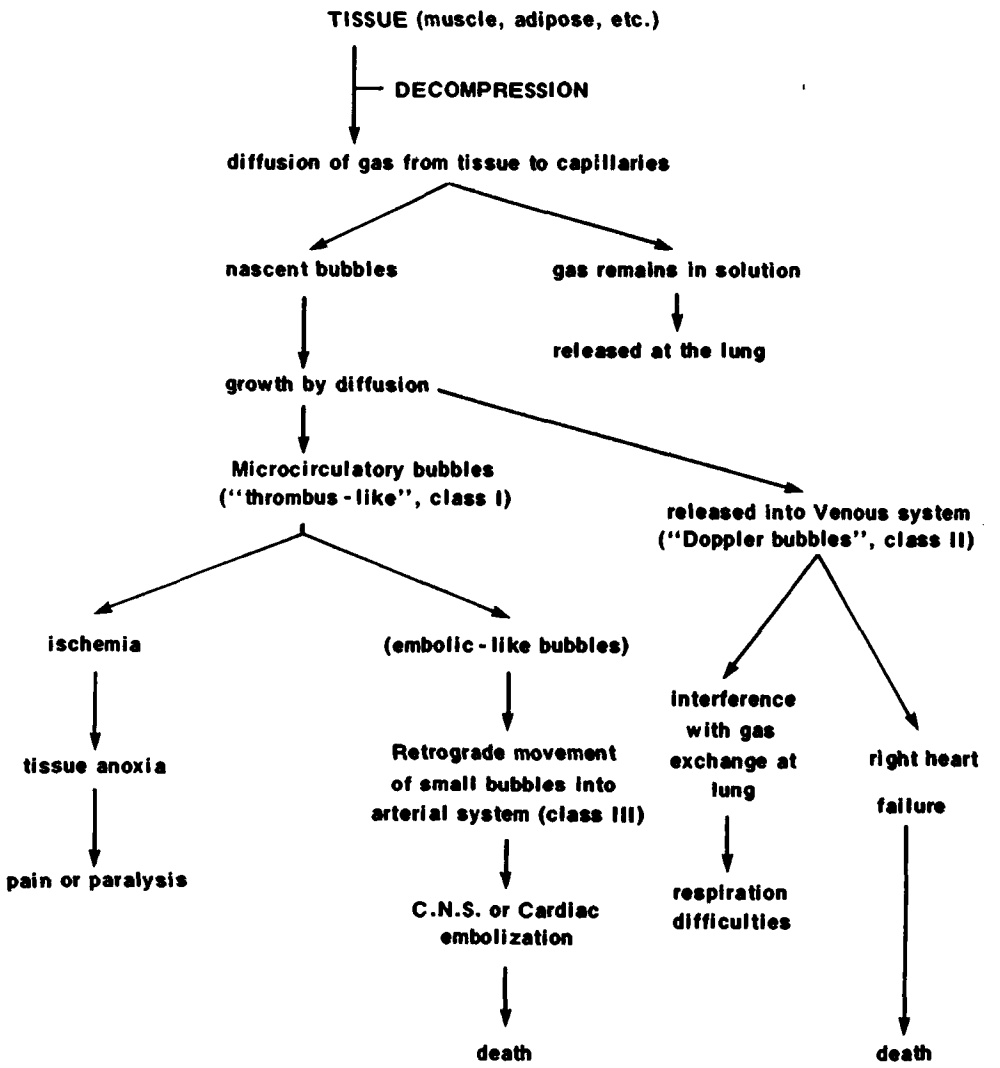

Fig. 1. Hypothesis to describe and explain the pathophysiological consequences of a gas phase in the body following decompression.

animals did not begin to display signs of decompression sickness until a Grade IV level of bubbles was reached. In these cases, approximately two-thirds of the animals with Grade IV bubbles later acquired some form of limb-bend decompression sickness.

An explanation for the difference in results among the gases nitrogen, helium, or neon is demonstrated in Fig. 1. The progression which has been hypothesized for the appearance of bubbles after decompression (5) can be seen in this figure. Bubbles responsible for decompression sickness are postulated to form first in the peripheral vascular system, principally in the microcirculatory system and more specifically, at the capillary level (9). These bubbles can grow (Fig. 2) to a point in which the circulation in that capillary is occluded. Further growth

Fig. 2. Gas phase in the microcirculatory system of rat muscle tissue. Photomicrograph of unprepared, whole tissue mount.

of the gas phase of the cylindrical bubble brings its edge to the point of bifurcation, so that adjoining capillaries can also be blocked. The subsequent blockage of circulation to adjacent microregions results in stagnation anoxia. Bubbles from these regions can later break off, enter into the larger venous system, progress eventually into the vena cava, and finally reach the heart, where they can be monitored with transcutaneous Doppler devices.

There is no good a priori reason to believe that bubbles form selectively only in those sites responsible for decompression sickness. Indeed, they probably form in all parts of the body to a greater or lesser degree. Tissues with short gas uptake half times, such as muscle, or those with high gas solubilities, such as adipose tissue, can be thought of as areas in which larger numbers of bubbles produce a background which "masks" those bubbles coming from the bends-producing tissues. With respect to adipose tissue, bubbles from this area could be significantly reduced by changing from high fat-soluble gases, such as nitrogen, to those of lower fat solubility, such as helium or neon. One would expect, if this hypothesis were correct, that

it would be easier to detect incipient decompression sickness bubbles when helium or neon was used as the compression gas instead of nitrogen. This was found to be true in the experiments with pigs.

Doppler Sounds and Decompression Sickness in Humans

Considerable research has been done in recent years on the pathogenic role of Class II bubbles in human divers, and their predictive value for determining decompression sickness (10, 11, 12, 13). The great majority of work has been conducted using air as the diving gas rather than helium or neon, and in no case, to our knowledge, has a blind study ever been conducted, i.e., a case in which divers were monitored but the decompression profile was not altered in response to the number of bubbles heard. The results of experiments conducted in a series of manned dives in which helium or neon mixtures were employed as the compression gas and in which the experiment was conducted, with respect to the Doppler monitor, in an essentially blind fashion, are reported here. The divers and chamber operators had no knowledge of the results with respect to the number of bubbles, nor was the profile changed according to the results or the gradation of bubbles heard or detected in human divers. These experiments with the Doppler monitor were conducted in conjunction with another series of manned dives made to compare neon with helium as a diving gas. It was not our intent to modify the dive profiles on the basis of the Doppler signals; indeed, such modifications could have biased the results with respect to the comparison tests.

Thirty-two monitoring sessions were conducted in 16 different diving experiments, and the divers were monitored at frequent but random intervals through the diving sequence. No attempt was made to increase monitoring during those times when divers had increased amounts of bubbling, to preclude the possibility that they might suspect that there was some deviation from normal. Instructions were only given to the divers to place the probes in the appropriate spot. No comment was ever made to give an indication of the number of bubbles being detected at the time of the monitoring session. Analysis of the tapes was conducted without the person doing the analysis knowing the final outcome of the dive series with respect to degree of decompression sickness.

The dives were carried out in two specific series; in the first, no oxygen breathing was used during the final decompression stops, and in the second series, oxygen breathing was utilized. The first series required a longer decompression time because an "oxygen window" was not present. When the dives in these series are divided, the numbers in each group are small, but it is believed that there is some validity in separating the test results into these two groups.

Table I shows data obtained from the low-oxygen breathing series of dives, those in which low oxygen partial pressures were used in the final portion of the decompression. Bubble grade is scored against the outcome of the dive. In this case, it can be seen that those divers displaying a Grade III or IV bubble, i.e., bubbles detected throughout each systole, had a considerably greater probability of developing decompression sickness later than divers with Grade I or II bubbles, i.e., those with most systoles bubble-free. Fully two-thirds of those with the higher grades of bubbles developed some form of decompression sickness. Divers with Grade IV bubbles were treated with their chambermates in 3 of 4 cases (shown by the asterisk in Table I). It can be conjectured that, if allowed to continue the decompression, they would also have encountered a problem.

TABLE I

BUBBLE GRADE AND DECOMPRESSION SICKNESS USING LOW Po_2's

Bubble Grade	Decompression Sickness Symptoms, % None	Slight	Moderate
0	—	—	—
I-II	17	—	6
III	6	6	11
IV	6 (17)*	6	28

* = Symptomless diver recompressed with chambermate.

Table II again shows bubble gradation scored against the dive outcome. In this series of dives with high oxygen partial pressure breathing during final decompression, approximately two-fifths of the divers suffered some form of decompression problems, ranging from niggles, or mild, barely discernible problems, to slight visual effects such as swirling vision accompanied by joint pain. As can be seen in Table II, problems were not encountered in the divers until Grade IV bubbles were detected, i.e., until bubbles appeared throughout each systole. In one of these cases, there were two divers, each with Grade IV bubbles, only one of whom, however, experienced decompression sickness at the time of monitoring. Both divers were recompressed together, and again it is difficult to predict whether the other Grade IV-bubble diver (shown by the asterisk in Table II) would have encountered decompression sickness if allowed to continue with his decompression schedule.

On the basis of this study it can be said that in dives requiring a decompression of 3 to 8 hours using helium or neon as the breathing mixture, no diver demonstrating Grade III bubbles or less is likely to encounter decompression sickness. Those with Grade IV bubbles should be watched particularly, because there is an approximately even chance that a problem will develop.

The difference between decompression sickness outcome and degree of venous bubbles in the two groups is perhaps explained by the role of tissue anoxia in the etiology of decompression sickness. In the presence of an elevated arterial oxygen tension, the degree of embolization in the microcirculation which tissues can tolerate is postulated to be greater, because collateral circulation can then supply anoxic tissues with oxygen over greater diffusion distances.

The results of these experiments illustrate a predictive, although not necessarily causal, re-

TABLE II

BUBBLE GRADE AND DECOMPRESSION SICKNESS USING HIGH Po_2's

Bubble Grade	Decompression Sickness Symptoms, % None	Slight	Moderate
0	7	—	—
I-II	7	—	—
III	29	—	—
IV	29 (7)*	7	14

* = Symptomless diver recompressed with chambermate.

lationship between the degree of bubbles in the venous system and the probability of decompression sickness.

Indeed, the fact that Grade IV bubbles are often found when animals are dived with nitrogen, compared to the fact that high grades are seen only in subjects who develop decompression sickness when helium or neon is used, would seem to argue against the implication that venous bubbles per se are the cause of decompression sickness insofar as limb-bend decompression sickness is concerned.

Cardiorespiratory Problems in Decompression Sickness

Recent work by Bove and his co-workers has indicated that large numbers of venous bubbles can produce an increase in right ventricular pressure and stasis in the azygos vein, which causes a reduction in nitrogen elimination in the spinal cord and results in paralytic bends (1, 2). To what degree an elevation of right ventricular pressure occurs in the less severe cases of decompression sickness, i.e., those which do not require recompression to prevent cardiorespiratory collapse, has been investigated using sheep as the experimental diving subjects. In these experiments, the animals were unanesthetized and free-standing during the entire experiment. After dives of different times and depths, bubbles in the venous system were monitored in the precordial region by means of the Doppler bubble detector. Bubbles entering into the systemic circulation were measured by Doppler cuffs surgically implanted either around the carotid artery or the abdominal aorta, and right ventricular pressures were measured by a catheter passed down the jugular vein into the right ventricle.

It was noted that even after dives in which there was a prolonged period of Grade IV bubbles, bubbles in the systemic circulation could not be found, and right ventricular systolic pressures did not rise more than 17% (Fig. 3). This would indicate that the pulmonary gas loadings found by Bove and his associates which produced elevations of right ventricular systolic pressure of 60% were considerably greater than those found in normal diving circumstances, even in those producing limb-bend decompression sickness. In our surface control experiments, in which a total of 100 cc of air was injected at 5-to 10-min intervals over a 30-min period, elevations in right ventricular systolic pressure of 70% were noted without any apparent change in the subject, as had also been found by Spencer and Oyama (14). Elevations of right ventricular systolic pressure of the same magnitude as those seen after the dive shown in Fig. 3 could be produced by injecting 30 cc of air over an 18-min period.

From these experiments it is concluded that the total gas load to the lungs is small after decompression from dives of short duration. Furthermore, a venous gas phase sufficient to produce Grade IV bubbles does not necessarily, of and by itself, result in limb-bend decompression sickness. The effects of chronic Grade IV bubbles, assuming that their occurrence is possible after a decompression, are not known. Acute Grade IV+ bubbles can result in pulmonary air embolism and death, and it is unfortunate that most researchers employing small rodents as subjects study this phenomenon rather than limb-bend decompression sickness.

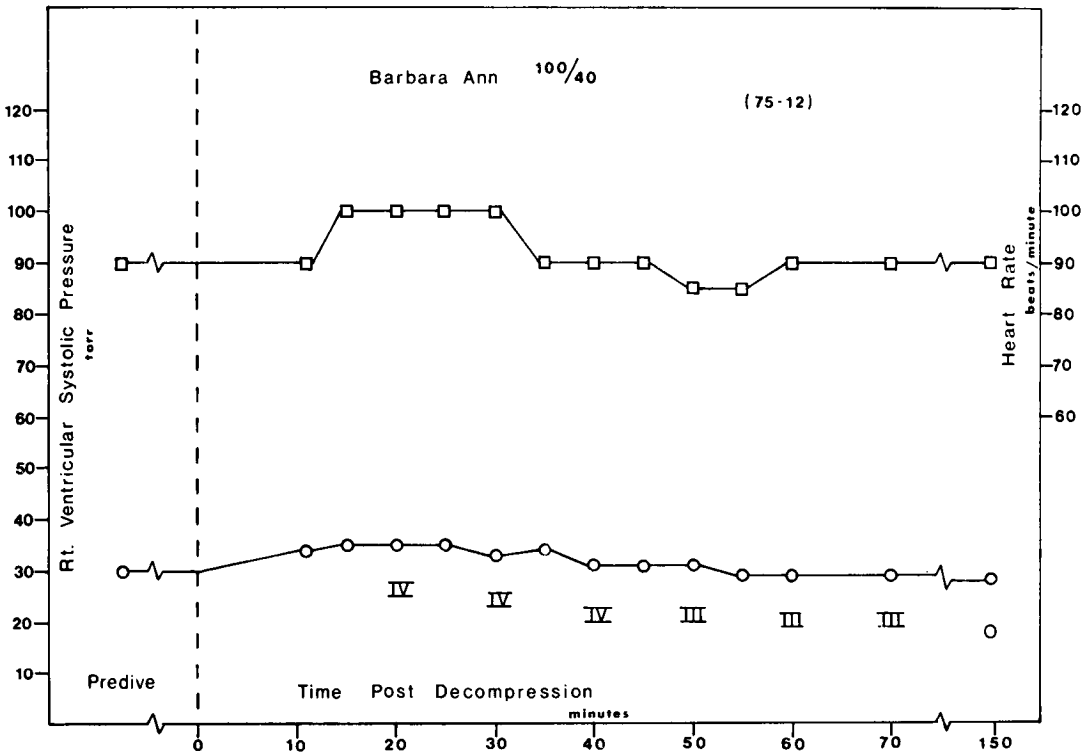

Fig. 3. Right ventricular systolic pressure and heart rate as a function of time following decompression. The roman numerals refer to precordial bubble grade (□—□ heart rate, O—O rt ventricular systolic pressure).

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DECOMPRESSION AND ISOBARIC SUPERSATURATION IN FLUID-BREATHING VERTEBRATES: TIMED RESPONSE VIA BIOASSAY, HEMATOLOGY, AND ULTRASONIC BUBBLE DETECTION

D. L. Beyer, B. G. D'Aoust, L. S. Smith and E. Casillas

This program grew from an interest in the discrepancy between the lethality of low level (120%) supersaturations to fish (4) and the "conceptual" tolerance of divers to supersaturations of 300% and 250% in 5- and 10-minute tissues, respectively, according to current U.S. models (11, 13).

The sensitivity of fish to low level supersaturations has been known and described in the literature since 1900, yet it has not been exploited by physiologists until recently.

The merits of using fluid-breathing organisms (salmonids in these tests) as comparative models for the study of decompression-related pathologies can easily be appreciated when the limitations in studies made with intact air-breathing animals are considered. Whatever the experimental decompression imposed on a mammal, gas elimination from the subject begins as soon as the ambient pressure is decreased; the results can therefore only be interpreted if the unique and unknown combination of gas elimination rate, saturation state, and decompression rate is kept in mind. It is impossible to extrapolate to estimate more precisely what supersaturations (ΔP) existed, for how long, and in what tissues, to produce the observed symptoms and/or signs. It is now accepted as probable that nitrogen (N_2) elimination after a dive does not parallel uptake (8, 9). Recently (6), it has been demonstrated that after a decompression from saturation at only 33 fsw in awake dogs, the tissue-to-blood N_2 elimination rate was greatly decreased as a result of the decompression. This indicated that N_2 was left in the animal, again raising the question as to whether or not the animal was in a supersaturated state.

By contrast, use of a closed system and fluid-breathing vertebrates narrows the interpretive gap between imposed conditions and observed results because the entire fluid system can also be supersaturated, thereby greatly reducing gas tension gradients between experimental animal and the environment after decompression.

There are other advantages in using salmonids for the study of bubble formation and growth (see Table I). The only way to achieve comparable experimental freedom with mammals is by imposing supersaturations through fluid breathing, which is itself an unphysiologic procedure.

Initial experiments (5) indicated that neither the excess gas tension (ΔP) nor the supersaturation ratio (P_i/P_f) in itself could be used as an index of decompression stress; solubility must also be included. Since diving currently involves the use of multiple gas mixtures, a unified concept of all factors promoting bubble formation and growth seemed desirable.

This paper reports studies of the relationship between decompressions externally imposed (as opposed to internally imposed supersaturations) (Fig. 1) with a number of different gases, and

TABLE I

ADVANTAGES IN USING SALMONIDS FOR DECOMPRESSION STUDIES

Fluid-to-fluid gas exchange, which allows both hydrostatic pressure and supersaturation to be varied independently.
Salmonids are physostomous (have a pneumatic duct) and can therefore be decompressed without bursting their swimbladders.
Supersaturation can be applied either internally or externally.
A large part of the fish is a poorly perfused, diffusion-limited system, i.e., the large white muscle mass.
Fish have a very low P_{CO_2} in their blood.
Absence of adiabatic temperature changes with pressure changes in water (approx. 1 degree/1000 atm).
The fish are readily available and relatively inexpensive.

hematological investigations after decompression stress. In addition, some preliminary studies using bubble detection with a Doppler ultrasonic catheter (12) in the dorsal aorta have been combined with direct measurements of supersaturation decay rates and the onset of symptoms. At this time the analysis is somewhat limited, but it provides some perspectives of direct relevance to the prevention and treatment of decompression sickness.

Materials

TEST ANIMALS

The fish used in these studies were coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*). Small coho (85–100 mm, 7.5–9.0 g) were used for the majority of the tests described, but adult trout (440–500 mm, 900–1400 g) were used for the Doppler studies. The possible effects of size on response to decompression are discussed elsewhere (2).

TEST CHAMBER

A 4-liter pressure chamber was used to establish the various test conditions. The chamber has temperature control, monitors for hydrostatic pressure and gas tensions, flow regulators, and a movable piston valve which can be used to divide the chamber into two separate parts. The monitor for gas tension (tensionometer) has been described by D'Aoust and co-workers (7). The chamber was designed for experiments in four major areas: (1) gas uptake and elimination rates in fish; (2) counterdiffusion; (3) response of fish to decompression and supersaturation; and (4) ultrasonic detection of bubbles as related to specific excess gas tensions. The first two of these topics are only briefly mentioned in this paper because the main emphasis in these investigations has been on the third and fourth areas.

Investigations

KINETIC STUDIES AND COUNTERDIFFUSION

Kinetic studies can be carried out in the chamber by means of the movable piston valve. With the valve, the chamber can be divided into two compartments which can be saturated

independently with dissimilar gases. By suddenly imposing a change in the volume ratio of the two compartments by moving the piston, and by analyzing the resulting gas concentrations over time, the uptake or elimination of a particular gas can be determined.

By saturating the entire chamber with one gas, and then suddenly switching to another gas of dissimilar solubility, the effects of counterdiffusion can be studied. Thus far, in limited experiments at 100 fsw and 200 fsw, the results of these counterdiffusion experiments have been negative, suggesting that certain maximal rates of gas mix transitions are without effect with helium, argon, nitrogen, and oxygen, at least with fish. The highest transient supersaturations directly measured by the tensionometer using this approach have been less than 33%.

Responses of Fish to Decompression and Supersaturation

These studies on responses of fish to decompression and supersaturation have used symptoms of decompression sickness, mortality, and hemostatic responses as end points. Figure 1 depicts the three ways of imposing a condition of excess gas tension on fish in this system, i.e., external supersaturation, internal supersaturation, and a combination of the two.

With external supersaturation, water in the chamber was saturated to a specific depth and then rapidly decompressed. Fish were then placed in the supersaturated water in the chamber, the hatch was closed, and the response was recorded. The response was based on a score (n = 10) of zero for no response, 1 for obvious signs of distress such as loss of equilibrium, and 2 for death. Although the initial level of supersaturation did not persist, the gas tension did remain above 1 atm for extended periods of time of up to one hour or more, as measured by the tensionometer.

With internal supersaturation, fish were placed in the chamber and the chamber was pressurized to depth for a certain length of bottom time. The fish were then decompressed to the surface, removed from the chamber, placed in "clean" water (at atmospheric saturation), and the response was recorded at a predetermined elapsed time.

The third approach was to place fish in the chamber and pressurize, as in the case of internal supersaturation. However, when the fish were decompressed to the surface, they were held in the chamber in the supersaturated water and thus the net excess gas exchange between the fish and the water was decreased compared to that of the two previous methods.

With the internal and external tests, normoxic mixtures of helium-oxygen, argon-oxygen and pure oxygen were used in addition to air, primarily to identify the relationship between solubility and bubble formation. Results of tests with these two modes of imposing supersaturation are detailed elsewhere (2). In general, however, the gases of higher solubility produced a greater response (or dive score) for any particular set of conditions of either bottom time or depth. Argon-oxygen was the most effective in eliciting a response; helium-oxygen and air were less effective. Pure oxygen gave responses lower than would have been expected based only on solubility. This was probably due to its respiratory function. It should be noted that the responses were elicited at lower minimum levels, e.g., 66 fsw with argon-oxygen in the external case than with internal supersaturations (100 fsw with argon-oxygen for minimum response). Salmonids thus appear to be more sensitive to externally imposed excess gas.

When the internal and external cases were combined, the response (with air only in these tests) was directly related to the length of time that the excess gas and the fish were held in the system after decompression. As the time of holding in the chamber in supersaturated water

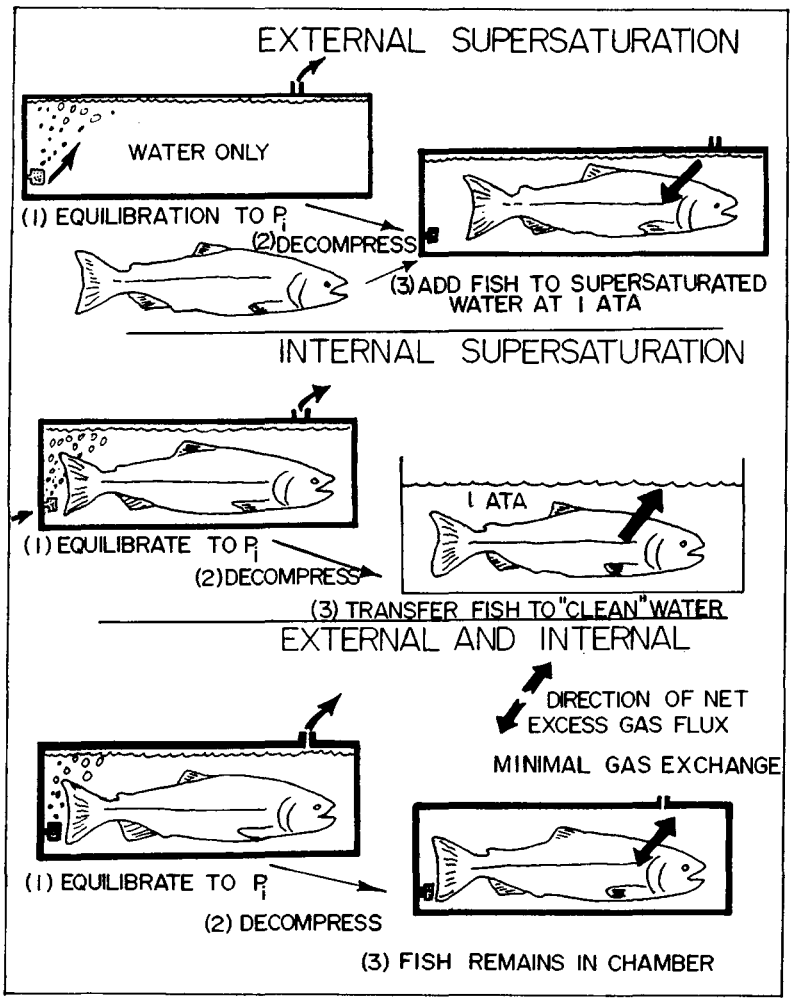

Fig. 1. Various ways of imposing an excess gas condition (supersaturation) on test animals in a fluid-breathing system.

after decompression was increased, the response increased. Thus, the time the gas stayed in the fish was highly critical, as might be expected. With the system used in these tests, this time can be controlled to a degree obviously not possible with an air-breathing test animal.

Pathological and Hematological Response to Decompression and Supersaturation

Most of the fish that died in these tests did so as a result of stoppage of blood flow at the bulbous arteriosus of the heart, which was often found to be distended due to bubble accumulation. Bubbles were also apparent in the sinus venosus, the atrium, and the coronary arteries. With blood stoppage in the heart, there was no flow to the gills and the fish died because of lack of oxygen. If the coronary arteries were blocked, the heart muscle may also have been anoxic. Bubbles were also readily visible in the internal organs, brain, and fins, but white muscle and fat, which are suspected to be poorly perfused with blood, showed a reduced

incidence of bubbles. Functional loss of the parts of the body affected by bubbles was probably important in contributing to the death of the fish, but ultimate blockage of blood flow through the heart and gills was probably the major contributor.

In addition to the pathological response, possible hemostatic responses were investigated. It has been proposed that release from a hyperbaric environment activates the hemostatic mechanism (1). Previous studies (3) have shown that the blood coagulation system of fish can become active in response to stress and that this activation may be part of the general stress syndrome in fish. Hence, it was decided to see if changes in the hemostatic mechanisms of fish coincided with the symptoms of decompression sickness. Results of this study are shown in Table II.

In this series of studies, small salmon were subjected to nonlethal (100 fsw) and lethal (200 fsw) decompression; several blood clotting parameters were measured before and at subsequent times after the event. The main effects were that both prothrombin and partial thromboplastin times were prolonged considerably after both lethal and nonlethal decompressions. Furthermore, fibrinogen levels decreased at various times after a nonlethal decompression, but especially after a lethal decompression. These changes suggest that a hemostatic response akin to consumptive coagulation had occurred. (Note that the speed and the degree of the response depended upon the severity of the decompression episode.)

Ultrasonic Detection of Bubbles as Related to Specific Excess Gas Tensions

Figure 2 shows the basic components of the ultrasonic Doppler bubble detection system, modified for studies with fish. The Doppler-tipped catheter was inserted into the dorsal aorta of a fish and secured to the tip of the snout. The other end of the catheter was threaded through the chamber endplate and attached to various monitoring devices, such as a tape recorder and/or headphones. In addition to the Doppler, a tensionometer which recorded the

TABLE II

Hemostatic Response of Salmonids to Decompression Stress

	Experimental conditions			
		Non-lethal		Lethal
Response	Control	100 ft, +1 min	100 ft, +60 min	200 ft, +1 min
TPP,	21.6	21.28	29.08*	22.9
mg/ml	±3.57	±3.05	±8.1	±4.08
Fibrinogen,	1.38	1.25	1.01	0.46**
mg/ml	±0.61	±0.56	±0.28	±0.49
PT,	34.9	42.0**	41.2**	57.7**
sec	±3.5	±5.0	±3.8	±18.0
PTT,	146.7	238.7**	316.7†	377.8†
sec	±58.4	±115.8	±200.3	±189.3

Values are means ± SD; TPP = total plasma protein; PT = prothrombin time; PTT = partial thromboplastin time. *Significantly different from controls ($P < 0.05$); **significantly different from controls ($P < 0.01$); †significantly different from controls ($P < 0.001$).

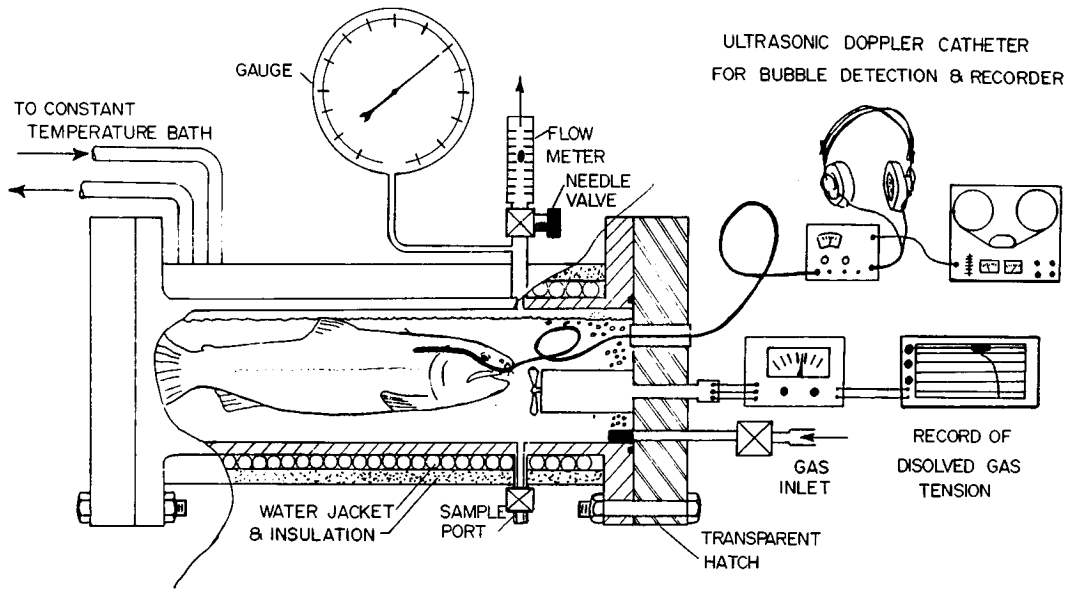

Fig. 2. Schematic drawing of chamber (*left*) and Doppler probe inserted in dorsal aorta of a fish. Probe is threaded through endplate and attached to a recorder. The endplate also contains the tensionometer (see text for details). A small propeller at tip of the tensionometer is used for circulating water and gases through chamber.

gas tension independently from the hydrostatic pressure was constructed. Basically, this device consisted of semipermeable Silastic tubing coupled to an electronic pressure transducer. The gas diffused across the membrane and the transducer responded to the pressure change. A small motor-driven propeller was implanted in the tip of the tensionometer to provide circulation in the chamber for both gases and water. The tensionometer was attached to a recorder, which permitted continuous monitoring of gas tensions throughout a dive.

Figure 3 depicts a recording of a 2-h, 100-fsw internal-external supersaturation dive with an adult trout. Also superimposed on this graph is a similar series of events from a typical external supersaturation dive at 200 fsw. At the beginning of the test, the gas tension increased rapidly but was slower than the hydrostatic pressure. Gas equilibration of the water was essentially complete in 15 minutes. At T_0, the fish was decompressed at 100 ft/min. The excess gas concentration (supersaturation) in the water decayed rapidly at first, but persisted at a lower level until the fish died, approximately 25 minutes after leaving the bottom. The ΔP's on the graph are the excess gas tensions in fsw (Fig. 3).

When the ΔP reached 14 fsw, the blood flow signal stopped, possibly because of temporary blockage of bubbles in the bulbous arteriosus or some undetermined physiological response. Approximately two minutes later, flow resumed and bubbles were monitored. The occurrence of these bubbles was most frequent at first but gradually declined, ending about 19 minutes after T_0. Extensive struggling by the fish was noted at 22 minutes after T_0 and death was believed to have occurred shortly thereafter, still in the supersaturated region. The struggle

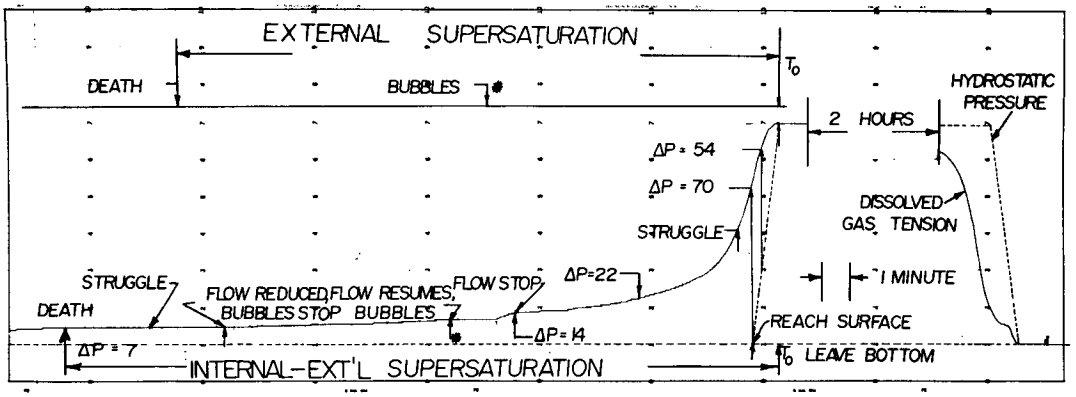

Fig. 3. Recording of gas and hydrostatic pressures as related to bubble formation during a 100-fsw internal-external supersaturation dive with an adult rainbow trout; an external supersaturation dive at 200 fsw is superimposed on the graph for comparison. ΔP's are in fsw (time moves from right to left in figure).

noted soon after T_0 was only temporary and the fish was calm throughout most of the time that bubbles were monitored.

With the external supersaturation, bubbles and death occurred at times similar to those of the internal-external supersaturation response. Although the results on this graph are associated with an initial external supersaturation of 200 fsw and are therefore not directly comparable to the internal-external supersaturation recording, there is remarkable similarity in time until death; these recordings demonstrate the obvious possibilities of studying the relationship between a given supersaturation level and time until bubble occurrence without the complicating factor of hydrostatic pressure change effects that is found with internal-external supersaturations.

Conclusions

Isobaric supersaturations are extremely promising as an additional tool for supersaturation-bubble interaction studies, for several reasons. First, no saturation at depth is necessary to elicit a response; bubbles occur as the excess gas in the system is taken up by the fish, thus giving a rather clear definition of initial conditions preceding bubble formation. Second, the external supersaturation is at least as effective as the internal, and the time period from exposure to response is short; a one-compartment system of well-perfused tissue, i.e., the blood, gills, and internal organs, is probably involved in external supersaturations. This reduces the complexities of additional tissues such as fat and muscle that might have to be considered in internal supersaturations; accordingly, use of a one-compartment system may facilitate definition of factors affecting bubble formation and growth.

Hemostatic changes indicative of disseminated intravascular coagulation are found in fish, and are much the same as the response found in mammals. Thus, the response to decompression appears to be similar in both cases, which warrants further investigation of possible blood-bubble interactions in fish, particularly at low level supersaturations ($< 150\%$).

The similarity in response time to internal and external supersaturations shown by the experiments utilizing fluid breathers and the Doppler-tensionometer combination suggests that

a perfusion-limited, critical tissue is involved. This shows definite promise for relating specific excess gas tensions to actual occurrence of bubbles to a degree not possible with air-breathing vertebrates.

ACKNOWLEDGMENTS

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PART IX. DETECTION, GROWTH, AND RESOLUTION OF BUBBLES

DISCUSSION

E. B. Smith, Chairman

Dr. D'Aoust: Dr. Lightfoot, I enjoyed your paper very much. Inherent in this optimization is a simple choice of how much you want to load the lung. It might be appropriate to point out that there has been some recent work on the hormonal role of the lung and the discovery that it can change vasoactive agents such as angiotensin I to II in one pass through the lung. I hope there is going to be some fairly careful physiology concerned with looking at what happens to the lung during embolization. Dr. Yount, it is fortunate that you are working at high pressure. I think the nucleation is probably not affected by this, but one slide did appear to show that gelatin was under negative pressure. I'm wondering if in any of the experiments where the gelatin was left for some time to equilibrate with a new gas, such as was reported in your *Science* paper, what effect the negative pressure in the gelatin might have on surface tension, exchange rates, growth, and so on?

Dr. Yount: We have tried a range of pressures, some of them below atmospheric, to determine the threshold for bubble formation. We find that if we mix gelatin at atmospheric pressure, the threshold for forming bubbles is -12 psig. This determines the radius of the largest nucleus present, and gives a value of about 1.2 microns. On the other hand, we also measure the numerical distribution of gas nuclei versus radius, which increases with decreasing radius. For example, we observe hundreds of nuclei having radii larger than .05 microns, and this number decreases as we go to larger radii. A whole range of nuclei are present.

Dr. Vann: A question for Dr. Yount. I noticed in your abstract that you say tribonucleation is essentially not a process which can lead to de novo nucleation. Given the experiments of Ikels in which he rolled steel balls down test tubes which he centrifuged before decompression, it would appear that he would have gotten rid of all the pre-existing micronuclei. How then can you say that tribonucleation is not de novo nucleation?

Dr. Yount: I think of tribonucleation and Reynold's cavitation as methods of applying a negative pressure. For example, tribonucleation occurs when a steel ball rolls down the side of a test tube: as the two solids separate, a negative pressure is induced. If there are nuclei present which have thresholds for bubble formation that are reached by this negative pressure, they will grow into bubbles. To my knowledge, no one has succeeded in producing a denucleated water sample, i.e., a sample that can withstand the homogeneous nucleation threshold. So I presume that almost any water sample that has ever been produced has had some nuclei in it.

Dr. Kuehn: This is a suggestion for Dr. Yount. Even though the homogeneous nucleation theory calls for very high supersaturations to produce bubbles, is it not possible that forces of short range in the liquid would act in some sense as "physical catalysts" to cause nucleation at specific sites within the liquid rather than on a homogeneous basis?

Dr. Yount: This is an interesting point that the process of nucleation by random molecular motion may be enhanced by, say, a two-step mechanism in which gas molecules first form small aggregates and these clusters collect together into larger aggregates. Any of these processes is characterized by a very short time constant of perhaps 10^{-10} seconds. So this is a way of producing at any location the possibility of forming a bubble. On the other hand, it doesn't produce stable nuclei. The nuclei we see in our experiments appear to be stable for periods on the order of years, since we get the same distributions for samples taken from a batch that has been frozen for a year. The random motion is a transient phenomenon and I don't think it would produce the stable gas nuclei that we see in our experiments.

Dr. Kuehn: But in the human or animal body, there is the possibility that this could take place. You have these very short-lived microbubbles which come into existence.

Dr. Yount: Yes, I agree with you; the process occurs.

Dr. Kuehn: A question for Dr. Lightfoot. Which equational method did you use to calculate your rate of growth of bubbles? Was it the Rayleigh equation or the Kurtzman equation?

Dr. Lightfoot: It is our own equation; I don't know how to describe it. I can assure you that I think it is the best approach. It involves a large number of approximations, all of which strike me as reasonable but which are very difficult to describe in detail orally. We use as a driving force the total gas pressure, including both inert and metabolic gases. We use the assumption of a spherical bubble, and we correct for surface tension in that way.

Dr. Kuehn: We have done similar calculations at DCIEM which, for examples similar to yours, would predict a bubble collapse rather than growth. Also, Dr. Berghage, I suggest that the excellent success of USN tables that you reported are a reflection of the reporting standards used by USN personnel on operational dives as well as the perhaps overly conservative selection of certain schedules.

Dr. Elliott: I think it is correct to say that Evans suggests that spontaneous nuclear fission is a bends-rate limiting factor. Can I ask you to comment on a hypothetical situation: if you have a tunnel full of workers, 98% of them will not have had a nuclear fission during that time under pressure. Are you saying that if there were an explosive decompression in that tunnel, there would only be a 2% death rate?

Mr. Evans: No, I'm saying that it is quite probable that if a tunnel full of men were explosively decompressed, rather a lot would have trouble and show bubbles. These bubbles would be due to many gas micronuclei which had been created in them by the spontaneous fission of uranium, possibly since birth. If the men have not been regularly exposing themselves to high pressure, which will tend to crush newly created nuclei out of existence, there is nothing to stop the nuclei forming a tough, fibrous, and effectively permanent organic skin. Therefore, to see how many nuclei each man might have in him you only have to count the number of 3-week periods since he was born.

Dr. Manley: I have a question for Dr. Lightfoot. In your calculations you started the bubble radius diameters at 1 micron. From one of the tables, with your calculations at what bubble size would you have obtained collapse of the bubble instead of growth?

Dr. Lightfoot: Roughly 1 micron. In other words, all of our growth calculations start with something very close to the critical bubble size.

Dr. Manley: All right. I have a comment about the last paper presented, the one which talked about requirements for decompression schedules. I represent the group of divers who are required to recover explosive debris that is left underwater after armed conflicts and who are severely limited by the lack of accelerated decompression schedules and lack of diluent gases other than helium. By the nature of the work they must operate as self-contained divers with minimal surface support. It would be very encouraging if someone would develop accelerated schedules that use gases with low thermal conductivities and provide perhaps 45 minutes on the bottom at intermediate depths on the continental shelf.

Mr. Evans: The LET of the β or γ emissions of technetium 99 or 99M is not high enough to nucleate bubbles in weakly supersaturated gaseous solutions.

Dr. Vorosmarti: I would also like to suggest that even though there may be fibrous nuclei in your body from uranium fission, every cc of your blood has somewhere on the order of 5 to 6 million fibrously covered nuclei which could cause bubble formation.

Mr. Evans: Yes, the red cells, but have you any reason to think there is a free gas phase in the red cell? I agree that there is a lot of gas in solution and in combination, but is there any free gas?

Dr. Vorosmarti: I'm saying they can form a nucleus, a place for a nucleus to form; I'm not saying there is free gas there.

Mr. Evans: You do need free gas before a bubble will arise.

Dr. R. A. Bennet: Dr. Evans, I don't know whether you know it, but Aberdeen is about the third most radioactive place in Great Britain. Do you know if there is any difference between the Aberdeen divers and those in any other place?

Mr. Evans: We haven't in fact gone into this; the idea is still in its formative stage. Certainly it would be interesting to compare men who have been born and bred in Aberdeen with those who come from a very low uranium area.

Dr. Schaefer: Dr. Berghage, you might have an explanation for CO_2 effects on the incidence of decompression sickness. Despite your probabilistic experimental approach, your data indicate specific effects of CO_2. Would you comment on that?

Dr. Berghage: There has been speculation in the past that CO_2 resulting from exercise produces high concentrations of CO_2 in very local areas within the muscle. This CO_2, because of its high solubility, forms the bubbles at

these local points. However, when the bubbles have been measured postmortem, the gas content has been shown to be primarily inert gas. Because of these postmortem data, researchers have speculated that the CO_2 was there for the formulation of the bubble, but diffused into solution as the bubble moved through the body prior to the death of the animal. I don't have a good theory for it.

Dr. Schaefer: CO_2 has a stimulating effect on metabolism and circulation in the concentration range used in your experiments, which results in an increased gas uptake under those conditions. This specific CO_2 effect could have caused the increased incidence of decompression sickness you reported.

Dr. D. A. Miller: Dr. Yount, you showed nicely that decreased numbers of gas nuclei are present in gelatin after administering a pressure spike, and Dr. Berghage, you reported a high bends incidence in saturation diving. I wonder if anybody would care to speculate about the possibility that in saturation diving, applying a pressure spike by means of a bounce dive might be a good preparation for decompression.

Dr. Yount: Dick Strauss will give a paper at the Hawaii Man-in-the-Sea Conference on the preparation of diving tables for humans based on information that has been learned from the gelatin model. In this method one takes into account the crushing of gas nuclei. The difference between humans and gelatin in this respect is that in gelatin there is no restoration of the gas nuclei: once they are crushed they remain crushed. In humans, on the other hand, there is evidence of restoration. I'm thinking of the experiments by Aggazzotti and Ligabue, in which they crushed dog gastrocnemius muscle and observed that the time required to restore the original volume was rather long, e.g., 30 to 70 minutes. This suggests that there is a recovery of gas nuclei in the case of humans, with a time constant that may be 30 to 70 minutes. If you take this into account, you can build into a human decompression table the effect of crushing and the restoration of the crushed nuclei after a period of time.

Dr. Berghage: I think that the distance you would have to drop back would be pretty substantial. Speaking from the standpoint of a behavioral scientist, I think you will get a lot of hesitation on the part of the divers to going back instead of up during decompression.

Dr. R. Smith: I have a question for Dr. Evans. It would seem that the distribution of a heavy metal such as uranium might be important as to where the disintegrations occur. Maybe these disintegrations, and thus nuclei, formed at the rate of one every three weeks since the diver was born, are in an irrelevant tissue, such as the lens of the eye. I wonder if you would like to comment on that?

Mr. Evans: They should not be in the lens of the eye. There are some in the soft tissues, which we don't think is interesting, but about 80% of the natural uranium in man is concentrated in the bones, which are of course associated with the symptoms of Type I decompression sickness. Furthermore, it tends to be concentrated at the ends of the bones, which are the sites at which aseptic necrosis of bone is frequently seen.

Dr. R. Smith: It seems that the correlation is exceedingly fortuitous, then.

Dr. Daniels: We have some data which may or may not be relevant to Prof. Walder's argument. We were concerned when we saw Drs. Evan's and Walder's paper in *Nature* on uranium 238 spontaneous fission to ascertain whether this process contributes to the variability observed in our studies of decompression sickness. So we treated a group of 10 mice chronically for 7 days with an intravenous injection of uranium 238, giving three injections, each one of which was sufficient to raise the level of activity due to spontaneous fission of ^{238}U by a factor of 100. These mice were then given a standard dive of 150 psi of nitrogen (Po_2 1 ATA), and we didn't observe any change in the incidence of decompression sickness.

Mr. Evans: Thank you for that information. I didn't know that anybody had done that experiment. I cannot work out on the back of an envelope from what you have told me if we would expect to have a fission during the relevant time or not, but I will be glad to talk to you afterwards about it.

Dr. Hills: Could I ask Dr. Daniels and our Chairman, perhaps, how they would interpret the fact that you can produce a nitrous oxide foam without any decompression at all by employing counterdiffusion? When you ventilate one lung with nitrous oxide and the other with oxygen, so that you have got counterdiffusion, you can produce this foam and not have any decompression. How would you correlate this with your hypothesis?

Dr. Daniels: Admittedly at the moment the experiment you suggest is not easily explained using our hypothesis. We hope to be doing some experiments in the very near future which are of a similar nature, with halothane ether vapors. Maybe when we have done these experiments, I might be able to come up with an answer for you.

Dr. E. B. Smith: Counterdiffusion intensifies the decompression flux; the two factors are probably contributory in that matter.

Mr. Krasberg: On our deep bounce dives, we have found that three out of four decompression incidents result from severe blows to the elbow during the work period. In response to one comment about all the bends being in the knees, we are finding them in the elbows and they are a result of a blow.

Dr. Lundgren: Dr. Berghage might like to add to the confusion some observations that we made and published

with Drs. Westling and Balldin several years ago. In goats, increased carbon dioxide load did not increase the incidence of bends, but when decompression sickness occurred it seemed to be more severe; there were more spinal hits.

Dr. Bove: A comment for Dr. Powell. First, I would like to congratulate him on a very nice study. There are, however, some apparent disagreements between their data and our data from a year or so ago, or rather their data in a sense complete some of the things that we didn't do. Our studies over several years have made clear a distinction between limb bends, that is peripheral evidence of decompression sickness, and central problems with chokes, spinal cord lesions, and vascular alterations. We have shown over the past few years that there are differences in platelet consumption, blood viscosity, hematocrit, and changes in plasma volume which are clearly present with chokes and spinal cord lesions but which are absent with limb bends. In our physiologic studies, since we required fairly complex equipment and had to travel to Duke for most of them, we ended up with a series of studies addressed only to spinal cord lesions and chokes. Our data on pulmonary artery pressures, pulmonary vascular resistance and cardiac output have to do with fairly severe decompression sickness, where chokes were evident in each case. Your data are addressed to the limb bends problem and I expect that we wouldn't find the significant circulatory alterations which we found in serious decompression sickness in limb bends. Your data complement our initial findings and I think complete the picture that there are significant alterations in many systems with serious decompression sickness, but these do not exist necessarily with limb bends; limb bends seem to be a more peripheral type of disease. One interim piece of information which is not apparent at this point concerns the problem of spinal cord lesions. There are findings in both humans and in some studies we have done that you don't need the serious circulatory alterations to have spinal cord lesions.

Prof. Walder: I would like to address some remarks to Dr. Cunnington.* I'm quite prepared to accept counter-diffusion and to believe that tissues can be saturated at ambient pressure, but what I find difficult to understand is how spontaneous bubble formation can come about as a result of this. Your first bit of evidence is that if you put a knife into the skin lesions, you see gas coming out. Of course, if you have a supersaturated tissue and you put a knife into it, you'll get bubbles because you will introduce gas micronuclei. Thereafter your experiments depend on the fact that you have a prepared animal, and of course during the preparation you have again introduced gas micronuclei into it. So, my question is, have you in fact found bubbles using a noninvasive technique (as for instance the Doppler technique) in an animal supersaturated in this way?

Dr. Cunnington: We have done some studies using the Doppler technique, but only with surgical implantation of the cuff. We have done studies without invasive techniques in which we simply place an endotracheal tube and have the animals breathe nitrous oxide while surrounded by helium. These animals die after a period of 2 to 5 hours. At autopsy the vascular tree, particularly the venous tree, is filled with bubbles.

Mr. Adams: In the Doppler technology per se, the simple fact that we do not hear bubbles does not preclude their presence, a fact that I tend to overlook and wanted to emphasize. It only means the bubbles may be smaller than the size limit of detection of the technology.

Dr. Spencer: Two questions, the first one directed to both Dr. Beyer and Dr. Cunnington. Could you speculate or do you have any experimental evidence on how the bubbles get into the blood? Does the gas pass into the blood and nucleate on blood element sites, or do the bubbles pass through the capillary wall from the tissues into the blood?

Dr. Beyer: We really haven't speculated on this in our fish experiments, primarily because ours are at such high levels. In low level supersaturations in the river, they are speculating on possible blood-bubble interactions such as this. But again, in our studies we haven't speculated.

Dr. Cunnington: We speculated on the same two possibilities, Dr. Spencer. The interesting thing about the single-layer diffusion-perfusion hypothesis which I presented just a few minutes ago is that you would expect the highest local partial pressure to be in the vascular system, the site at which the nitrous oxide pressure and helium partial pressures are greatest. So we assume that bubbles form in the microvasculature itself, on nuclei which we presume must already be present. But in truth, we have no experimental evidence.

Dr. Spencer: Do the skin lesions develop obviously before the bubbles appear in your collection chamber?

Dr. Cunnington: The skin lesions are usually seen first; these are the white lesions. The erythema nearly always appears in close association with the vascular bubbles, and as I said we think this erythema is the result of stasis caused by the bubbles. As I pointed out, only 80% of our animals had intravascular bubbles, but all of our counter-diffused animals did have the white raised lesions which have always been shown at autopsy to contain subcutaneous gas bubbles. We always get subcutaneous gas, but not always intravascular gas. That presents a problem to our most recent hypothesis.

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*The paper presented by Dr. Cunnington has not been included in this volume.

Dr. Spencer: I'm interested in the phenomenon of chokes as it might relate to massive blood bubbles or foam in the blood returning to the pulmonary vasculature and raising the pulmonary artery pressure and producing the symptoms of chokes. I think Dr. Powell's findings were very interesting, the first I have seen in which bubbles have been monitored in a patient with chokes.

Dr. E. B. Smith: If gas elimination is homogeneous, as in the Haldane or other mechanisms, then obviously a monotonic decompression schedule must be optimum. In other words, you must go continuously towards the surface. But if there is gas transport involving bubbles, then it is by no means clear that the decompression schedule needs to be monotonic. An undulating mechanism where you allow the bubbles to grow and compress them to transport them through the capillaries could well be optimum. I wonder if anybody has tried diving profiles that are not monotonic?

Dr. K. H. Smith: I would like to comment on Dr. Cunnington's statement that only 80% of his animals had intravascular bubbles. They only found bubbles in the vascular system of 80% of the animals, and there is a big difference with respect to what you say about this type of profile. When we recompress an animal after a decompression, we get a shower of bubbles. That means bubbles are being freed from somewhere and we can show it very nicely every time we recompress an animal. After the spike of bubbles the count drops off to a lower level, but we do get the spike.

Dr. E. B. Smith: We observed this with multiple dives with small rodents and I wonder if this may be the optimum way of bringing people out. If bubbles are important in gas transport then perhaps one should overshoot the stages, get the gas into the bubbles and then compress them to make them less damaging during their transport. This might be an idea to bear in mind.

Dr. Beyer: I want to add a comment in partial answer to Dr. Spencer. We know pretty well that the bubbles are forming in the gill capillaries or more remote capillaries. We can kill a fish in 7 minutes with no change in ambient pressure just by putting him in extremely supersaturated water with a soluble gas. That pretty well indicates that it is unlikely that there is any gross transport of bubbles across membranes or any such model.

Dr. Hills: I would like to follow up on the very good point which Professor Walder made to Dr. Cunnington. I think that the counterdiffusion supersaturation interpretation of Graves for the actual choke effect is most ingenious, but in reading Graves' paper, in the first publication at least, you had to place bubbles at the interface before you saw them actually growing. Is that true?

Dr. Cunnington: Yes, ground glass nuclei were required.

Dr. Hills: So you had to nucleate the interface before you got bubbles growing; this is a key point that Professor Walder brings out.

Dr. Cunnington: This is quite true in the in vitro model. The explanation for what is going on is far from clear and certainly I'm not competent to speculate as to why it is occurring. All I can say is that I have studied the process myself in vivo for a long time and there is no doubt in my mind that when you are getting 67 cc per hour of gas coming out of the hind limbs in the inferior vena cava, in a system in which you have done nothing more than give the animal an ordinary hospital anesthetic and taken away the room air and replaced it by helium, it is a pretty impressive result. I would certainly welcome explanations because explanations are difficult at this point.

Dr. P. B. Bennett: Just after we found this effect at Duke, we did try subjects in air and breathing N_2O and O_2, and we couldn't produce this.

Dr. Cunnington: Helium is ideal. We have done the experiment with N_2O and helium and have seen the linear rise of gas volume with time. Nearly as soon as you stop the helium and return the animal to a nitrogen environment, the bubbles stop.

Dr. Behnke: At what pressure is it safe to breathe air when surrounded by helium without bubble evolution?

Dr. Cunnington: We have studied this only moderately; in pigs surrounded by helium and breathing air at 1 ATA, symptoms are relatively few, but we have noticed the white hard lesions which we believe are the first sign of counterdiffusion bubbles on some occasions. However, if you go to 2 or 3 ATA you do get the full-blown syndrome. At 1 ATA it takes 6-8 hours to get the premonitory signs. I cannot imagine why it should take so long. Obviously the uptake and elimination of gases must have reached a steady state long before that. It is intriguing that in our system, say with helium and nitrous oxide, the steady state of gas transfer must be virtually complete within an hour and yet our gas accumulation may not even start or certainly not be steady state until, say, 3 hours. Obviously there is something very significant going on here and this relates to Dr. Walder's point on the difference between the production of supersaturation and the production of bubbles. But there is no question that there are bubbles.

Dr. Behnke: If oxygen is inhaled and the body is surrounded by helium, you would not expect bubble evolution at any pressure, would you?

Dr. Cunnington: I wouldn't.

Mr. Evans: In connection with the Chairman's remarks about the possible benefits of cyclic decompression, there

is already some information on this subject which comes from the technique known in the diving world as surface decompression and in the compressed air world as decanting. The subject comes in a quick pull from pressure to the surface, where he goes into a chamber in which he is recompressed to the original working pressure and then decompressed, apparently as if nothing had happened, if this is done within five minutes. Now the experience at the Tyne tunnel shows that the decompression sickness rate among those men who were decanted is rather lower than the decompression sickness rate among the men who were not decanted. So it could well be that it has a beneficial effect, as you suggest.

Dr. Mackay: As I mentioned at the movie on ultrasonic imaging of decompression bubbles, we routinely see showers of 1 micron and larger bubbles during recompression of intact subjects, as has been hypothesized. The existence of bubbles presents a problem when one is measuring the amount of gas being carried by some part of the blood stream. For example, a probe with a diffusion membrane feeding a mass spectrometer will indicate the partial pressure of a gas dissolved in blood in a vein but will not indicate the possibly greater quantity of gas being carried in the gas phase if true bubbles form. Large errors result if both phases are not monitored, and bubbles must be carefully noted during routine measurements.

Part X. DECOMPRESSION SICKNESS AND THERAPY

CLINICAL AND RADIOLOGICAL FEATURES OF 88 CASES OF DECOMPRESSION BAROTRAUMA*

D. H. Elliott, J. A. B. Harrison and E. E. P. Barnard

During the last 20 years there have been 127 incidents associated with buoyant ascent training in the Royal Navy. In such an incident, the criteria for a definite diagnosis of pulmonary barotrauma are the recent history of a decompression from an exposure to pressure within the no-stop limits, and some objective evidence of associated pulmonary or neurological damage.

Of the 88 cases of pulmonary barotrauma reviewed here, 79 cases were in this category, and nine others who had neurological symptoms but no physical signs were included with a presumptive diagnosis of barotrauma. Among the 39 remaining incidents, there were 16 cases of decompression sickness, most of which occurred in the training staff after dives or in those who had been attendants during therapeutic recompression. The remainder of cases fall into no neat diagnostic category, but consist of those reported to have had some transient manifestation after decompression.

All the cases of pulmonary barotrauma occurred in apparently fit young men; all but four occurred after buoyant ascents which approached a terminal velocity of 8 ft/sec (2.5 m/sec) through the water. More than half of these cases (45) occurred after ascent from the greatest training depth (100 ft; 30 m), and one was from the shallowest depth (less than 10 ft; 3 m). Of the four cases which did not occur after buoyant ascents in the water, one followed a slow free-ascent through the water, two followed the conventional diving rate of ascent (60 ft/min; 20 m/min) after a chamber dive to 100 ft (30 m), and one followed a more rapid decompression from a similar depth in a chamber.

Some respiratory problem occurred in the water in at least 50% of the cases; 13 of these patients reported that they had experienced chest pain during the ascent. While the presence of similar problems in the remainder cannot be excluded by a retrospective examination of the original reports, it can be stated that the 88 cases included reports of several individuals who were reported to have been exhaling correctly and who claim to have experienced no problems during the ascent.

The majority of cases were recompressed to 50 m (165 ft; 6 bar) within seconds of the onset of their disorder. Immediate and rapid recompression of all those with neurological manifestations is, of course, routine in submarine escape training.

In two cases recompression was withheld. In each case this was because there were only mild chest symptoms but no neurological manifestation. In one case these symptoms were asso-

ciated with a pneumothorax, and in the other with a mediastinal emphysema. Both cases were confirmed radiologically and treated conservatively, and were without complications.

Of the 86 cases of decompression barotrauma treated by recompression, 65 demonstrated some neurological manifestation before recompression. Thirty of these patients had collapsed, unconscious, within seconds of surfacing, 20 had become confused, disoriented, or incoordinated after emerging from the water, and 15 had presented with a paresis. Six cases presented with an upper monoparesis, and five with a hemiparesis.

A total of 81 cases were recompressed to 50 m (165 ft; 6 bar). On arrival at that depth, 47 patients were already completely rid of their manifestations. However, this was not necessarily indicative of therapeutic success, and only 26 of this group remained symptom- and sign-free during subsequent decompression. Of the 21 patients developing some further sign or symptom, 13 were given another recompression; in this latter category there was one fatality.

Among the 34 cases not fully symptom-free on arrival at the therapeutic depth, manifestations persisted in 31, and 3 patients were declared dead. The majority of these patients were sign- and symptom-free by the time their decompression from 165 ft began, but a few began the decompression with persistent manifestations even after 2 hours at depth. Among those with manifestations that had not cleared on arrival at depth, 10 required a further recompression, and one subsequently died.

It is possible that the duration of the stay at 165 ft (60 m; 6 bar) may influence the success or failure of the subsequent decompression. The 60 reports in which the duration of treatment at maximum depth was recorded showed that 22 patients subsequently required another recompression. Although the numbers are too small to be of statistical significance, it is worth noting that all cases which required an additional recompression were in the group which spent 60 min or less at 165 ft. No patients who spent more than 60 or up to 120 min at depth subsequently required recompression for a relapse.

Complete neurological recovery was achieved in all but the 5 fatalities. The postrecompression chest X ray was reported to be normal in 54 cases, including 3 of the 10 patients in whom signs of subcutaneous air over the thoracic inlet had been detected. There were only 16 patients for whom the postdecompression films were reported as abnormal.

Case No. 35

The subject, aged 39 years, had been prompted to breathe out several times by the instructors during his 100-ft buoyant ascent. He arrived on the surface red-faced and rigidly gripping the ladder. He was immediately recompressed to 165 ft, where the medical officer found him to be alert and free from clinical manifestations. Pressure was reduced to 100 ft when subcutaneous emphysema was noticed in the region above the right clavicle. The percussion note was hyper-resonant at the right lung base posteriorly, and there was a prolonged expiratory phase. These were interpreted as being the signs of a right pneumothorax. Since there were no abnormal neurological signs, pressure was reduced in 10-ft increments, and although the surgical emphysema extended across both sides of the neck and face and across the right shoulder and pectoral region down to the nipple, there was no subjective or objective worsening of the pneumothorax. The surface was reached 70 minutes after the original incident. The X rays (Fig. 1) showed no pneumothorax, but there was extensive mediastinal emphysema with air extending retroperitoneally, outlining the right kidney with remarkable clarity (Fig. 2).

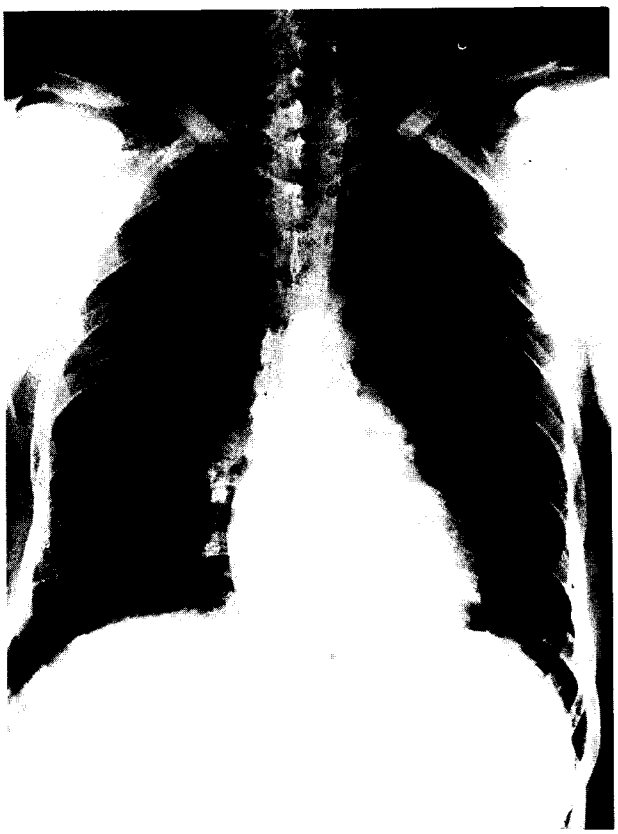

Fig. 1. *Case 35*; radiogram shows no pneumothorax.

CASE NO. 57

This 19-year-old made an apparently normal ascent from a depth of 30 ft. He had some difficulty when climbing the ladder out of the water and had to be assisted. As soon as he was standing on the deck, he was seen to go limp and was immediately placed in the recompression chamber. At this stage the patient had become unconscious. He was pale and his legs were trembling, but the trembling ceased during the compression. Both pupils were dilated, central, and nonreacting. As compression continued, he recovered consciousness and the pupils became normal. After 1 minute at 165 ft, he had fully recovered and had no complaints apart from "ears." At this stage he remembered feeling a sensation of tightness in his chest during the ascent. Clinical examination was entirely normal except for evidence of bilateral otitic barotrauma. Decompression was begun on a therapeutic table (Royal Navy Table 5B, equivalent to U.S. Navy Table 2A). Apart from slight chest discomfort, the subsequent decompression was uneventful.

In common with all trainees, this patient's pre-ascent chest X rays were normal; the particular features of the X rays after treatment (Fig. 3) included cystic areas at the right and left lung bases. There was also some mediastinal emphysema and bilateral pneumothoraces with

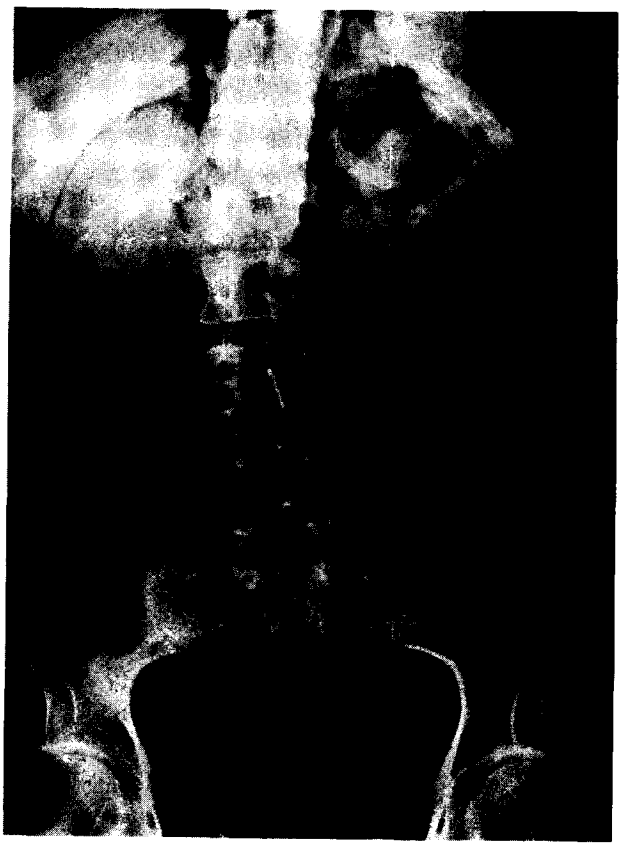

Fig. 2. *Case 35*; radiogram shows extensive mediastinal emphysema with air extending retroperitoneally.

slight displacement of the mediastinum to the left. The cysts, which can be interpreted to be caused by local air trapping, diminished slowly over the next eight weeks.

CASE NO. 63

This 19-year-old made a 100-ft ascent from the Single Escape Tower, using the hooded suit. On arrival at the surface he was unable to climb out of the water and did not respond to commands. He was lifted out of the water and placed in the recompression chamber. During this time he was conscious but seemed confused, and later stated that he felt "unable to move." On arrival at the therapeutic depth, 165 ft, there were no abnormal manifestations. He was decompressed on standard diving tables, but at 20 ft it was learned that the attendant had dived previously and required longer decompression stops, so the pressure was restored to 60 ft. Five minutes later, 30 minutes after the original ascent, the patient complained of blurring of vision. Pressure was increased to 165 ft and after 30 minutes it was reduced to 140 ft (Royal Navy Table 5D; U.S. Navy Table 4). At this time vision had improved, but there appeared to be some loss of visual fields. When the patient's vision again deteriorated with blurring of peripheral vision, he was again recompressed to 165 ft and maintained there until he regained vision subjectively. During the subsequent decompression, again on Table 5D, he complained

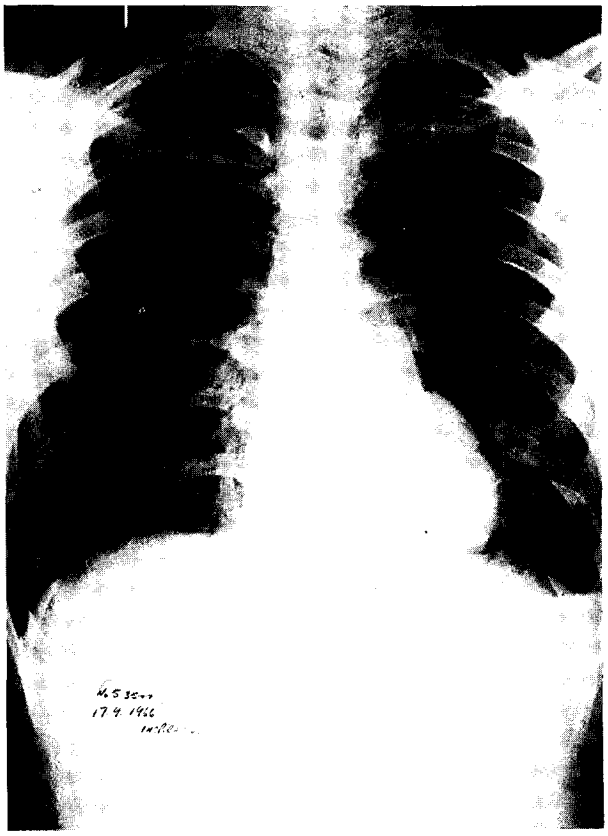

Fig. 3. *Case 57*; post-treatment radiogram shows cystic areas at right and left lung bases and some mediastinal emphysema.

of retrosternal discomfort spreading up towards his laryngeal region and, on examination, some subcutaneous emphysema was found about 1 inch below each clavicle. This had completely disappeared before he reached the surface, and there were no residual visual manifestations.

The X rays (Fig. 4) showed considerable mediastinal air and interstitial air at the root of the neck (Fig. 5) (X rays were taken with a portable machine).

CASE NO. 69

This patient, aged 25 years, did not undergo any ascent training in the water. He had completed the preceding compression chamber dive to 100 ft which is undertaken by trainees to familiarize them with ear-clearing. Approximately 5 minutes after returning to the surface at the standard diving rate of ascent, he was noticed to be having difficulty in putting his left arm into the sleeve of his jacket. He later said that he could move his arm but not direct it. He was observed to be repeating a cycle of trying to put his arm in his left sleeve and then brushing back his hair. He then became unable to stand unsupported and was obviously confused. He was immediately recompressed to 165 ft, where he gradually recovered a full range

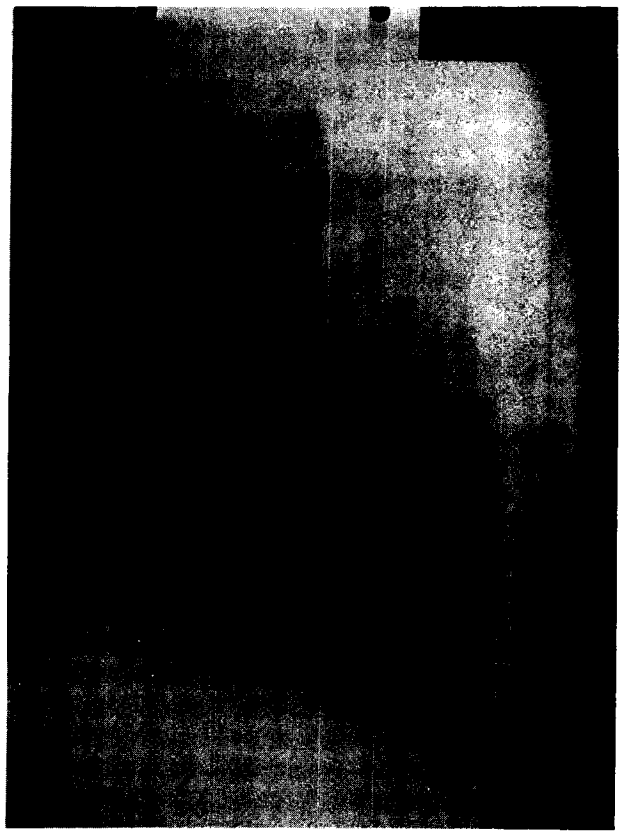

Fig. 4. *Case 63*; radiogram shows considerable mediastinal air.

of movement and power. His level of consciousness returned to normal and, after 30 minutes, decompression began on therapeutic Table 5D (U.S. Navy Table 4).

Immediately on arrival at 60 ft he vomited and presented a left-sided Jacksonian seizure. Pressure was reapplied to 200 ft, and then stabilized at 165 ft until the patient recovered consciousness some 30 minutes later. The subsequent decompression, while the patient remained in a postepileptic state of drowsiness, was slow. At 120 ft he had another major seizure and, after sedation, a very slow decompression was continued. During the subsequent decompression he gradually recovered completely. Chest X-ray examination was normal on arrival at the hospital.

CASE NO. 80

This 27-year-old completed a 100-ft buoyant ascent and, about 1 minute later, complained of pain and a feeling of fullness in his chest. He was not in respiratory distress and there were no neurological manifestations. The diagnosis of a pneumothorax was made and it was decided that recompression was not indicated. His chest X ray (Fig. 6) showed a large pneumothorax which re-expanded over the next few weeks without intervention.

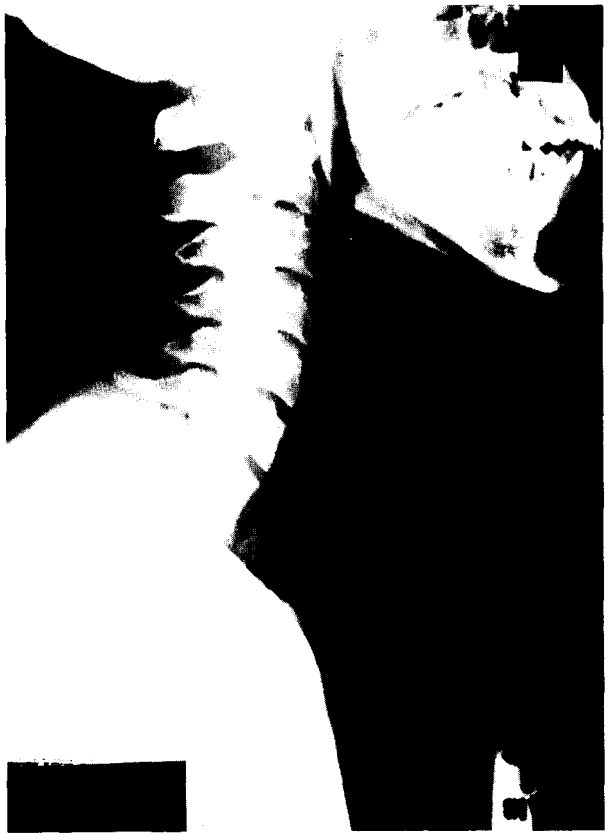

Fig. 5. *Case 63*; radiogram shows interstitial air at base of the neck.

Discussion

These cases have been selected to illustrate some of the clinical features of decompression barotrauma seen in the Royal Navy during the last 20 years. As a result of experience gained from the management of these cases and others, the present principles of prevention and treatment have evolved.

Candidates for buoyant ascent training must be under the age of 35 and, in addition to having no evidence of any pulmonary disease, must have a FEV/FVC ratio of $>75\%$. Nevertheless, it is acknowledged that no physiological test is likely to detect local areas which could predispose to pulmonary barotrauma. Full-plate chest X-ray films in full inspiration and full expiration taken within three months of training must have been read as normal. The criteria for exclusion of trainees on careful consideration of a single PA chest film of good quality are difficult to define precisely, since the earliest radiological signs of local altered lung compliance due either to fibrosis or bullous areas are very difficult to detect. It is felt that the expiratory films enhance the chance of detection, and in at least one case, bullae in the cardiophrenic angle were detected on an expiration film. These were unsuspected and had not been apparent

Fig. 6. *Case 80*; radiogram shows a large pneumothorax.

on serial PA chest films, but were confirmed by subsequent tomography. Any firm conclusion on the value of taking this additional view awaits further experience. It seems likely that should it prove of value, refinement of radiographic positioning in the inspiration and expiration film will be required, and an AP film may be of value. If this technique is adopted, it seems likely that chest X rays using a high kV technique should be employed, with exposure actuated by lung inflation pressure.

The treatment of cerebral air embolism based on the tables of Van der Aue, Brinton, Kellar and Behnke (1) is immediate recompression to 50 m (165 ft). While other authorities may use U.S. Navy Tables 5A and 6A successfully with just 15 to 30 minutes at depth, our experience is that a recurrence is less likely to follow if the patient is retained at maximum depth for a longer period of time. If fully rid of manifestations, decompression may begin after 60 minutes using R.N. Table 55 (previously R.N. Table 5D; U.S.N. Table 4). If not completely cured, the patient is kept at depth for the full 120 minutes and decompressed on the same table. This table is now used with one minor modification which was introduced on the occasion of Case 69, the patient who was unconscious until leaving maximum depth and who then had epileptiform convulsions during decompression. Since the underlying lesion precipitating the seizures

may have been the expansion of a trapped intravascular bubble, it was felt that linear rates of ascent (R.N. Table 71), rather than stoppages, might be an advantage, especially at the shallower depths.

The accumulation of clinical experience is, fortunately, a slow process: there have only been 88 cases in 20 years from some 200,000 trainee-ascents and an unknown but similar number of instructor-ascents. No statistically valid conclusions can yet be drawn on the effectiveness of insisting on a minimum duration of 60 minutes at 50 m (165 ft) for all cases of cerebral air embolism. While the subsequent long decompression is more arduous for the compression chamber staff than the shorter tables, we believe it is justified by potential benefit to the patient. Certainly it is unlikely to be less beneficial than the more rapid decompressions used elsewhere, and the figures so far accumulated tend to support our view.

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DECOMPRESSION SICKNESS AFTER SATURATION DIVING*

R. de G. Hanson, J. Vorosmarti, Jr. and E. E. P. Barnard

During the years 1971-1974, a series of saturation dives was carried out in the Deep Trials Unit (DTU) at the Royal Naval Physiological Laboratory (RNPL), Alverstoke. The purpose of these dives was to develop empirically a decompression schedule suitable for use after exposure to a pressure equivalent to 250 meters of seawater (26 bar) for one week. The development of these tables has already been described by Barnard (1) (*dives 1 to 39*) and Vorosmarti (11) (*dives 40* on). Seventy-two dives were carried out during these years; of these, 48 were involved in the development of a decompression schedule.

During the dives the atmosphere was controlled to maintain a P_{O_2} of 0.22 bar and a P_{CO_2} of <0.005 bar; the nitrogen content was <0.02 bar. The chamber was vacuumed before filling with the oxygen-helium diving mixture. The chamber temperature was maintained between 27 and 31 °C, depending on the depth of the dive. The relative humidity varied between 60% and 80%.

For this survey, only cases where the reapplication of pressure was used to relieve symptoms have been included. Because the numbers are relatively small and the variables numerous, it was felt that any statistical analysis would be valueless or misleading, so none has been attempted. In addition to the cases included in the survey, there were numerous cases of mild pain or niggles which did not require treatment. These latter symptoms usually appeared almost immediately after a pressure drop and disappeared within a few (<10) minutes. Divers also occasionally reported feeling "something moving" in the joints, and in one case one diver had a substantial amount of gas in his knee joints (6). A similar case has also been reported after a dive in Marseille (unpublished data). During the course of the schedule development dives, there were 36 cases of decompression sickness. These occurred during 25 of the 48 dives. It is interesting to note how close this figure is to the 50% bends rate predicted by Barnard for his original experimental design. It is also reassuring to note that as yet there have been no bends in the "proving" dives, though occasional niggles warn that these dives were not too far to the safe side of the line.

Variation in Decompression

The decompressions used can be divided into three phases; typical dive profiles are shown in Fig. 1. The first, which included *dives 1–27*, consisted of 24-hr stages with large pressure

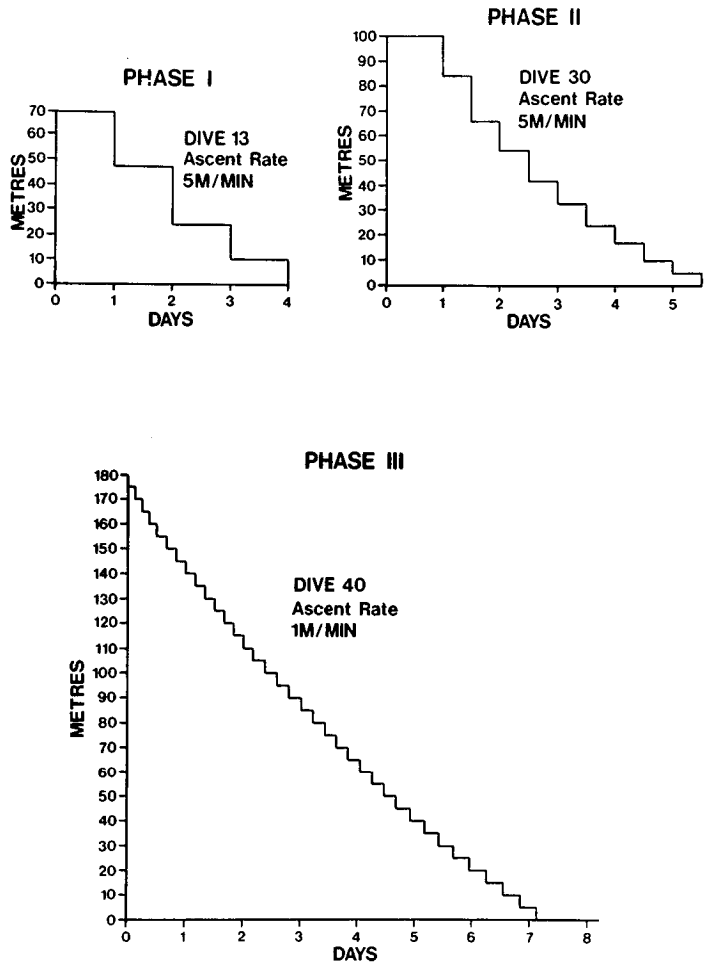

Fig. 1. Typical dive profiles for three phases of development dives.

drops. These large drops were based on ratios of 2:1 from 10 m to the surface, 1.4:1 for drops from 100 m to 69 m, and 1.5:1 for 108 m to 69 m. As can be seen, this meant pressure drops of not less than 10 m and not greater than 39 m. The rate of change of pressure during the pressure drops was 12 m/min for *dives 1* to *8*, i.e., for dives down to 50 m, and 5 m/min for *dives 9* to *27*, which included dives down to 100 m. The second phase, *dives 28* to *34*, from 100–138 m, while still maintaining relatively large pressure drops and lengthy stages, reduced the stages to 8 or 12 hr, and the pressure drops ranged from a minimum of 5 m near the surface to a maximum of 17 m; the majority of the pressure drops ranged between 10 and 12 m. The rate of change of pressure during the drops remained 5 m/min. The last phase of the development dives, *dive 35* on, consisted of dives where the drop in pressure was 10 m or less; after *dive 36*, the drops were 5 m or less and the stages varied from 2 to 7 hr. The changes of pressure between stages during this last phase were carried out at a rate of 1 m/min. As Table I shows, the short, intermediate phase of development dives did not give

TABLE I

CASES OF DECOMPRESSION SICKNESS IN EACH PHASE OF DEVELOPMENT DIVES

Phase	No. Dives	Dives Giving Decompression Sickness	Cases
1	26	13	17*
2	7	0	0
3	15	12	15
Total	48	25	32

*Excludes 3 cases of knee pain which arose during therapy.

rise to any cases of decompression sickness which required treatment; there is thus a direct comparison of cases occurring after large pressure changes after a 24-hr stage, and cases which developed after a small pressure change following a much shorter stage.

TYPES OF DECOMPRESSION SICKNESS

The cases of decompression sickness were mainly of the joint-pain type. However, there were cases in which there was central nervous system involvement (Table II). The same table shows that 28 cases involved the lower limbs only, while 3 cases involved the upper limbs only. Both upper and lower limbs were involved on two occasions. This finding is in contrast to the findings of Kidd and Elliott (7), Rivera (9), and Slark (10), who were dealing with nonsaturation dives, but it agrees with observations of decompression sickness in caisson workers (8) and in deep oxygen-helium diving (7). It may be noteworthy that the cases of upper limb involvement occurred during the early shallow part of the series, while in the deeper dives only the lower limbs were involved. Except for the two cases of eighth nerve involvement, the central nervous system symptoms consisted of tingling or paresthesia in association with joint pain. There was one incident which occurred that is worthy of separate note. A diver complained of headache and visual disturbance 2½ hr after arriving at a 60-m stop during a dive from 180 m. The oxygen was increased from 0.2 to 0.4 bar, and he was immediately recompressed to 65

TABLE II

SITE OF DECOMPRESSION SICKNESS

Site	Phase 1	Phase 3
Lower limbs	13	15
Upper limbs	3	0
Both limbs	2	0
Eighth nerve	2	0
Other central nervous system	3*	0**

*Associated with joint pain; **see text for details.

m, with complete relief of symptoms at 61 m. Subsequent decompression was uneventful. On surfacing, this diver was referred to the hospital for assessment and was diagnosed as suffering from migraine. The fact that he had no previous history of attacks and appeared to be relieved by immediate reapplication of pressure and increase of the oxygen partial pressure may be fortuitous. However, a combination of scintillating scotomata and headache has been reported as symptomatic of dysbarism, especially in the hypobaric field (4, 5). Since it is difficult to be certain of the diagnosis, this incident has been excluded from the summary. Unfortunately, the patient has now left the Navy, and it has not been possible to follow up this case.

One of the cases of eighth nerve involvement has already been reported by Coles (3); it showed mixed auditory and vestibular involvement. The other case which demonstrated only auditory symptoms presented as sudden onset of sensorineural deafness 1 hr after the diver dropped to 47 m from 69 m, where he had been for 48 hr. He was recompressed to 75 m, and showed a marked improvement in his hearing after 69 m was reached. At the time of the incident, the diver was suffering from otitis externa, which may account for a certain degree of conductive deafness. Over the next few months he showed further recovery, but a residual sensorineural deafness of 35 dB over the middle frequencies persisted.

Treatment

During the course of the experiments, three types of treatment were used, as shown in Fig. 2. In addition, Royal Navy Treatment Table 61 was used for cases which occurred after surfacing and occasionally for those which occurred near the surface. The first of these treatments, (A), was based on a bleed ratio of 1.3:1 over 5 hr, as suggested by Barnard (1). This was used for

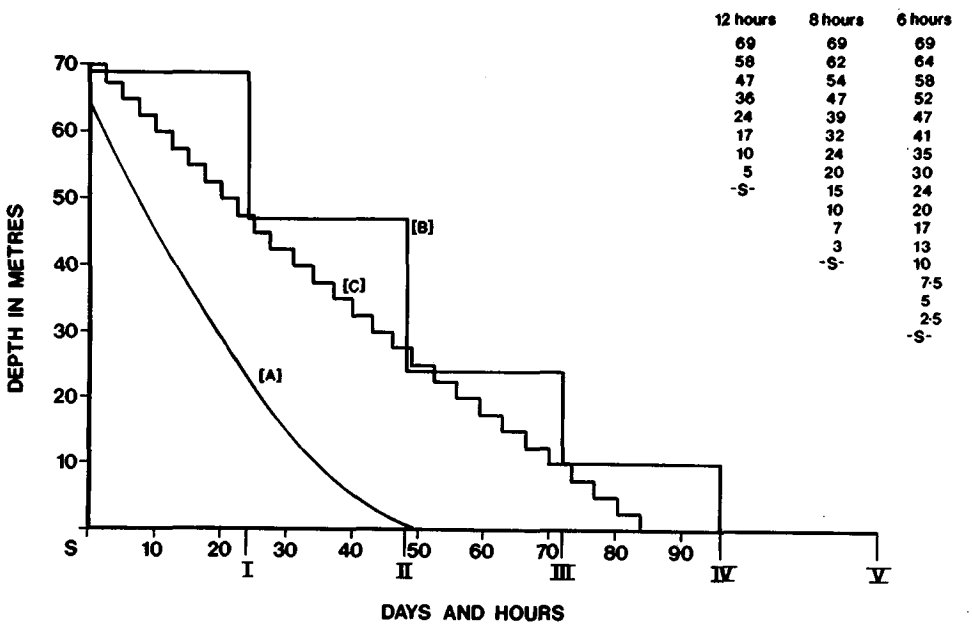

Fig. 2. Three types of treatment schedule used in development dives.

the first cases but was abandoned after *dive 15* due to the high incidence of recurrence in the diver and the occurrence of bends in the diver's companions during therapy on this treatment schedule. The second treatment, (B), was based on the success of a series of 24-hr stages from a depth of 69 m, which had been reached by *dive 15*. These stages are shown in Fig. 2. This schedule was used in the following way. The medical officer on duty at the time of the incident decided whether to use a series of 6, 8, or 12-hr stages (see Fig. 2). After *dive 27*, the treatment employed, (C), was to raise the oxygen partial pressure from 0.22–0.4 bar, recompress in 5-m stages to the depth of relief, hold for 6 hr, and then rejoin the original schedule while holding the oxygen at 0.4 bar and halving the pressure drops from 5 m to 2.5 m. This meant that the decompression after treatment varied with the experimental program.

There was no correlation between the depth of the original dive and the ΔP required to relieve the symptoms in cases of bends following either the large drops or those following the small drops. Neither was there any correlation between the depth of the bend and the ratio of pressure required to relieve the symptoms. These relationships can be seen in Table III. The mean ratios required to relieve the symptoms were 1.4 for the pain-only bends and 1.7 for bends with central nervous system signs. These ratios compare with those of 1.3 and 1.6 cited by Barnard (1) and 1.5 and 1.9 cited by Kidd and Elliott (7).

Results of using the three types of treatment (A, B, and C) are shown in Table IV. As this table shows, there were occasional complications which arose during treatment. These consisted either of recurrence of symptoms in the affected diver or symptoms arising de novo in one of his companions. At one time it was felt that the recurrence of symptoms was due to leaving the depth of relief too soon. However, Table IV shows that this was probably not the complete answer, since over half of the cases with recurrences had spent 6 or more hours at the depth of relief. It is of interest to note that of the 11 recurrences, 8 occurred during 6 treatments when large drops were being used, and only 3 occurred when using treatment C. The three instances of bends arising in the affected diver's companions during the treatment schedule arose during the early treatments (Table IV).

The treatment of these recurrences, and of new cases which occurred as a rule at the shallower depths and were minor in degree, consisted of a slight recompression or pause in the ascent followed by smaller drops, and was usually accompanied by spells of oxygen breathing at depths less than 18 m.

Discussion

The anatomical site of the pain in the bends cases is of interest because there was such great disparity in the number of times that the lower limbs were affected compared to the upper limbs (Table II), a distribution similar to that found in caisson workers rather than divers. A possible explanation for this may be the exercise routine which was carried out by the subjects. Ferris and Engel (4) point out that the site of bends pain can be markedly influenced by straining exercises. These authors were discussing findings in hypobaric decompression sickness, but it is felt that the same rationale could well apply to these cases. The divers' exercise schedule consisted of a morning and afternoon session of 1½ hours' duration each. The exercises consisted of the three divers taking turns with a bicycle ergometer, a rowing machine, and a form of chest expander. As can be seen, these exercises involved the lower limbs to a much greater extent than the upper. Another factor which may have played a part is that, like caisson workers, the diver in the compression chamber is in a weight-bearing condition, in

TABLE III

RELATIONSHIPS AMONG DEPTH OF DIVE, ONSET OF SYMPTOMS AND RELIEF OF SYMPTOMS

Dive	Symptom	Relief	ΔP	Relief/Symptom Ratio
Pain-Only Symptoms				
6.1	3.4	4.3	0.9	1.3
6.1	1.0	2.8	1.8	2.8
8.5	5.1	5.4	0.3	1.1
8.3	3.4	4.2	0.9	1.2
8.3	3.4	4.0	0.6	1.2
7.9	2.0	2.5	0.5	1.3
11.0	2.0	3.0	1.0	1.5
11.0	2.0	2.7	0.7	1.4
10.2	2.3	2.8	0.5	1.2
10.2	2.3	2.8	0.5	1.2
7.4	3.2	3.0	-0.2	0.9
11.8	6.1	6.6	0.5	1.1
11.8	6.1	6.6	0.5	1.1
11.8	6.1	7.0	0.9	1.1
7.4	2.0	2.2	0.2	1.1
6.8	3.4	3.9	0.5	1.1
19.0	1.0	2.8	1.8	2.8
18.7	5.5	5.6	0.1	1.0
19.0	8.5	11.5	3.0	1.4
19.0	9.0	11.0	2.0	1.2
19.0	0.3	0.5	0.2	1.7
19.0	7.5	8.0	0.5	1.1
19.0	7.5	8.0	0.5	1.1
22.0	3.5	3.7	0.2	1.1
22.0	2.5	2.6	0.1	1.0
22.0	2.3	2.5	0.2	1.1
22.0	2.0	2.5	0.5	1.3
22.0	2.0	2.1	0.1	1.1
19.0	2.5	3.0	0.5	1.2
19.0	1.5	2.0	0.5	1.2
CNS Symptoms				
6.7	2.5	5.5	3.0	2.2
6.1	3.4	4.3	0.9	1.3
8.5	5.7	6.5	0.8	1.1
10.2	5.7	7.7	2.0	1.4
11.0	5.7	7.9	2.2	1.4

Values are depths in bars; mean ΔP's for pain-only and central nervous system bends were 0.7 and 1.78, respectively, and mean ratios were 1.3 and 1.5, respectively.

TABLE IV

COMPARISON OF TREATMENT SCHEDULES

Schedule	No. Times Used	No. Patients	Failure of Treatment	Causes of Failure	
				Recurrences	New Cases
A	3	5	2	3	1
B	9	13	4	5	2
C	12	14	2	3	—
61	3	4	—	—	—

TABLE V

COMPARISON OF TIME SPENT AT DEPTH OF RELIEF

Recurrence	No Recurrence
2	2
1	2
1	0.75*
6	8
6	6
4	6
9	6
9	0.75*
----------	0.75*
6	9
6	10
4	8
	8

	0.75*
	6
	6
	6
	2
	4
	6
	6
	6
	6
	6
	Mean (excluding
Mean 4.73	Table 61) 5.95
	Mean 5.08

Values are hours at relief depth; * = treatment on R.N. Table 61; dotted line indicates boundary between large and small pressure drops.

contrast to the diver in the water, who is weightless. The upper limb bends and central nervous system decompression sickness which did occur happened only in those dives with large pressure drops. It may be that central nervous system involvement tends to occur at a greater tissue ΔP than does simple pain. Both the cases of eighth nerve involvement occurred after pressure drops of 22 m which had been preceded 24 hours previously by drops of 23 m and 31 m, respectively.

The correlation of the findings in this series with regard to the pressures required for the relief of symptoms with those reported previously by Barnard (1) and Kidd and Elliott (7) is also of interest. Not only are the ratios of these pressures very similar, as has already been mentioned, but the absolute pressure change required for the relief of pain was also similar (Table VI). This pressure differential is remarkably small when compared to the pressure used for treating cases which occur on the surface after diving. In the present series there was little difference in the ΔP required for the relief of pain following large drops and those following small drops. In fact, the mean ΔP required after the large drops was 0.63 bar compared to 0.73 bar after the smaller drops. However, it is felt that this small difference between the two is not significant. The fact that a greater ΔP was required for the more serious cases was to be expected, but there is a marked discrepancy between the mean pressure differential required in this and in Kidd and Elliott's series compared with that of Barnard's.

A comparison of the treatment schedules (Fig. 1) and the results of using them (Table IV) shows that the original scheme of treatment A, which was based on a 1.3:1 ratio over 5 hr, was too rapid. Schedule B, though longer than most schedule C treatments, gave rise to a greater number of recurrences and bends in the attendants. However, it must be remembered that schedule C used a Po_2 of 0.4 bar while schedule B used a Po_2 0.2 bar on all except the last two schedule B treatments, when the Po_2 was raised to 0.4 bar. During these last two occasions, there were no recurrences. If the cases treated on schedules B and C are combined and then divided according to whether the Po_2 was 0.2 bar or 0.4 bar, the figures are as follows: 0.2 bar was used 7 times and failed 4 times; 0.4 bar was used 14 times and failed twice. This Po_2 difference may be of more relevance than the difference in the ΔP of the drops of the treatment schedules. Table IV shows the difference between the two schedules, and from these and the figure quoted above, it can be seen that a successful treatment is more likely when a Po_2 of 0.4 bar and small pressure drops are used.

TABLE VI

MEAN ΔP REQUIRED FOR RELIEF OF SYMPTOMS

	Pain-only		CNS	
	No. Cases	ΔP	No. Cases	ΔP
Present series	30	0.7	5	1.78
Kidd and Elliott (1)	17	0.63	17	1.11
Barnard (2)	17	0.86	6	5.46

Values are mean ΔP's in bars.

Conclusions

It is difficult to draw hard and fast conclusions from a series where the individual dives, and even the basic experimental plan, were constantly altering. However, certain points which emerge should be remembered because they may help to lend weight to findings of others studying the problems of decompression sickness. These points are:

(1) The site of bends during saturation diving is similar to the site of bends in caisson workers, rather than those found in "bounce" diving;
(2) Large pressure changes appear more likely to cause central nervous system symptoms than small ones; and
(3) Treatments using a P_{O_2} of 0.2 bar were not satisfactory.

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STUDIES OF DECOMPRESSION SICKNESS IN JAPANESE DIVING FISHERMAN

K. Hayashi, M. Kitano, M. Kawashima, T. Torisu and S. Matsuoka

The sea is said to cover about 70% of the surface of the earth, and it has attracted the interest of mankind since the beginning of time. In Japan, which is surrounded by the sea, there are many professional divers, some of whom have had 20–30 years of diving experience. The Kyushu Rosai Hospital (Kyushu Labor Accident Hospital) was equipped with hyperbaric chambers for therapeutic and experimental purposes in 1966. From 1966 to 1974, 176 patients with decompression sickness, or so-called "diver's disease," were hospitalized. This article will present results obtained from the clinical and statistical analysis of 176 cases of the disease, including pathological findings from three autopsied cases.

Subjects

Age ranged from 19 to 62 (average, 34.5 years). Seventeen of the 176 cases were female. In regard to the diving method used by the subjects, 34.1% used a helmet, 28.4% used a mask, and 26.7% employed SCUBA (Table I). As for diving experience, the subjects had from less than 1 year to more than 25 years; 80% of the cases had between 1-14 years' experience.

TABLE I

Age Distribution and Diving Method of 176 Hospitalized Patients

Age	Method of diving				Total	Percentage
	Helmet	Mask	Aqualung	Unknown		
Less than 20	0	0	1	0	1	0.6
20-24	10	1	5	1	17	9.7
25-29	11	8(2*)	14	6	39(2*)	22.2
30-34	11	8	9	5	33	18.8
35-39	13	13(4*)	10	5	41(4*)	23.2
40-44	8	10(6*)	5	1	24(6*)	13.6
45-49	0	6(3*)	1	1	8(3*)	4.5
More than 50	7	4(2*)	2	0	13(2*)	7.4
Total	60 (34.1)	50(17*) (28.4)	47 (26.7)	19 (10.8)	176(17*) (100.0%)	100.0%

Mean age = 34.5 yr; * = female; figures in parentheses below totals are percent of series.

Results

Upon admission, clinical findings revealed that the most frequent symptom was localized pain (41.7%), referred to as bends or the musculoskeletal type of decompression sickness. Motor involvement (paralysis or paresis) was present in 13.1% of cases, and urinary disturbance was noted in 11.9% of the cases which were diagnosed as spinal cord injuries. In addition, dizziness and vertigo (4.2%), tinnitus (2.9%), nystagmus (0.6%), and nausea or vomiting (2.6%) were observed. These symptoms may have been due to Ménière's syndrome, a labyrinthine disturbance which frequently accompanies auditory involvement. A few patients (2.2%) showed unconsciousness, perhaps secondary to brain damage rather than caused by shock. Finally, dyspnea, cough (1.6%), and chest pain were observed secondary to cardiopulmonary lesions (chokes) (Table II).

Table III shows a symptomatologic classification, i.e., brain (1.1%), spinal cord (27.3%), Ménière's syndrome (5.7%), cardiopulmonary (chokes) (2.3%), and musculoskeletal or bends (63.6%). Analysis of the site of bends pain revealed that the most frequently involved sites were shoulders (41.5%), knee joints (31.3%), and elbow joints (13.6%).

The 48 cases of spinal-cord decompression sickness showed different clinical features from traumatic spinal cord lesions. Only 15 of these patients demonstrated complete transverse lesions, para- or tetraplegia, rectourinary or bilateral sensory involvement.

Sensory involvement was noted in 42 cases. Incidence and level of cord injury were 33.4% at D9, 16.7% at D5-6, 14.3% at C2-3, 9.5% at D1-2, and 9.5% at L5 (Table IV).

Lumbar puncture was performed on the eight most recent cases of this type, and six of the eight cases showed some abnormal findings, i.e., increased pressure in three cases, pleocytosis in one case, and increased protein content in four cases. These findings may have been due to edema of the spinal cord.

TABLE II

FREQUENCY OF SIGNS AND SYMPTOMS PRESENTED IN 176 CASES

Signs and symptoms	No. of cases	Percentage
Localized pain	130	41.7
Anesthesia or hypesthesia	45	14.4
Paralysis or paresis	41	13.1
Urinary disturbance	37	11.9
Dizziness or vertigo	13	4.2
Headache	10	3.2
Tinnitus	9	2.9
Nausea or vomiting	8	2.6
Unconsciousness	7	2.2
Dyspnea or cough	5	1.6
Auditory disturbance	3	1.0
Visual disturbance	2	0.6
Nystagmus	2	0.6
Total	312	100.0

TABLE III

TYPES OF DECOMPRESSION SICKNESS IN HOSPITALIZED PATIENTS

Type	Year									Total (%)
	'66	'67	'68	'69	'70	'71	'72	'73	'74	
Brain	0	0	1	0	0	0	1	0	0	2 (1.1)
Spinal cord	1	1	6	4	5	4	6	10	11	48 (27.3)
Ménière's	0	2	1	1	0	2	0	1	3	10 (5.7)
Chokes	0	1	1	0	0	0	0	1	1	4 (2.3)
Bends	3	26	34	16	4	6	6	12	5	112 (63.6)
Total	4	30	43	21	9	12	13	24	20	176 (100.0)

TABLE IV

LEVELS OF SPINAL INVOLVEMENT
(MOST DISTAL UNINVOLVED SEGMENT)

Level	No. of cases	Percentage
C2-3	6	14.3
D1-2	4	9.5
D5-6	7	16.7
D6-7	3	7.1
D8	1	2.4
D9	14	33.4
D12-L1	3	7.1
L5	4	9.5
Total	42	100.0

Cerebrospinal fluid findings in a 26-year-old male patient with spinal-cord decompression sickness 3 days after onset were as follows:

Nonne-Apelt (+); Pandy (+ + +); cell 239/3; protein 98.0 mg/dl; sugar 64 mg/dl; Cl 141 mEq/liter; pressure 75 mmH_2O; and Queckenstedt prompt. At 9 days after onset, findings were Nonne-Apelt (−); Pandy (+); cell 29/3; protein 50.3 mg/dl; sugar 66 mg/dl; Cl 130 mEq/liter; pressure 170 mmH_2O; and Queckenstedt prompt. At 8 months after onset, findings were: Nonne-Apelt (−); Pandy (−); cell 2/3; protein 18.0 mg/dl; sugar 55 mg/dl; Cl 123 mEq/liter; pressure 270 mmH_2O; and Queckenstedt prompt.

Although severely abnormal fluid measurements (except the pressure measurement)

returned to normal in a relatively short period, no clinical improvement was obtained. It is our impression that the nature of the CSF findings in this type of decompression sickness may help in determining the prognosis, but more cases need to be added before further analysis.

Table V shows the latency period of each disease type. All of the cases, except bends, evidenced symptomatology within three hours, while about 45% of the bends cases showed an asymptomatic period of more than three hours.

Table VI shows the effects of recompression therapy for decompression sickness of the cases in our study. Recompression therapy was remarkably effective, since 125 of the 161 cases for which follow-up information was available recovered completely; the divers resumed their previous diving work. Even in cases of the spinal-cord type, 20 of the 41 showed complete recovery.

Various kinds of medical examination and tests were carried out. The bone X-ray findings have already been described in other papers (1, 8). ECG examination revealed 48 cases of

TABLE V

LATENCY PERIOD OF DECOMPRESSION SICKNESS

Type	During dive	During ascent	Within 30 min	30 min. to 1 hr.	2-3 hours	Total	4-6 hours	7-12 hours	13-24 hours	More than 24 hr.	Grand Total
Brain	0	0	1	0	0	1	0	0	0	0	1
Spinal cord	1	9	23	5	4	42	0	0	0	0	42
Ménière's	2	0	1	0	6	6	0	0	0	0	9
Chokes	0	0	1	0	0	1	0	2	0	0	3
Bends	2	11	17	5	17	51	6	2	1	33	94

TABLE VI

EFFECT OF RECOMPRESSION THERAPY

Type	Status before recompression	No. of cases	Status after recompression: Returned to previous diving	Able to do easy ground work	Incapacitated	Dead	Total
Brain	Monoplegia (r. upper extremity)	1	1	0	0	0	2
	Semicoma	1	0	1	0	0	
Spinal cord	Complete lesion	14	0	5	7	2	41
	Incomplete lesion	27	20	6	1	0	
Ménière's	Tinnitus, hearing loss, vertigo	10	8	2	0	0	10
Chokes	Loss of consciousness	2	0	1	0	1	3
	Chest pain	1	1	0	0	0	
Bends	Arthralgia, myalgia	105	95	9	1	0	105
	Total	161	125	24	9	3	161

sinus bradycardia in 153 cases. In other examinations such as peripheral blood count, urinary analysis, and serum analysis, no remarkable changes were found.

Autopsied Cases

Three fatal cases of decompression sickness of divers were autopsied. The correlation between clinical observations and autopsied pathological findings were as follows.

Case 1

A 38-year-old male helmet diver dove to a depth of 40 meters for 4 hours and surfaced in 20 minutes; within the next 20 minutes in the decompression chamber, he lost consciousness and died.

Autopsy was performed seven hours later. There were numerous bubbles in the blood in the right ventricle of the heart and in the superficial vessels of the liver, gastrointestinal tract, and brain. In addition, the blood seemed somewhat concentrated. Congestion and edema of visceral organs were also noted. There was microscopically characteristic vacuolization in the bone marrow cavity, brain (Fig. 1), spinal cord, and other organs. This vacuolization was assumed to be a result of air bubbles formed during decompression because (1) these vacuoles were unstainable by all dyes used in the laboratory, and (2) the tissues adjacent to the vacuoles looked as though they had been compressed by the bubbles.

Case 2

A 36-year-old male SCUBA diver had a history of diving four times to a depth of 60 meters for 30–40 minutes each time, with an interval between dives of 10–15 minutes. He complained of pain in both legs immediately after surfacing from the last dive, and 20 hours later, was

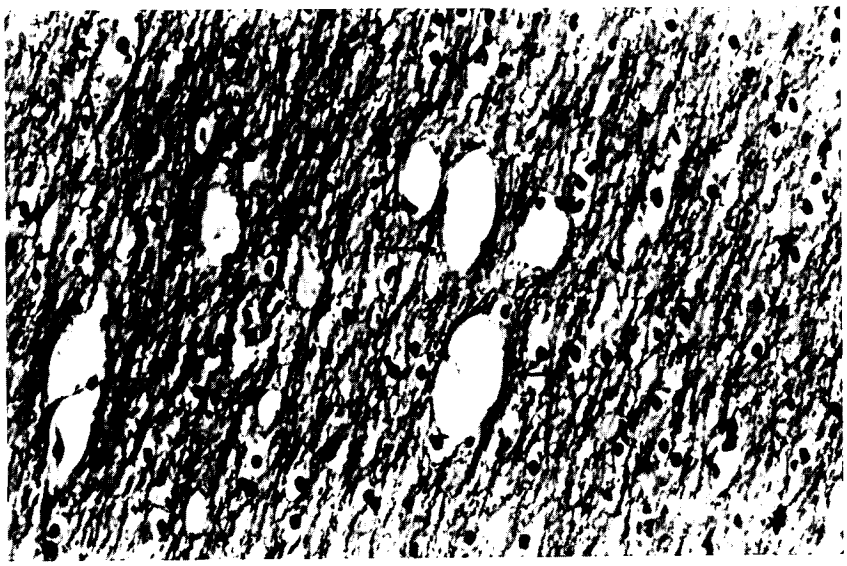

Fig. 1. Vacuolization (shown by the arrow) in the white matter of the brain (Case 1).

transferred to our hospital. Physical examination on admission showed tetraplegia and sensory loss at the C4 level, and this diver died within 5 days despite recompression therapy.

At autopsy, slight crepitation was felt during skin incision, but no bubbles were visible in the blood vessels. Congestion and edema of visceral organs were also noted. Histologically, there were rather widespread necrotic foci with coincidental edema, congestion, and hemorrhage in the brain and the spinal cord, especially in the dorsal segment (Fig. 2). These foci might be called "spongy in appearance," as Haymaker (7) described. The lower dorsal segment of the cord was the most affected part of the cord. Venous thrombi were also found in the dorsal cord.

Case 3

A 20-year-old male SCUBA diver had a history of diving to 40 meters and surfacing in 1-2 minutes; immediately after surfacing, he complained of numbness of both legs and was transferred to our hospital 12 hours later. Physical examination on admission showed tetraplegia and sensory loss at the C4 level. He showed some improvement during recompression therapy on Table 4, but 14 days later he became unconscious and died in cardiac arrest.

Autopsy revealed congestion and edema of the visceral organs. Intravascular bubbles were not visible. Focal edematous necroses with hemorrhage in the spinal cord were found, especially in the cervical segment. These changes were similar to those in Case 2. Pulmonary bleeding was also seen.

Oil-red-O staining of frozen sections was performed, and a few fat emboli were found in the lungs of the three autopsied cases.

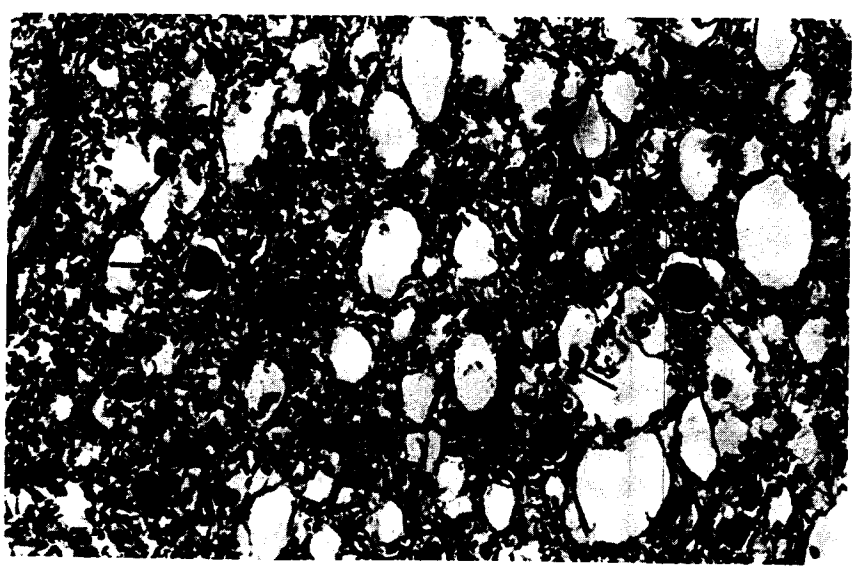

Fig. 2. Spongy appearance of the spinal cord; swelling of the axon (shown by arrow) is remarkable (Case 2).

Discussion

Symptomatology of Decompression Sickness

Decompression sickness has various symptoms, according to the site of the damage. However, certain factors, such as the fat content in the tissues and the blood supply to the tissues, vary with different symptoms and sites. The simple and often-used classification of decompression sickness into Type I and Type II cases, a classification used by Golding et al. (5) and many other authors, was not adequate for the cases reported in this paper. In these cases, the spinal-cord type of decompression sickness manifested remarkable pathognomonic symptoms, and differential diagnosis was not difficult in any of these cases.

Etiology of Decompression Sickness

In 1955, Haymaker (7) reported that he had found fat emboli in the vessels of the lungs at autopsy of victims who had died of altitude decompression sickness. Haymaker and other authors (2, 3, 4) stressed that fat emboli may play an important role in decompression sickness, but only a few fat emboli were found in any of the three autopsied cases reported here. This difference may arise from the fact that death in their cases was caused by decompression to high altitude, and this paper's cases died from decompression from the sea bottom.

Hallenbeck et al. (6) pointed out that venous obstruction followed by the direct and indirect action of bubbles may have an important role, especially in spinal-cord decompression sickness injuries.

Venous thrombi were found in the spinal cord of Cases 2 and 3 of this series. These conclusions agree with Hallenbeck: circulatory disturbances, especially in the venous system, are an important etiologic factor in this disease.

Summary

A clinicopathological study was performed on 176 cases of decompression sickness in divers during the past nine years. Based on the predominant signs and symptoms, these cases were classified as follows: (1) brain; (2) spinal cord; (3) Ménière's syndrome; (4) cardiopulmonary; and (5) musculoskeletal. Among these, the spinal-cord cases showed specialized and characteristic features of this disease. Results of the three autopsies showed macroscopic bubbles in Case 1, microscopic vacuolization in the brain, spinal cord, and bone marrow cavity in all of the cases, venous thrombi associated with edematous necrosis in the spinal cord in Cases 2 and 3 and congestion and edema of the general visceral organs in all of the cases.

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STUDIES ON THE HEMOSTATIC SYSTEM OF PROFESSIONAL DIVERS AFTER SHORT-TERM HIGH PRESSURE EXPOSURES IN A DIVING SIMULATOR

H. Beeser, H. Oser, H. Nass and S. Kessler

To prevent severe incidents from causing permanent injury to professional divers during the decompression phase of a dive, pilot studies using these trained subjects may contribute substantially to finding optimal decompression conditions. Periodic investigations for several days after a dive might reveal when minor physiologic changes, which can also occur under apparently safe decompression conditions, again reach normal predive levels. Defects of the hemostatic system known as disseminated intravascular coagulation (DIC) have been found to be regularly associated with severe decompression sickness (4, 7, 12, 13, 15, 19) and to play a role in its pathogenesis (13). The actual changes in the hemostatic mechanism after decompression may therefore be advantageously used as a sensitive indicator to verify the quality of decompression profiles.

Very little data on the influence of decompression on the hemostatic system of human subjects who have performed 100-200 meter dives are presently available. This paper will investigate the practicability of using safe and economic short decompression profiles for these depths instead of existing profiles, which provide considerably longer times but are still not always reliable. The profiles are shown in Fig. 1. In use, these profiles proved to be practically free of symptoms, except for three cases of Type I decompression sickness. Changes in the hemostatic system were used as another parameter to define a safe dive, especially since it has been proven that dysbaric osteonecrosis is caused by frequent, cumulative, discrete episodes of the hyper-coagulable state (15, 21).

Methods

As Table I shows, 18 healthy male professional divers were studied after simulated dives in a dry chamber, partially in a wet pot, at a pressure in the range from 14.5 to 21 bars, with bottom times varying from 20-60 minutes. The breathing gas mixtures used during the dives can be seen in Fig. 1. The following hemostatic parameters were determined before the dive (as a control), immediately after the dive, at 24 and 48 hours after the dive, and in some men at 72 and 96 hours after decompression: platelet count, thrombelastogram, prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), antithrombin III, euglobulin lysis time, and the concentration of the clotting factors I, II, V, VII, VIII, IX, and X. Additionally, the packed red cell volume (PCV), red blood cell counts (RBC), and the hemoglobin concentration (Hb) were measured. Blood samples were collected carefully by alternate antecubital venous punctures.

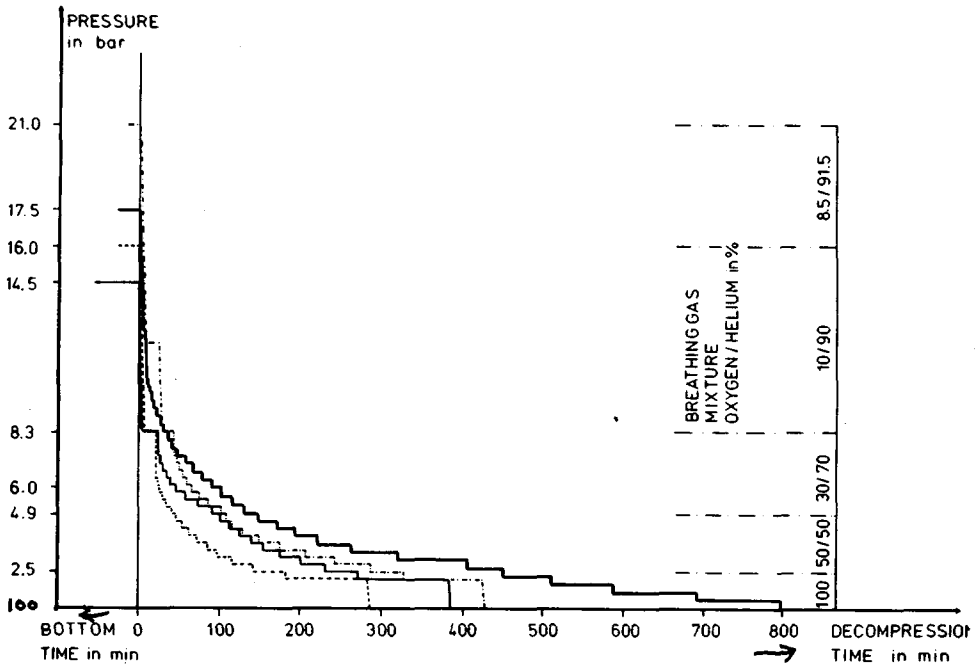

Fig. 1. Deutsche Forschungs-und Versuchsanstalt für Luft-und Raumfahrt (DFVLR) decompression profiles (dashed lines) compared to typical profiles used in the field (solid lines).

Results

As has been mentioned previously, the tested profiles showed a low incidence of decompression sickness, with pain-only bends in three subjects (Nos. 7, 14, and 17); all these cases could be treated easily and adequately with short recompression therapy. For obvious reasons, it was not possible to keep the divers under standardized conditions for long periods after decompression, so that comparable statistical results were not available. These findings are therefore presented as trend analyses (see Tables II-VI).

Table II summarizes the platelet counts of all divers after decompression compared to predive control values. It shows a nearly uniform trend of a drop in platelet count immediately after the dive which is independent of the maximum depth, bottom time, and decompression profile. The degree and duration of this tendency are dependent on the individual and especially on the kind of dive: Subjects 1-6 had the slightest changes and shortest duration of platelet decrease after a 30-minute, 16-bar dry chamber dive. Four subjects of this group showed a tendency for the platelets to increase within 24 hours. The severest platelet decreases were found in Subjects 7-14, all of whom were involved in a wet-pot dive with a longer and heavier work load. Subjects 7-10 had undergone the same bottom times and profiles as those of Subjects 1-6. Three subjects of the 7-10 group showed a distinct tendency of the platelets to fall even 24 hours after the dive. All four subjects had increasing platelet counts 48 hours after the dive, but values had still not reached control levels. Subjects 11-14 differed from the previous group, because they had undergone a 60-minute, 14.5-bar wet-pot dive with longer

TABLE I

SUBJECT, DIVE, AND BLOOD SAMPLING DATA

Subject No.	Date	Pressure, bar	Bottom Time, min	Decompression Time, min	Breathing Mixture, O_2/He	DCS	Blood Samples (hours) Predive	0	24	48	72	96
1	12/2/74	16.0	30	319		−	+	+	+			
2	12/2/74	16.0	30	319		−	+	+	+			
3	12/3/74	16.0	30	319		−	+	+	+			
4	12/3/74	16.0	30	319		−	+	+	+			
5	12/5/74	16.0	30	300	10:90	−	+	+	+			
6	12/5/74	16.0	30	300		−	+	+	+			
7	1/21/75	16.0	30	300	30:70	+ +	+	+	+	+		
8	1/21/75	16.0	30	300	50:50	−	+	+	+	+		
9	1/25/75	16.0	30	303		−	+	+	+	+		
10	1/25/75	16.0	30	303	100:0	−	+	+	+	+		
11	1/23/75	14.5	60	428		−	+	+	+	+		
12	1/23/75	14.5	60	428		−	+	+	+	+		
13	1/24/75	14.5	60	428		−	+	+	+	+		
14	1/24/75	14.5	60	428		+ +	+	+	+	+		
15	4/3/75	21.0	20	374	7.5:92.5	−	+	+	+	+	+	
16	4/3/75	21.0	20	374	30:70	−	+	+	+	+	+	+
17	4/4/75	21.0	20	374	50:50	+ +	+	+	+	+	+	
18	4/4/75	21.0	20	374	100:0	−	+	+	+	+	+	

+ + = Pain-only bends in Divers 7, 14, 17.

decompression times. In two of this group there was still a considerable tendency of the platelets to fall even after 48 hours. In Subjects 15-18, who performed a 20-minute, 21-bar dry chamber dive, there was a noticeable tendency for the platelet count to increase in three subjects immediately after the decompression. This tendency was still evident in Subject 18 as late as 24 hours after the dive. The 48-hr postdecompression samples revealed a platelet drop; there was a more rapid decrease in the 72-hr samples. This tendency persisted in the 94-hr sample of Subject 16.

It is worthy of note that the maximum decrease of the platelets in comparison to the predive controls varied from 10,000 to 100,000/mm^3. The total platelet count dropped in three subjects for a short time to slightly pathological values of 90,000-100,000 thrombocytes/mm^3.

It can be seen from Table II that nearly all subjects, immediately after the decompression, showed a reduced maximal thrombus elasticity (mξ) as determined by the thrombo-elastograph (TEG), which can be considered an index to thrombus solidity; however, after 24 hours a counter-regulation was observed. This parameter also showed pronounced changes in that group which performed the dive in the wet pot.

The reaction of factor IX activity to decompression is especially interesting because of its surface sensitivity, which in this context would involve the intravascular bubble-liquid inter-

TABLE II

PREDIVE PLATELET COUNTS, TEG, mξ AND FACTOR IX ACTIVITIES AND POSTDECOMPRESSION TENDENCIES

Subj. No.	Platelets, $\times 10^3/mm^3$						TEG, mℰ					FIX, %					
	I	II	III	IV	V	VI	II	III	IV	V	VI	I	II	III	IV	V	VI
1	170	↓	↑				↑	↓↓↓				72	←	↓↓			
2	215	↓	↑				↑↑↑	↓↓				40	↑↑	↓↓			
3	159	↓	↓				↓↓	↑↑				45	←	↑↑↑			
4	147	↓	↑↑				↓↓	↑↑				74	↑↑	↓↓			
5	212	↓	↓↓				↓	←				195	↓↓	↓↓			
6	190	↑	↓↓				↑	↑↑				45	↑↑↑	↑↑			
7	250	↓↓↓	↓↓	↑↑↑			↓↓↓	↑	↓			77	↓	↓↓	↑↑↑		
8	218	↓↓	↓↓↓	↑↑			↓↓	↓	↑			100	↑↑	↓↓	↓		
9	135	↑↑	↓↓↓	↑↑↑			↓↓	↑↑↑	←			250	↓↓	↓	←		
10	142	↓↓	↑↑	↑			↓↓		↑↑			125	↑↑	↑↑↑	↓↓↓		
11	164	↑	↓↓	↓↓				↑↑↑				74		↑	↓↓		
12	195	↓↓	↑↑	↑			↓↓		↑↑			150	↑↑↑	↓	↓↓		
13	250	↓↓	↓↓	↑↑			↓↓	↓↓↓	↑↑↑			77	↑↑	↓	↓↓		
14	218	↓↓↓	↑↑	↓↓↓			↓↓↓	↑↑	↑↑			100	←	↑↑	↑↑↑		
15	205	↓↓	←	↑	↓↓		↑	←	↑↑	↓↓		175	↓↓↓	←	←	↓↓↓	
16	230	↑	↓↓	↓	↓↓	↓↓	↑↑	↑	←	↓	↓↓	85	↑	↓	↑	↑↑↑	←
17	140	↑	↓	↓	↓↓		↓	↑	↑	↓		84	↑↑	↓↓	↑	↑↑	
18	150	↑↑	↑↑	↓	↑		↓	↑	↓	↓		55	↓	↑	↓	↑↑↑	

I = predive values; II = immediate postdive values; III = 24 hr postdive; IV = 48 hr postdive; V = 72 hr postdive; VI = 96 hr postdive. One arrow = moderate tendency; two arrows = pronounced tendency; three arrows = strong tendency; direction of arrow indicates direction of tendency.

faces. In the majority of divers, this activity was found to be considerably increased immediately after surfacing. This tendency continued 24 hours postdive in six of the divers.

In the 200-m dives (Subjects 15–18), it was remarkable that the increase of Factor IX activity was most pronounced 72 hours postdive. The Factor IX activity increase, in comparison to the predive control, varied from 10 to 230% of the normal level (mean of 60%). The 230% activity increase was observed in Subject 14 after a 60-min/14.5-bar wet-pot dive. This subject developed Type I bends 3 hours after surfacing.

Noticeably high Factor IX activities were found in the predive control samples of Subjects 5, 9, and 15. In contrast to the others, these subjects showed a considerable decrease in activity of Factor IX after the decompression, in the range of 45–100% of normal.

Table III lists the changes in the activity of Factors V, VIII, and X, and of antithrombin III. Clotting activities V, VIII, and X showed no uniform tendency to change. The variations noted should be considered individual regulation processes, which are disregarded in this paper. It should be mentioned that Factors V and VIII revealed high activities in a few of the

TABLE III

PREDIVE ACTIVITIES AND POSTDIVE TENDENCIES OF FACTORS V, VIII, X AND ANTITHROMBIN III

Subj.	F V,%					F VIII,%					F X,%				Antithrombin III,sec			
No.	I	II	III	IV	V	I	II	III	IV	V	II	III	IV	V	II	III	IV	V
1	91	↑	→			88	→	→			↓	→			↑	↓↓		
2	99	↑	→			100	→	↑			↑	↑			↑	↑↑		
3	120	→	↑↑			98	↑	↑↑			↓	↑↑			↓	↑↑		
4	155	↓↓	↓			140	↑↑	↓↓↓			↑↑	↓↓			↑↑	↓		
5	99	↑	↓			100	→	↑			↑↑↑	↓↓			↑	↓		
6	125	↓↓↓	↓↓			115	→	↑↑			↑↑	→			↑	↓		
7	170	↓↓↓	→	↓↓		200	↓↓↓	↑	↓		→	↓	↑↑		↓	↑	↑	
8	94	↑	↓	↑		94	↓	↑	↓		↑↑	↓↓	→		↑	↓↓	↑	
9	115	↓	↓↓	↑		110	↓	↓	↑↑		↓	↓	↓		↑	↓	↑	
10	135	↓↓↓	↓↓	↑		200	↓↓↓	→	↓		→	↓	↑		↑	↓	↑	
11	68	↑	↑↑	↓↓		90	↑↑	↓	↓↓		→	↑	→		↑	↑	↓	
12	84	↓↓	↑↑	↓		100	↓	→	↓		↓	↑	↓		→	↓	↑	
13	170	↓↓↓	↑	↓		200	↓↓↓	→	↑		→	↑	↑		→	↑	↑↑	
14	94	↓	→	↓		94	↓	→	↑↑		↑	↓	↑		↑	↓	↓	
15	120	↓	→	↑	→	135	↑	↓	↑	↑↑	→	↓	→	↑	→	↑	↑	↓
16	150	↓↓	↓	↑	↑	220	↓↓↓	↑	↑↑	↑	↓	↑	↓↓	↑	↓	↓	↓	↑
17	85	→	↓	↑	↓	88	↑↑	→	↑	↓↓	↓	↓↓	↑	↑↑	↑	↓	↑	→
18	69	↑	↓	↑↑	↓	93	→	↑	→	↑	↑	↓	↓	↑	↑	↓	↑	↑↑↑

Symbols are same as in Table II.

subjects before the dive. Antithrombin III activity was moderately increased in nearly all the subjects after decompression, but the increase only lasted longer than 24 hours in a few cases.

Table IV shows the postdive euglobulin lysis time, fibrinogen concentration, and thrombin time. The euglobulin lysis time was reduced in the majority of the divers immediately after surfacing. Some of the subjects already had reduced predive lysis times. The most pronounced changes were again found in Subjects 7-14, the group which worked in the wet pot during the dive. There was a general tendency to regain normal values within 24 hours after the dive.

In the "wet-pot" group there was a distinct rise in fibrinogen concentration which lasted approximately 24 hours. Thrombin time was generally slightly reduced immediately after decompression.

As may be seen from Table V, the activities of Factors II and VII, and prothrombin time (PT) and partial thromboplastin time (PTT) were only moderately changed in all the divers after decompression.

Twenty-four hours postdive, the samples showed a slightly reduced prothrombin activity. As a screening test of extrinsic clotting activation, PT was slightly diminished immediately after the dive. At the same time, PTT indicated a moderately prolonged intrinsic coagulability.

TABLE IV

POSTDIVE TENDENCIES OF FIBRINOGEN CONCENTRATION, EUGLOBULIN LYSIS AND THROMBIN TIMES

Subj. No.	Fibrinogen, mg% II	III	IV	V	VI	Euglobulin lysis, hours II	III	IV	V	VI	Thrombintime, sec II	III	IV	V	VI
1	↓	↓				↓	↑				↓	↑			
2	→	↑				↓	↓				↑	↓			
3	↓	↓				↓	↑				↓	↓			
4	↑	↓				↓	↑				↓	↑			
5	→	↓				→	→				↓	↑			
6	→	→				↓	↓				→	→			
7	⇑	⇑	→			⇓	⇑	↓			↓	→	↑		
8	⇑	→	↑			⇓	↑	↓			↓	→	→		
9	⇑	→	⇓			→	→	↑			↓	↑	↑		
10	⇑	⇑	⇓			→	→	→			⇓	↑	↑		
11	→	↑	↓			→	→	↓			↓	→	→		
12	⇑	→	↓			⇓	↑	↓			→	↓	↑		
13	⇑	⇑	⇓			⇓	⇑	↓			↓	→	→		
14	⇑	↑	↓			⇓	⇑	→			↓	→	→		
15	↓	→	→	↑		⇑	→	↓	→		↓	↑	↑	↓	
16	↑	↑	↓	→	→	↑	⇑	↓	→	⇓	↑	→	↓	↑	→
17	→	↑	↓	→		↓	↑	⇑	⇓		→	→	→	→	
18	↑	↓	→	↑		↑	→	⇑	⇓		→	→	→	→	

Symbols are same as in Table II.

The postdive values of PCV, RBC, and Hb concentration are listed in Table VI. A slight or moderate increase in PCV immediately after the dive indicated a tendency towards hemoconcentration. The Hb concentration showed parallel tendencies, and RBC showed a slight tendency to fall up to 48 hours postdive.

Discussion

As has been well established in the literature, the postdecompression platelet drop was the most striking finding in our investigations (2, 4, 8, 10, 13, 15, 16, 20). The adhesion of platelets to the surface of intravascular bubbles (which exist in nearly every decompression) resulting in the formation of platelet aggregates is the most likely cause of the observed drop in platelet count (14, 15).

It is very likely that silent bubbles will occur during the first extensive pressure reduction when these profiles are used. The unavoidable cooling which occurs during the initial part of the decompression may lead to peripheral vasoconstriction, with consequent reduced inert gas elimination, which again would predispose to bubbles. Further support for this assumption is

TABLE V

POSTDIVE TENDENCIES OF CLOTTING FACTORS II AND VII AND PROTHROMBIN (PT) AND PARTIAL THROMBOPLASTIN (PTT) TIMES

Subj. No.	$F II_s$%				$F VII_s$%				PT_s%				PTT_s sec			
	II	III	IV	V	II	III	IV	V	II	III	IV	V	II	III	IV	V
1	↑	↓			↑	↓			↓	↓			↑	⇈		
2	↓	↓			↑	↑			↓	↓			↓	⇊		
3	↑	↑			↓	⇈			↓	↑			↑	⇊		
4	–	↓			–	⇊			↑	–			↓	↑		
5	↓	↓			↑	↓			↑	↓			↓	⇈		
6	↑	↓			↑	↑			↑	↑			↑	↓		
7	↓	↓	↓		↓	⇊	↑		↑	↓	↑		⇈	↓	↑	
8	–	↓	↑		–	↓	↓		↓	↓	↑		↓	⇊	↑	
9	↑	↓	–		⇈	↓	↓		–	–	↑		↑	–	↓	
10	–	↓	↓		–	↓	↓		↓	↓	↑		⇈	↓	↓	
11	–	–	↓		↓	↑	↑		↓	–	↓		↓	⇊	↑	
12	↓	↓	↑		↑	↓	–		↓	↑	↓		↑	↓	–	
13	↓	↓	↓		⇊	↓	–		↓	↓	↑		⇈	↓	↑	
14	–	↓	↓		–	↓	↓		↓	↓	–		↓	↑	↑	
15	–	↓	↑	–	↓	–	↑	↑	–	↑	↓	–	↑	↓	↑	⇊
16	↓	↓	↑	↑	↓	↓	↓	↑	↓	↑	⇊	↓	⇈	↓	↑	↓
17	↓	–	↑	↓	⇊	⇊	↑	↑	↓	↑	–	–	↓	⇊	↑	↓
18	↓	↓	↑	↑	↓	↑	↑	⇈	↓	–	↑	–	↓	↓	↑	↓

Symbols are same as in Table II.

that the most pronounced platelet decrease was found in those divers who spent their bottom time in the wet pot and who were therefore coldest at the beginning of decompression.

A latent hypercoagulability which could account for the platelet drop can be excluded because of the findings relating to the activities of the clotting system, especially the reduced maximal thrombus elasticity and the missing activation of thrombin-sensitive Factors V and VIII after decompression.

The functional changes in the thrombocytes and the activation of the hemostatic system are apparently discrete enough for an early blockade to be initiated. The physiological regulation processes involved are characterized by the introduction of antithromboplastins and antithrombins within the reaction sequence of the clotting system. In this connection, the immediate postdive antithrombin III increase becomes important, because rapid neutralization of thrombin traces which might have resulted from possible clotting activation inhibits severe derangement of the clotting system in the direction of hypercoagulability.

The remarkably increased Factor IX activity found immediately after the decompression speaks also for latent activation of the clotting process. It is possible that the blockade in the reaction sequence happens at this point. Because Factor IX is activated by the contact product

TABLE VI

PREDIVE PACKED CELL VOLUME (PCV), RED BLOOD CELL COUNT (RBC), AND HEMOGLOBIN CONCENTRATION (HB), AND POSTDIVE TENDENCIES

Subj.	Packed-cell volume, %						Red cells, x10^6						Hemoglobin, %					
No.	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
1	35	↑	↓				5,2	↑	↓				92	↑	↓			
2	41	↑	↓				5,4	↑	↓				102	↑	↓			
3	36	–	↓				5,2	–	↑				94	↑	↓			
4	40	–	↓				5,8	↑	⇓				104	↑	↓			
5	38	–	↑				5,4	–	↑				92	↑	↑			
6	41	↑	⇓				5,6	↓	–				94	↑	↑			
7	41	–	⇓	↑			5,6	↓	–	–			101	–	–	↓		
8	37	⇑	⇓	–			5,7		↑	↓			104	↑	↓	–		
9	38	↑	–				5,2	–	–				94	–	↑			
10	36	⇑	–				5,5	–	–				103	↓	↑			
11	36		↓	↓			5,1		↑	↓			91		↑	↓		
12	37	↓	↓	↑			5,3	↓	↓	↑			95	↓	–	–		
13	41	⇑	⇓	–			5,6	–	↓	↑			101	↑	↓	↑		
14	37	⇑	↓	⇓			5,7	–	↓	↓			104	–	–	↓		
15	36	↑	↓	↓	⇓		5,3	↑	↓	↑	↑		99	↑	↓	↑	↓	
16	39	↓	⇓	⇑	↑	↑	5,5	–	–	–	–	–	109	↓	↓	↓	⇑	↓
17	31	⇑	⇓	↓	↑		5,4	–	↓	↓	↑		93	⇑	⇓	⇑	⇓	
18	37	↓	↓	↓	⇑		5,0	↓	↑	–	↑		96	↓	↑	↓	↑	

Symbols are same as in Table II.

of surface Factors XII and XI (which could not be determined for technical reasons), the hypothesis that high postdive Factor IX activity may occur in close relation to the surface of the intravascular bubbles (22) may be postulated. This assumption could be important for dives in excess of 200 meters because high Factor IX activity could be demonstrated in these experiments even 72 hours after decompression. This might indicate that silent bubbles still exist in the organism even after such a long postdive period, an idea which may be supported by the finding of a 230% Factor IX increase in Subject 14 in connection with pain-only bends occurring three hours after surfacing.

The reduced euglobulin lysis time should be considered an indicator of increased fibrinolytic activity. Some of the divers had already shown an increased euglobulin lysis activity in their predive samples. We suggest, therefore, as has been stated elsewhere (10, 11), that there is a strong psychosomatic, stress-related component even in experienced divers. The high predive Factor VIII activities in some divers (see Table III) may also lead to the conclusion that stress factors may cause increased clotting activity (5, 6, 9, 17, 18).

The distinct and immediate postdive fibrinogen increase in Subjects 7 to 14 may be inter-

preted in relation to the moderate hemoconcentration observed in this group. The approximately 7-hr abstinence from fluid intake, the dry breathing gas in the closed-circuit system, a loss of pulmonary transpiration and an extensive transpiration during the compression and initial part of the bottom phase may account for the temporary hemoconcentration. There was no positive correlation between the three cases of Type-I decompression sickness and changes in the hemostatic system, a finding which agrees with those of other authors (3).

Conclusions

These decompression profiles have been proved to be safe to a high degree (3 cases of Type I decompression sickness in 18 simulated dives). A minimal interval of 72 hours between dives should be observed because of the discrete activation of the hemostatic system and the decrease in platelet count, to prevent the organism from cumulative effects which could lead in the worst case to manifest hypercoagulability. This is especially important for dives in excess of 200 meters. To prevent a labile status of the hemostatic system caused in part by the considerable postdive platelet decrease, the prophylactic application of aggregation-inhibiting substances such as pyrimido-pyrimidine derivates or acetyl-salicylic acid should be considered (1, 19).

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CERTAIN COAGULATION FACTORS IN NONTRAUMATIC DECOMPRESSION, AND PREVENTIVE ACTION OF MEDICATION ON DECOMPRESSION SICKNESS IN THE DOG

B. Gardette, F. Sicardi and X. Fructus

Certain factors involved in blood coagulation show variation following asymptomatic decompression (15). To determine which factors were most interesting as indicators, a nontraumatizing decompression schedule was experimentally developed for the dog, and a series of blood samples was performed before and after each dive (9). These results were compared to the evolution of the same parameters after very traumatic dives. Finally, the effect of Migristene in preventing decompression accidents was tested, following the work of Chryssanthou et al. (4) and our own earlier research (7, 8).

Experimental Protocol

Male dogs weighing between 20 and 30 kg were used. The dogs were placed, two at a time, in a hyperbaric chamber and were exposed to dives on air to 60 meters (7 ATA) lasting 26 minutes (compression in 6 minutes and 20 minutes at depth). The chamber was rinsed with gas to keep the carbon dioxide (CO_2) at an acceptable level.

The following decompression schedules were used.

Decompression Table D_4

Ascent from 60 m to 12 m in 4 minutes
stop at 12 m for 3 minutes
stop at 9 m for 4 minutes
stop at 6 m for 7 minutes
stop at 3 m for 18 minutes
Total ascent time: 36 minutes

Decompression Table D_{18}

Ascent from 60 m to 9 m in 2 minutes
stop at 9 m for 4 minutes
stop at 6 m for 4 minutes
Total ascent time: 10 minutes

Blood samples were taken before each dive, 5 and 24 hours after each dive, and in some cases 2 and 3 days later. The blood was analyzed at the Centre de Transfusion Sanguine in

Marseille. Variation in the following parameters was measured: coagulation time (by thrombelastogram), platelet count, hematocrit, fibrinogen, plasminogen, and fibrin degradation products (FDP).

To test its preventive action against decompression accidents, the medication dimethothiazine methano-sulfonate (Migristene) was used according to the method of Chryssanthou (4). It was administered intravenously. Two dosages were used: 4 mg/kg injected just before the dive, and 8 mg/kg given in one 4 mg/kg injection the day before the dive and in a second 4 mg/kg injection just before the dive.

Results

Clinical Observations

Decompression Table No. 4—(Table D_4)

This decompression table was tested on seven dogs. For each dog, the number of decompression accidents per number of dives is shown in Table I. Only dogs A and B incurred decompression accidents. Two cases of posterior hemiplegia and three bends were observed in dog A, and two cases of bends in dog B. These accidents occurred between 10 and 30 minutes after the end of decompression. Bends were distributed equally among the anterior and posterior right and left legs of the dogs. Dogs indicate a bend by retracting the leg; there is also stiffness of the joint.

Decompression Table No. 18 (Table D_{18})

Results obtained with this table are given in Table II. A very high percentage (75%) of decompression accidents was observed, all of the bends type.

Effect of Migristene

The 4 mg/kg dose had no significant effect on the percentage of bends on Table D_{18}. However, with the 8 mg/kg dose no decompression accidents at all were noted.

Blood Modifications

Decompression Table No. 4

Figure 1 shows the mean platelet concentration before and after diving. These results do not include the platelet modifications obtained after decompression accidents. Taken together, the seven dogs showed a drop in platelet count of 14% 5 to 10 minutes after the dive, 13% after 5

TABLE I

Number of Decompression Sickness Attacks per Number of Dives

	Dogs							
	A	B	C	D	E	F	G	Total
Decompression Table No. 4	5/9	2/13	0/6	0/6	0/4	0/3	0/1	7/42

TABLE II

NUMBER OF DECOMPRESSION SICKNESS ATTACKS PER NUMBER OF DIVES

Decompression table			
No. 4	No. 18	No. 18 + Migristene (4 mg/kg)	No. 18 + Migristene (8 mg/kg)
7/42 (16%)	3/4 (75%)	2/3 (66%)	0/8 (0%)

hours, and 6% 24 hours after the dive. The student's paired t-test, applied to the before-dive and 5 min-after-dive results, showed a significant difference at the 99.8% level. After this initial important drop in platelet count, 24 hours were necessary before the count regained its initial value.

The other parameters studied (coagulation time, hematocrit, fibrinogen, plasminogen and FDP) did not show any significant variations.

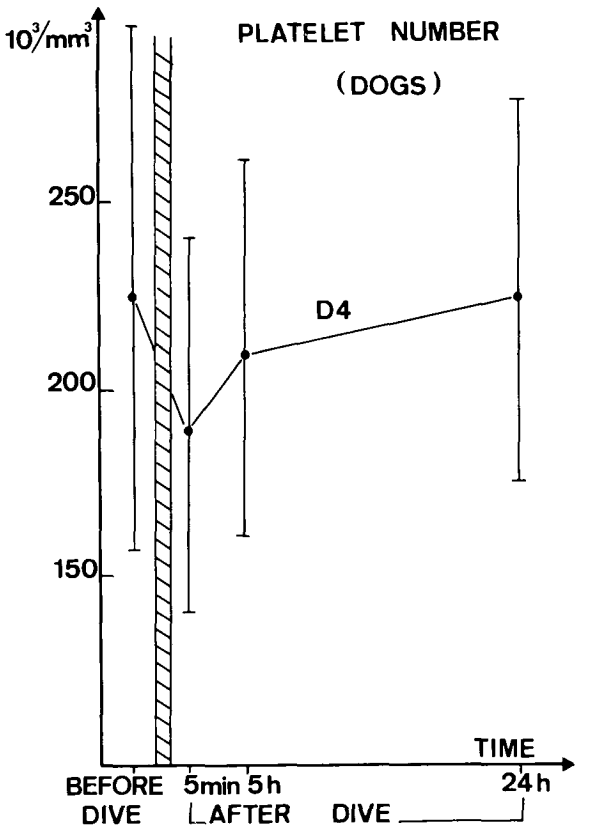

Fig. 1. Mean platelet concentration before and after diving on Table No. 4.

Decompression Table No. 18

For this decompression schedule, platelet counts for two dogs during 4 dives are shown in Fig. 2. A fall in platelet count of 24% is evident one day after diving. For the 4 mg/kg Migristene dose, the same 24% decrement was observed. A decrease in platelet count of 40% was measured for the 8 mg/kg Migristene dose.

The drop in platelet count (with or without Migristene) lasted until three days after the dive and averaged 40%. The count returned to normal only after five days.

The other blood parameters studied showed the following variations.

Coagulation time diminished by 30% in the days after unmedicated dives. With 4 mg/kg Migristene, diving produced no change in coagulation time, while 8 mg/kg Migristene augmented coagulation time by 20%. Also with 8 mg/kg, the dynamic thrombelastographic constant diminished 20%.

The hematocrit diminished by roughly 20% during the hours following decompression, with or without Migristene. Blood fibrinogen diminished by 10% a few minutes after the dive, with or without Migristene. This factor then showed a compensating effect by rising to a value of 40% above normal, and continued at this level until the fourth day after the dive.

Plasminogen showed no variations, and blood FDP sometimes appeared after a dive, but their presence could not be correlated easily with decompression.

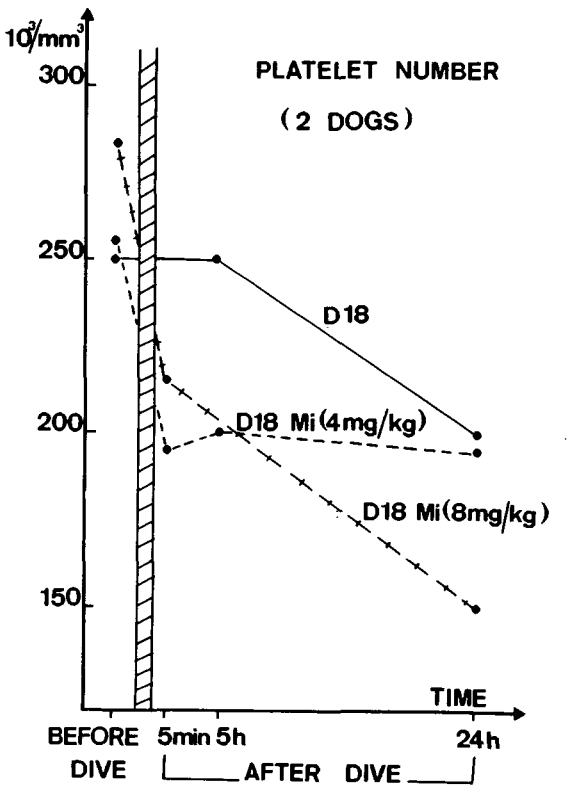

Fig. 2. Platelet counts for two dogs on four dives on Table No. 18.

Discussion

Decompression Tables

It is evident that decompression table No. 4 was nontraumatic. The only accidents observed while using it occurred during the early phases of experimentation when chamber ventilation was not fully satisfactory. After a more complete ventilation program was introduced, no further decompression accidents were produced. The two incidents of paralysis in dog A appear to have been related to some physiologic anomaly.

On the other hand, decompression table No. 18 was quite a traumatic schedule, producing bends in 75% of the cases.

Blood Modifications

With table D_4, platelet count was the only one of the measured parameters which showed significant modifications. With table D_{18}, platelet count modifications were more prolonged, continuing to decrease during the days following the dive.

This fall in platelet count has already been documented by several authors. Ehm et al. (6) observed a continuous drop four hours after a dive of 22 minutes at 3 ATA in rabbits, and Broussolle et al. (2) reported a 30% decrease in the rat on dives without a decompression accident, and a 50% decrease after such an accident. In man, the work of Martin and Nichols (12), and Ackles et al. (1) showed a maximal 15% lowering of platelet count three days after a symptom-less 4-ATA experimental dive. Ricci and Tiepolo (16) reported an average decrease of 23% in amateur divers 12 hours after diving. After a helium saturation dive, Sicardi (17) noted a 37% fall in three divers. Platelet grouping was observed by Clay (5) in a dog's blood vessels after decompression. Philp (14) reported the same observations in the rat. Moreover, this author showed that platelet clumps surround intravascular bubbles formed during the decompression (15).

The fall in platelet count in the minutes which follow a nontraumatic decompression can therefore be explained by an agglutination of platelets around circulation bubbles, whose presence has been documented by Spencer and Oyama (18), Guillerm et al. (10, 11), and Masurel et al. (13), with the aid of an ultrasound sensor. These platelets encircling the bubbles would be rapidly entrained by the reticulo-endothelial system of the lungs, without being destroyed. This explains how the platelet count returns to normal 24 hours after the dive. In the case of a traumatic dive, the quantity of circulating bubbles is much greater and the fall in platelet count is therefore more marked. In this case the decrease lasts three days after the dive, meaning that an actual destruction of platelets exists with the formation of new platelets. Moreover, different substances such as serotonin, histamine, bradykinin and SMAF (smooth muscle activating factor) would be activated (3). These substances could play an important role in the genesis of decompression accidents. It must be added, also, that after a traumatic decompression, fibrinogen decreases in the blood, and coagulation time as measured by thrombelastography is diminished. These occurrences form a true pathology of consumptive coagulation, known as disseminated intravascular coagulation, even in the case of traumatic decompressions.

Migristene Effects

Administered in a preventive fashion, this drug appears to diminish and even prevent

decompression accidents. These results confirm those of Chryssanthou (4) in the mouse and those obtained after a dive rendered traumatic by exercise (Fructus et al., 7, 8). Nevertheless, a large dose of the drug is necessary (8 mg/kg).

At this concentration of the drug, the sedative effect of Migristene cannot be avoided, and nitrogen is therefore dissolved in the tissues under modified conditions. However, Migristene appears to lengthen coagulation time and diminish the value of the dynamic thrombelastographic constant. In this connection, it acts as an inhibitor of esterizing enzymes, and works therefore by inhibiting the activation of serotonin, histamine, and bradykinin (Chryssanthou et al. (4)).

However, Migristene does not prevent the aggregation of platelets. The fall in platelet count is equally important with or without Migristene, after a traumatic decompression.

Conclusions

Of the blood parameters studied here, only platelet count showed variations in the case of nontraumatic decompressions. In addition, the decrease in the number of platelets seems to be proportional to the degree of trauma produced by the decompression. Monitoring of this parameter could therefore be of interest during the testing of decompression tables. It could also permit the establishment of a method of judging the time necessary for the complete physiologic recuperation of a man after diving.

Our results also show that Migristene has a true preventive action against decompression accidents. However, its sedative effect constitutes a contraindication for use in diving. Despite this, the drug ought to be added to the list of products available for the therapeutic treatment of decompression sickness.

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EFFECTS OF INDOMETHACIN ON PLASMA VOLUME LOSS IN EXPERIMENTAL DYSBARISM

C. H. Wells, J. G. Hilton and A. Rosenbaum

The development of large, clinically significant reductions in plasma volume in severe experimental or accidental dysbarism is well documented. Brunner has reported plasma volume losses of 20% or more in cases of human dysbarism (2). Losses of 25 to 40% have been observed in dysbaric experimental animals (6, 23). Hemoconcentration, which may also be considered indirect evidence of plasma volume loss, has been observed by numerous investigators in experimental dysbarism (3, 7).

The possible roles of vasoactive substances in the development of this plasma loss have not been thoroughly explored. Studies by Chryssanthou and his co-workers (4) suggest that bradykinin is involved in the pathogenesis of dysbarism. The effects of this agent on vascular permeability are well established. It is reasonable to wonder if the mechanisms responsible for dysbaric plasma volume losses do not also involve other vasoactive substances. Studies of fluid shifts in response to turpentine or carrageenan indicate that several vasoactive compounds are involved in the extravasation process (8, 9). Under these experimental conditions, the initial phases of fluid extravasation appear to result from the effects of kinins, histamine, and serotonin. Prostaglandins appear important in later stages of plasma extravasation.

There is reason to suspect that prostaglandin release occurs in dysbarism as well (14, 16). This study was undertaken to investigate the effects of pharmacologic blockade of prostaglandin production by indomethacin upon plasma losses in experimental dysbarism.

Methods

Two groups of mongrel, sodium-pentobarbital-anesthetized dogs ranging in body weight from 8 to 16 kg were used for this study. One femoral artery and one femoral vein of each animal was catheterized with polyethylene tubing (Clay Adams PE-220) for sample collection and ^{131}I-tagged albumin injection, respectively. Three plasma volume determinations were carried out on each animal: one before compression, one beginning 10 minutes after decompression, and one beginning 60 minutes after decompression. For the first determination, 0.9 μCi of ^{131}I-tagged human serum albumin was injected into the vein, and arterial blood samples were collected at 10, 20, 30, and 40 minutes after injection. The plasma volume at the time of injection was determined by standard tag dilution principles employing retrograde extrapolation to the time of injection from the blood determinations. Subsequent determinations were similarly conducted using doses of ^{131}I-tagged albumin of 1.8 and 2.7 μCi for the second and third determinations, respectively.

All animals were placed in the Bethlehem hyperbaric chamber after completion of the first plasma volume determination, and were compressed to five atmospheres' absolute pressure in a mixture of 5% oxygen, 95% nitrogen. After 60 minutes' exposure at this pressure, the animals were decompressed. Compression and decompression were conducted at 10 psi per minute, without staging. The chamber was flushed at a rate of 10 liters per minute throughout the compression period.

Animals of the experimental group (n = 9) received a total dose of 25.0 mg of indomethacin orally 30 minutes before compression. The control group (n = 7) received no drug.

Results

Figure 1 presents the mean plasma volume for each group of animals at each period in the experiment. The small bars represent the standard error of the group mean. The mean plasma volume in the untreated animals prior to compression was 55 ± 4 ml per kilogram of body weight. That of the animals receiving indomethacin was 52 ± 3 ml per kilogram of body weight. Plasma volumes determined 10 minutes after decompression were found to be 50 ± 2 ml

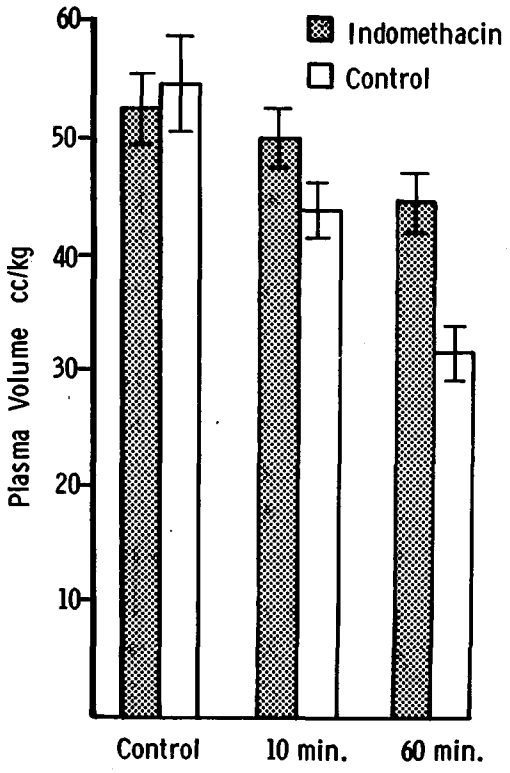

Fig. 1. Plasma volume of treated and untreated dogs prior to and following decompression. Control = prior to compression; 10 min = 10 minutes after decompression; 60 min = 60 minutes after decompression. Shaded bars = animals receiving indomethacin, 25 mg/kg, prior to compression; open bars = untreated animals. Small bars = standard error.

per kilogram of body weight for the indomethacin-treated animals, and 44 ± 2 ml for the animals in the untreated group. One hour after decompression, the plasma volumes were 44 ± 3 ml per kilogram of body weight for the indomethacin-pretreated group of animals and 31 ± 2 ml per kilogram body weight for the untreated group of animals.

Prior to compression, differences between the plasma volumes of the two groups of animals were slight and statistically insignificant. After decompression, the mean plasma volume of each group was significantly ($P < 0.01$) less than corresponding precompression values.

The decrease in plasma volume in ml/kg observed in the untreated and indomethacin-pretreated animals is shown in Fig. 2. The loss in the treated animals was significantly ($P < 0.01$) less than that of the untreated animals 10 minutes after decompression. One hour after decompression, the indomethacin-pretreated animals' average plasma volume loss was 9 ± 2 ml per kilogram body weight, which was substantially less than that of the untreated animals (23.5 ± 3.5 ml/kg). This difference was also statistically significant ($P < 0.01$).

Discussion

These studies reveal a loss in plasma volume after decompression, determined by serial radioactively tagged serum albumin dilution techniques, that was substantially less in indo-

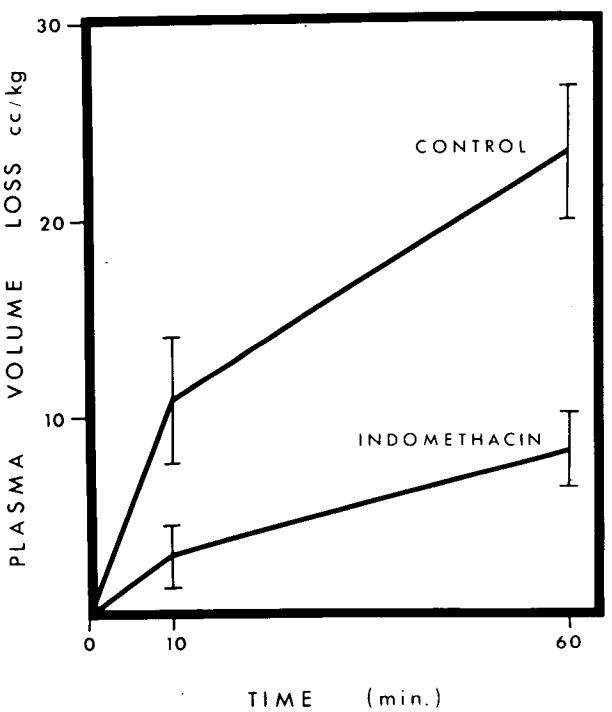

Fig. 2. Plasma volume loss in the dog following rapid decompression. *Ordinate:* plasma volume loss in cc/kg; *abscissa:* time postdecompression. Control = untreated animals ($n = 7$). Indomethacin = indomethacin-treated animals ($n = 9$). Small bars represent standard error.

methacin-pretreated animals than in animals receiving no drug treatment. All experimental animals were subjected to identical compression and decompression schedules.

This observation suggests that prostaglandins are, in large part, responsible for the plasma losses that occur during the first hour after decompression. The dose of indomethacin used in these studies has been shown to be highly effective for the inhibition of prostaglandin production (10, 23), with relatively little effect upon serum levels of other vasoactive substances such as bradykinin, serotonin or histamine (8, 22).

Prostaglandin release could occur in dysbarism through a number of different mechanisms. First, prostaglandin-like substances have been demonstrated in pulmonary venous blood following the injection of bubbles in the pulmonary artery (14). Evidence derived from direct and microscopic observations (17) and ultrasonic bubble monitoring (17, 18, 21) has repeatedly revealed bubbles in the systemic venous system of human subjects and experimental animals after decompression. Second, prostaglandin release has also been demonstrated following the injection of artificial microemboli into the pulmonary artery (16). Emboli formed by platelet aggregates, (13, 15) coalesced serum lipid masses (7, 11), and even occasionally bone marrow fragments (14), have been demonstrated in dysbarism. Third, prostaglandins E_2 and F_2 may also be released during the coagulation process (19, 20). Numerous studies indicate the probable presence of a subclinical disseminated intravascular coagulation-like syndrome in dysbarism (12).

While the results of this study indicate that indomethacin substantially reduces the plasma losses observable in the first hour after decompression, its failure to block these fluid shifts completely suggests that this is not solely a prostaglandin-mediated event. It is unlikely that the inability of indomethacin treatment to prevent dysbaric plasma losses completely occurred as a result of functionally incomplete blockage of prostaglandin release. As indicated earlier, indomethacin in the dosage used in these studies is a highly effective inhibitor of prostaglandin synthesis.

This study offers no evidence of the nature of dysbaric plasma loss mechanisms other than those that can be blocked by indomethacin. However, the additional involvement of other vasoactive substances deserves consideration.

Carrageenan- and turpentine-induced edema appear to be mediated in the first two hours after challenge by a combination of serotonin, histamine, and vasoactive kinins (8, 9, 22). The effects of indomethacin blockage on edema formation becomes evident after the second hour (9). There is little evidence of possible roles of histamine or serotonin as mediators of the plasma losses of dysbarism, although serotonin administration has been shown to increase the severity of the disorder (5).

Chryssanthou (4) and his collaborators have accumulated substantial evidence that bradykinin is involved in the pathogenesis of dysbarism. While there is little evidence that this substance plays a role in the genesis of dysbaric plasma losses, evidence of its presence in dysbarism and its well-known vascular properties indicate that it is likely to be involved in some aspects of the fluid loss mechanism.

This study was designed to investigate one of several possible mechanisms of plasma loss in dysbarism. The effectiveness of indomethacin in reducing these losses suggests that prostaglandin synthesis and release play a substantial role in this fluid loss mechanism. The possible therapeutic implications of this finding remain to be demonstrated. These studies utilized pretreatment before decompression. No effort was made to assess the efficacy of this agent on plasma loss of dysbarism when given after the animal was subjected to decompression stress.

Prior studies of the prostaglandin-synthesis-inhibiting substances, indomethacin and aspirin, failed to establish the usefulness of these agents in dysbarism (1, 14, 24).

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BLOOD FLOW STUDIES DURING SPINAL-CORD-DAMAGING DECOMPRESSION SICKNESS IN DOGS

J. M. Hallenbeck and L. Sokoloff

Over the course of the past several years, a series of studies has been reported supporting the general hypothesis that epidural vertebral venous system (EVVS) obstruction by bubbles is the major etiologic factor in spinal cord damage due to decompression sickness (7, 9, 11).

To investigate this hypothesis further, two additional types of experiments were performed. Cinephotomicrography has been undertaken to monitor by direct vision dog EVVS and pial vessels during safe dives and dives leading to spinal-cord-damaging decompression sickness. Also, studies of brain and spinal cord blood flow are being done in control dogs and dogs with cord-damaging decompression sickness by means of the 14C-antipyrine autoradiographic blood flow technique (18). The purpose of these studies is to measure the degree and distribution of central nervous system blood flow change during paralytic decompression sickness. The results of the cinephotomicrography experiments are reported in greater detail elsewhere (13).

Laminectomies were performed on 31 conditioned male mongrel dogs weighing 18–30 kg. The first 12 dogs in the series were anesthetized with pentobarbital, 25 mg/kg, and the subsequent 19 dogs were anesthetized with chloralose, 100 mg/kg, so that evaluation of the dogs' neurologic status was possible. The animals were intubated with a cuffed endotracheal tube and an esophageal thermistor was placed to monitor core temperature. Core temperature was maintained near 38°C with heating pads. Various intravascular and intracardiac pressures were monitored in 18 dogs. Aortic pressure catheters were placed via the right femoral artery in all 18 animals. In 17 of this group, a central venous pressure catheter was inserted through the right femoral vein. A third catheter was added in the last seven dogs of this group. It was inserted into the left femoral vein and positioned in the right ventricle in five dogs and in the pulmonary artery in two dogs. Cerebrospinal fluid pressure was monitored by percutaneous cisternal puncture in 12 of the 18 dogs.

Following a laminectomy in the lower thoracic, upper lumbar region which unroofed 4–6 vertebrae, the animals were suspended by their axial skeletons at two points. Crutchfield tongs supported the pelvis and several size 2 braided silk sutures were tied to an upper thoracic dorsal spinous process.

Filming was done with an Arriflex® 16-mm movie camera and a Leitz Ortholux® microscope equipped with a Leitz Ultropak® at magnifications of 20× and 40×. Cinephotomicrography of the EVVS and dorsal pial vessels was performed both predive, after the animal was fully prepared, and postdive, when continuous microscopic observation revealed anything noteworthy. Arterial samples for pH, P_{CO_2} and P_{O_2} were obtained both predive and postdive during cinephotomicrography in 10 dogs.

All dives were to a simulated depth of 220 feet in a pressure chamber with air as the breathing medium. The rate of descent was 75 feet per minute and the rate of ascent was 60 feet per minute. Bottom times ranged from 5 to 60 minutes and five dogs received two separate dives.

An assessment of neurologic function was possible in dogs anesthetized with chloralose. In these animals, spinal cord damage could manifest as loss of the triceps or patellar reflexes, extensor rigidity of an extremity, paralysis of intercostal muscles and diaphragm (requiring respiratory assistance), or by loss of the segmental panniculus reflex.

The brains and cords of 14 dogs were removed after the respective experiment for gross pathologic analysis.

During the control period and after dives that did not produce decompression sickness, the sinuses were visible at magnifications ranging from 20× to 40× as large, thin-walled vascular trunks expanding with expiration and partially collapsing with inspiration. Individual red cells were not resolved at the magnifications used, so analysis of the rate and direction of flow awaited the appearance of bubbles as particulate intravascular markers. During incipient decompression sickness, single bubbles and small groups of bubbles were noted to move rapidly in a cephalad direction, and the cyclic engorgement and emptying of the sinuses that accompanied respiration became more pronounced. The flow in the EVVS at this point was not the slow ebb and flow with frequent changes in direction that has been described in the resting state (1), but was instead very rapid and resembled the flow change demonstrated cinevenographically in the EVVS during the early stages of incremental pulmonary air embolism in dogs (7). If the decompression sickness progressed toward cord damage, bubbles continued to appear in the EVVS in increasing numbers and the rate of flow steadily decreased until clumps of bubbles that did not occlude the lumen were observed moving about but making no net progress. Finally, through a process of accumulation, growth and coalescence, the EVVS became completely choked and occluded by a population of heterogeneously sized bubbles in frothed blood.

As flow in the EVVS slowed, flow in the pial vessels became sluggish and sludging became pronounced. Bubbles in pial veins became stagnant and hemorrhage appeared on the pial surface of the cord. In the end stage, flow in all pial vessels nearly ceased, although a very sluggish flow of sludged red cells often continued in pial arteries.

The EVVS became blocked in 24 animals. The obstruction was generally bilateral but in several animals, only one sinus was blocked. Of these 24 animals, 18 were examined neurologically and/or pathologically and all showed evidence of spinal cord damage.

In 7 animals exposed to nine 220-foot dives ranging from 5 to 50 minutes in duration, the EVVS never became fully occluded by bubbles. Three other animals did not incur EVVS obstruction after the first dive but developed EVVS obstruction after the second dive. Thus, the EVVS did not become fully occluded in a total of 12 dives made by 10 animals.

It was possible to correlate the absence of EVVS obstruction with either gross cord pathology or signs of spinal cord damage in 10 dives. After 9 of these dives, there was no clinical or pathologic evidence of cord damage. However, one animal with a considerable number of EVVS bubbles manifested cord damage remote to the area of observation. He developed diaphragmatic and intercostal muscle paralysis after his second dive, implicating the upper cervical region.

The dura was open in 24 of the 31 animals. In 11 animals with the dura incised, the order of appearance of bubbles in the various vessels could be distinguished and in all of these animals EVVS bubbles preceded pial vessel bubbles. Bubbles were never observed in the pial

vessels in the absence of EVVS bubbles. When the EVVS was obstructed, pial veins were either blocked with bubbles or showed very slow sludge flow with prominent red cell and platelet aggregates. Small hemorrhages often developed on the surface of the cord when the pial veins became blocked. When the EVVS was not congested by bubbles or blood, the circulation in pial vessels either appeared normal or showed minimal sludging. Pial arterial bubble emboli were noted occasionally but were rare in comparison to venous bubbles and were absent early in the evolution of the lesions.

Average predive pressures were: aortic pressure (AoP) 150/108 mmHg, central venous pressure (CVP) −0.6 mmHg, and cerebrospinal fluid pressure (CSFP) 5.1 mmHg. Postdive, the corresponding maximum pressures averaged: AoP 196/126 mmHg, CVP 4.4 mmHg, and CSFP 18.2 mmHg. Predive and postdive CVP and CSFP both differed significantly by paired *t*-test ($P < 0.005$). Pre- and postdive right ventricular pressure (RVP) and pulmonary artery pressure (PAP) averaged 24/18 and 47/36, and 29/0 and 53/3 mmHg, respectively. The blood gases were in the normal range for dogs and did not differ significantly pre- and postdive.

Three dogs developed EVVS obstruction without any preceding rise in central venous pressure, right ventricular pressure or pulmonary artery pressure. They were exposed to dives of 220 feet for 25 minutes, dives which are at the threshold for producing paralytic decompression sickness in anesthetized dogs.

These studies demonstrate conclusively that bubbles accumulate, coalesce, and grow in the EVVS during the course of spinal-cord-damaging decompression sickness and ultimately cause widespread obstruction of these venous channels. In addition, the observed correlation between the presence of EVVS obstruction and the presence of clinical and/or pathologic signs of spinal cord damage was excellent. Eighteen of the 18 dogs with EVVS obstruction manifested damage to the spinal cord. The correlation between the absence of EVVS obstruction and the absence of clinical or pathologic signs of cord damage was also good. Signs of cord damage were absent after nine of ten dives in which EVVS obstruction by bubbles did not occur. After one dive in this group, signs of cord damage remote to the area of observation developed.

Since EVVS bubbles clearly preceded bubbles in the pial vessels when this distinction could be made and bubbles in pial vessels were never seen without the concomitant presence of numerous bubbles in the EVVS, it is likely that the pial circulation is not a major source of EVVS bubbles. The epidural space is fat-filled (15) and, being influenced by the negative intrapleural pressures of the chest, is periodically at a negative pressure (8). These factors would be expected to predispose to local bubble formation, particularly when the breathing medium is air, since nitrogen is about five times more soluble in lipid than in water (5). In addition, the EVVS is a valveless venous lake which has rich communications with intracavitary veins (2) and accordingly could receive bubbles from other vascular beds such as those of the retroperitoneal fat.

The fact that EVVS obstruction occurred without any preceding rise in central venous pressure, right ventricular pressure, or pulmonary artery pressure in three dogs exposed to marginally unsafe dives indicates that acute pulmonary hypertension and central venous congestion are not absolutely essential prerequisites for epidural venous occlusion. This is very interesting because clinical experience with human decompression sickness cases indicates that pulmonary symptoms are often mild or absent and that spinal cord dysfunction can occur without other symptoms. It is also clear from human decompression sickness cases that the development of "chokes," with its attendant cardiopulmonary disturbances, is not sufficient cause for the production of spinal cord damage. "Chokes" is a fairly common form of decom-

pression sickness among aviators, while spinal cord damage is a rare complication of hypobaric decompression (4, 3). However, the consistent experimental observation that pulmonary hypertension and central venous congestion develop in dogs exposed to dives that produce early involvement of the spinal cord suggests that central cardiovascular and pulmonary changes may facilitate early EVVS obstruction, although they are not absolutely essential for its ultimate occurrence.

To date, blood flow studies have been performed on three control animals and three animals with paralytic decompression sickness. In these dogs, catheters were placed under nitrous oxide anesthesia, and the animals were then allowed to recover for two hours. Two catheters were inserted into a femoral artery, one directed proximally into the aorta and the other distally further down the femoral artery. These catheters functioned as an external shunt for rapid sampling of arterial blood when later joined through a Y-connector. A catheter was also placed in the right ventricle via the femoral vein.

All animals received a chamber exposure. Control animals were placed in the chamber for 15 minutes during which a surface ventilation was carried out with both the supply and exhaust valves open so that pressure did not build up. The experimental (or paretic) animals were compressed with air as the breathing medium to a simulated 220 feet at a rate of 75 feet per minute. After a bottom time of 25 to 30 minutes, they were decompressed at 60 feet per minute. When early signs of decompression sickness developed on the surface, they received a second compression to 70 feet followed by a decompression at a variable rate. This permitted control of the decompression sickness process and facilitated the production of spinal cord lesions.

In all animals, arterial blood was drawn for pH, P_{CO_2}, and P_{O_2} both before and after the chamber exposure. Systemic arterial pressure was also measured at these times using the aortic catheter.

Both before and after the chamber exposure, a neurologic exam was performed on animals in both groups.

After completing the chamber exposure and the operations that have been described, a 14C-antipyrine autoradiographic blood flow procedure (17) was done and the animals were killed with a 20-cc saturated solution of potassium chloride injected as a pulse through the right ventricular catheter. The brains and cords were quickly removed and frozen in liquid freon suspended over liquid nitrogen.

Later, 20-μ frozen sections of the tissue were incubated with Kodak SB 54 X-ray film to make autoradiograms. By including standards of known 14C concentration with each film, the concentration of 14C in various areas of tissue could be determined by means of a photodensitometer.

Local blood flow was calculated for numerous anatomic areas in the central nervous system by the following formula:

$$C_i(T) = \lambda k_i \int_0^T CaC^{-k_i(T-t)}dt$$

where $C_i(T)$ = the concentration of tracer substance in the tissue at time T; λ = the tissue-blood partition coefficient for the tracer material; k_i = the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that tissue; and Ca = the concentration of the tracer substance in the arterial blood.

The AoP averaged 130/85 before chamber exposure and 130/85 before blood flow measurement in the control group. The AoP averaged 152/83 before chamber exposure and 170/95

before blood flow measurement in the paretic group. Values for pH at corresponding times were 7.44 and 7.48 for controls and 7.44 and 7.42 for paretic animals. Values for P_{CO_2} at corresponding times were 42.6 mmHg and 31.7 mmHg for controls and 35.6 mmHg and 38 mmHg for paretic animals. Values for P_{O_2} at corresponding times were 94.4 mmHg and 90.8 mmHg for controls and 91.1 mmHg and 100.9 mmHg for paretic animals.

The average values for blood flow in ml/100 gm/min for various anatomic areas of brain and *unaffected* segments of cord were very similar in both the control and paretic groups. Some representative examples, with the control group value preceding the paretic group value, are: visual cortex 85, 82; sensorimotor cortex 74, 65; centrum ovale 19, 16; internal capsule 22, 24; cerebellar cortex 74, 73; cerebellar white matter 14, 17; vestibular nuclei 61, 62; cervical cord gray 49, 42; cervical cord white 14, 17; thoracic cord gray 39, 45; thoracic cord white 14, 13; lumbosacral cord gray 50, 42; and lumbosacral cord white 14, 14.

In *affected* segments of cord, flow disruption was profound, with flow rates generally in the range of 0 to 5 ml/100 gm/min. The reduction in flow uniformly affected gray and white matter in a cross section of cord, and encompassed the whole cord rather than conforming to the territory of distribution of a spinal artery or spinal artery branch (Fig. 1).

The presence of severe cord ischemia at levels that were appropriate for the neurologic deficits observed in paretic animals indicates that the myelopathy of decompression sickness is a vascular disease. The pattern of flow disruption in this vascular disease is of some interest. Reduction of flow as measured in cord autoradiograms uniformly involved the whole cord rather than small circumscribed areas in the territory of distribution of a spinal artery or spinal artery branch. This pattern is considered most compatible with impairment of cord venous drainage by obstruction at the level of the epidural veins. The major arterial pathways to the spinal cord include posterior branches of intercostal arteries, spinal arteries, and anterior and posterior radicular arteries. Bubbles in decompression sickness are small enough not to be expected to block vessels of this size. The radicular arteries join intraspinal arteries such as the anterior spinal, posterior spinal, and circumflex arteries. These vessels in turn ramify to supply their respective territories within the parenchyma of the spinal cord. Bubbles embolizing systemically could conceivably lodge in one or more segments of the terminal vascular bed of intraspinal arteries. However, this would be expected to give a mottled dis-

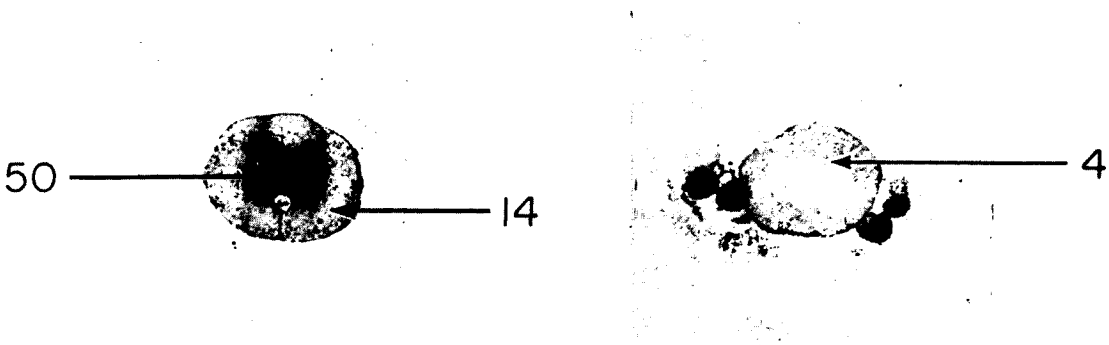

Fig. 1. Two autoradiograms from ^{14}C-antipyrine autoradiographic blood flow studies in two animals. Autoradiogram on *left* illustrates normal cord blood flow, with flow rate characteristically higher in central gray matter than in surrounding white matter. Autoradiogram on *right* is from an affected segment of cord in a paralyzed animal, and shows a profound depression of cord blood flow which is uniform and encompasses the whole cross-sectional area of cord.

tribution of interrupted flow in the territory of the affected intraspinal arteries. For bubbles that should disseminate widely through the systemic circulation to concentrate at only several segments of the longitudinal extent of the spinal cord by entering appropriate spinal arteries bilaterally, traversing anterior and posterior radicular arteries bilaterally, and uniformly obstructing vessels in the territory of each intraspinal artery bilaterally, at each affected level, would appear to be a miraculous series of events. The possibility of this occurrence is made even more remote by the total absence of discrete areas of flow disruption in the brain. However, bilateral obstruction of the epidural sinuses, which has been repeatedly shown to occur, would uniformly interfere with total venous drainage from an area of cord, much as constriction of a renal vein would uniformly reduce a kidney's blood flow.

We would now revise our previous overview of the sequence of derangements leading to spinal cord damage in decompression sickness (7, 10, 11) and arbitrarily divide the sequence into peripheral and spinal stages with central cardiopulmonary changes classed as a nonessential but facilitatory parallel process. Initially, bubbles arise peripherally in regions of the systemic microcirculation and interstitial spaces, where they begin to exert direct mechanical effects and also indirect effects due to activity at gas-blood interfaces. The gas-blood interface as discussed by Lee (16, 17) is a 40–100 Å zone of electrokinetic forces which tends to alter the secondary and tertiary configuration of blood proteins, leading to activation of the clotting system, platelet aggregation, release of vasoactive substances, and disturbance of blood rheology due to plasma loss and red cell clumping. Depending on the extent of bubble nucleation, the process could follow one of several courses. Elimination of gas, bubble resorption, and inactivation of products of bubble surface activity could cause the process to subside. Failing this, the process could continue as a local phenomenon and reach the clinical horizon as perhaps pain in the region of a joint, a "limb-bend." In more extreme cases, bubbles begin to accumulate in the EVVS, thus initiating a process which can lead to the spinal stage. Some of the bubbles may arise locally and others may enter the system from neighboring or distant vascular beds. These bubbles are permitted by the peculiar physiology of the EVVS to accumulate, coalesce, and grow without being swept away. As a temporally parallel occurrence, many bubbles and products of bubble surface activity may enter the larger veins from various systemic vascular beds and migrate to the lungs. This causes the pulmonary artery pressure to rise acutely and the cyclic fluctuations of intrathoracic pressure that accompany respiration to increase in amplitude, becoming more negative during inspiration and more positive during expiration (7). These changes are followed by central venous congestion and a rise in central venous pressure which is reflected back into the EVVS, promoting stasis and congestion and facilitating the process by which bubbles contained in this epidural venous reservoir attain sufficient size and number to effect complete occlusion. However, even without the facilitatory occurrence of central cardiovascular and pulmonary changes, the process of accumulation, coalesence and growth of bubbles in the EVVS may proceed to widespread regional obstruction of epidural venous channels. The bubbles activate the hemostatic process (14), and if stasis is maintained for about the length of the silicone-clotting time of the blood (6), fibrin deposition occurs, reinforcing any obstruction. When the EVVS is blocked over a longitudinal extent that is sufficient to compromise the drainage of critical radicular veins, the resulting interference with spinal cord venous drainage synergizes with the generalized rheologic disturbance attending severe decompression sickness (19) to produce an area of infarction.

In summary, cinephotomicrography has substantiated previous work indicating that the EVVS becomes obstructed during paralytic decompression sickness, and ongoing studies of spinal cord blood flow in the disease indicate a causal role for this obstruction.

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EXPERIMENTAL CEREBRAL AIR EMBOLISM AND ITS RESOLUTION

D. C. Grulke and B. A. Hills

Occlusion of the cerebral arterial system by gas emboli has long been implicated in many of the more serious manifestations of inadequate decompression. It is, therefore, particularly interesting to see how carefully calibrated microbubbles of known frequency and dimensions behave when introduced into the cerebral circulation, since it is unlikely that gas emboli formed by decompression will be the same as those derived from the bolus injection of air used in previous studies (1, 2, 3, 8).

A forcible air injection of 1-2 ml/kg in the carotid artery of dogs produces a characteristic response, consisting of a transient rise in blood pressure for 1-2 minutes associated with tachycardia, and a brief period of apnea (3). This characteristic response can also be produced by bolus injections into the vertebral arteries supplying the brainstem, but is eliminated with carotid injections if silver clips are placed on the Circle of Willis to isolate the posterior-inferior circulation from the carotid supply. This suggests that the bolus injection may actually force air in a retrograde direction into the vertebral-basilar artery system against the direction of blood flow. Moreover, a forcible gas injection produces an unknown distribution of bubble sizes in the arterial circulation which could range from finely dispersed bubbles to totally gas-filled vessel segments. Therefore, the "bolus injection" is felt to be a somewhat unselective approach for experimental studies, so a study has been undertaken to investigate the behavior of microbubbles of known size and frequency when these are continuously infused into the cerebral arterial system.

Animal Preparation

Guinea pigs of the Duncan-Hartley strain and various mixed strains weighing approximately 500-700 gm were anesthetized with an intraperitoneal injection of Nembutal (30 mg/kg). With the animal in the supine position, a tracheal tube was inserted and the left common carotid artery was cannulated with 2 catheters, the infusion catheter (cephalad direction) and the blood pressure cannula (retrograde direction). Blood pressure, respiration, and heart rate were monitored throughout the infusion experiment. A rectangular cranial window (approximate dimensions = 1.5 × 0.7 cm) was prepared with the animal in the prone position. The exposed brain surface was continuously bathed with isotonic saline warmed to body temperature and covered with a glass coverslip to improve photographic results.

Microbubble Infusion

Microbubbles were produced outside the animal by controlled gas injection through fine

hypodermic needles (.001″ ID) in an isolated fluid compartment containing surfactant solution (3% Teepol in saline). Bubble size could be selected by varying a number of parameters (7) to give stable patterns which showed narrow size distributions for any diameter within the range 45-210 μm, gas flow rates ranging from less than 0.6 ml/hr to 12 ml/hr. Microbubbles rising from the fluid compartment were carried into the prone animal in suspension by means of a saline infusion stream (see Fig. 1). The size distribution of bubbles collected from the infusion stream was determined with a Coulter Counter® modified for bubble measurement (7). Bubbles reaching the pial arteries were also measured photographically using a 500-μm grid placed over the cranial window. Events in the vascular bed were observed through a binocular dissecting microscope (7-40×) and recorded with sequential photographs.

Bounce Compression

For bounce compression experiments, microbubble infusion was continued until the exposed pial arteries were almost completely air-filled. Animals in the treatment group were then compressed to 75 psig (6 ATA) on air over a 3-5 minute period. The total time at the bottom depth was less than 1 minute, and decompression to 1 ATA was completed over 2-3 minutes. This procedure was used to study the effectiveness of mechanical volume reduction in clearing gas emboli from the cerebral circulation. The extent of gas removal was evaluated both by visual inspection of the cranial window vessels shortly after the bounce compression procedure, and by measurement of the residual gas volume in the excised brain using a tissue compressibility technique.

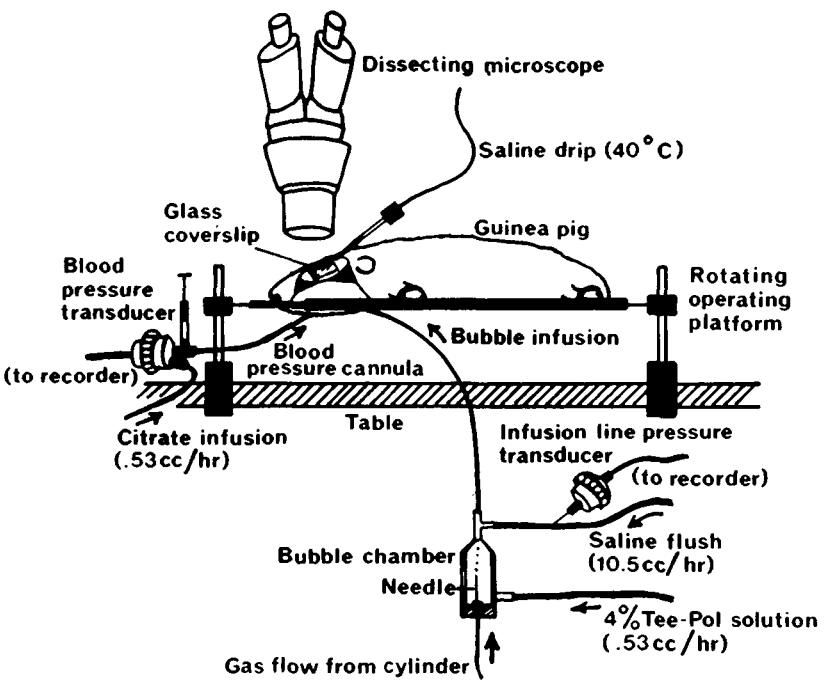

Fig. 1. Microbubble formation and infusion system.

Oxygen Microbubbles

Microbubbles produced with 100% oxygen (medical grade; B.O.C.) were infused into four animals to determine the extent of emboli resolution with a change in the gas composition.

Oxygen Breathing During the Infusion of Air Microbubbles

Oxygen breathing at 1 ATA was used to study the effects of an enhanced driving force for gas dissolution without recompression. A breathing mixture of 95% O_2/5% CO_2 was supplied to the animal by means of a flow-through breathing bag attached to the tracheal cannula. Oxygen breathing was started during preparation of the cranial window and was continued throughout microbubble infusion.

Results

Arterial Bolus Injections

Air bolus injections of 0.5, 1.0, 1.5, and 2.0 cc/kg were given in the common carotid artery of 17 guinea pigs. The acute response to bolus injection was similar to that described in a previous study using dogs (3), and consisted of a transient rise in blood pressure of 20-40 mmHg sustained for 2 minutes and associated with a period of apnea ranging from 30 to 90 seconds. The same response was observed with a bolus injection of mercury (1 cc), but no response was observed with a saline bolus injection. Examination of two brains at postmortem

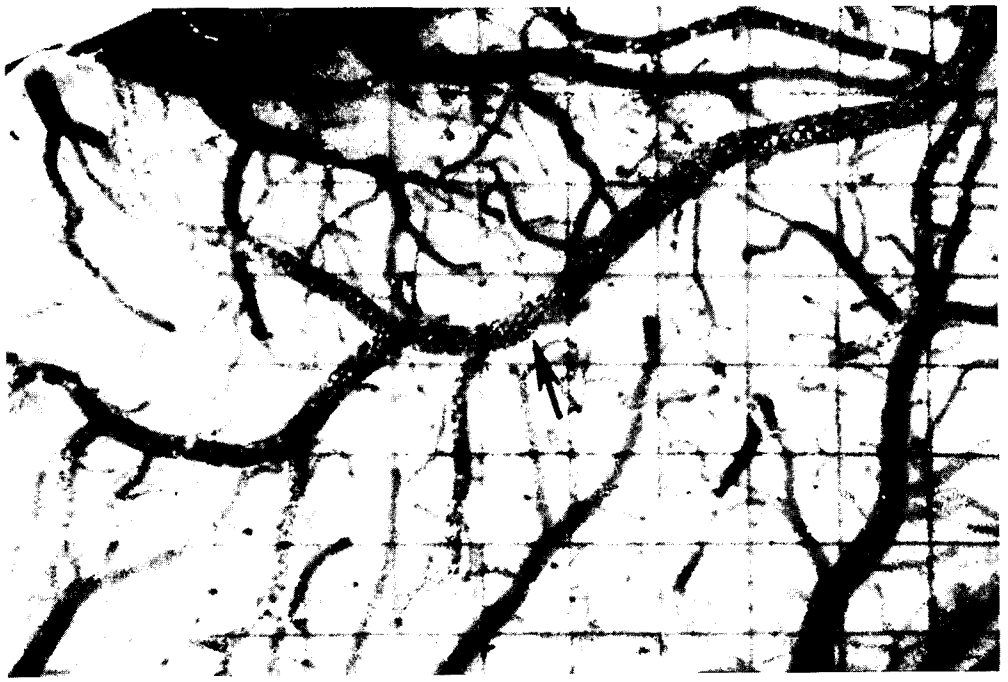

Fig. 2. A cerebral artery after infusion of large numbers of microbubbles.

showed air plugs filling the Circle of Willis and proximal cerebral arteries, with gas extending into the basilar artery. The basilar artery supplying the brainstem was also occluded in the animal which received the mercury bolus.

Basic Microbubble Infusion Studies

Twenty-three animals were used for basic infusion studies of the carefully calibrated microbubbles. Bubbles infused when the animal was in the prone position were distributed primarily in the left middle cerebral artery distribution. Although bubbles less than 50 μm in diameter usually passed into the penetrating vessels out of view, larger bubbles were retained in the surface arteries at points where the vessel diameter was similar to that of the bubbles. These microbubbles tended to accumulate into 'strings' (see Fig. 3); accumulations of more than several bubbles in a given vessel segment tended to *coalesce*, to form a short, cylindrical gas plug or a longer gas column. These intermediate gas 'slugs' had a very characteristic form (see Fig. 4). Coalesced gas formations produced marked local distention of the vessel wall, and showed only limited advancement in the vascular bed. Increases in systemic blood pressure to maximum physiological limits produced some redistribution of gas formations in the surface arteries, but no apparent clearance of gas through the capillary bed. In the untreated state, venous gas was noticeably absent except in several experiments which were felt to represent unusual cases.

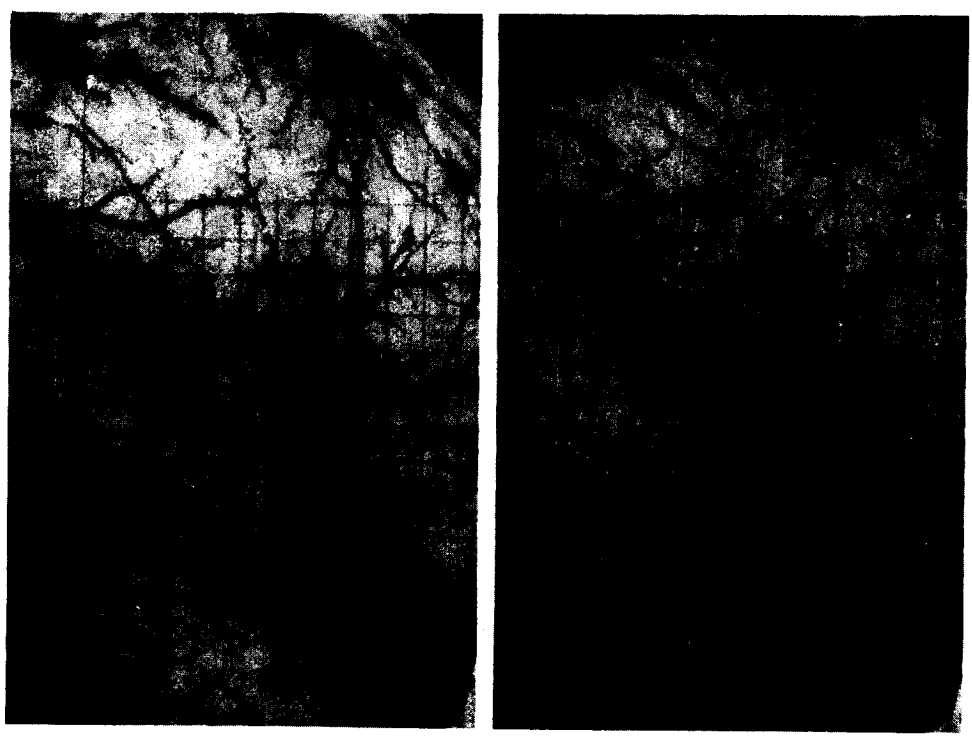

Fig. 3. Chains of microbubbles congregating in a small artery after infusion.

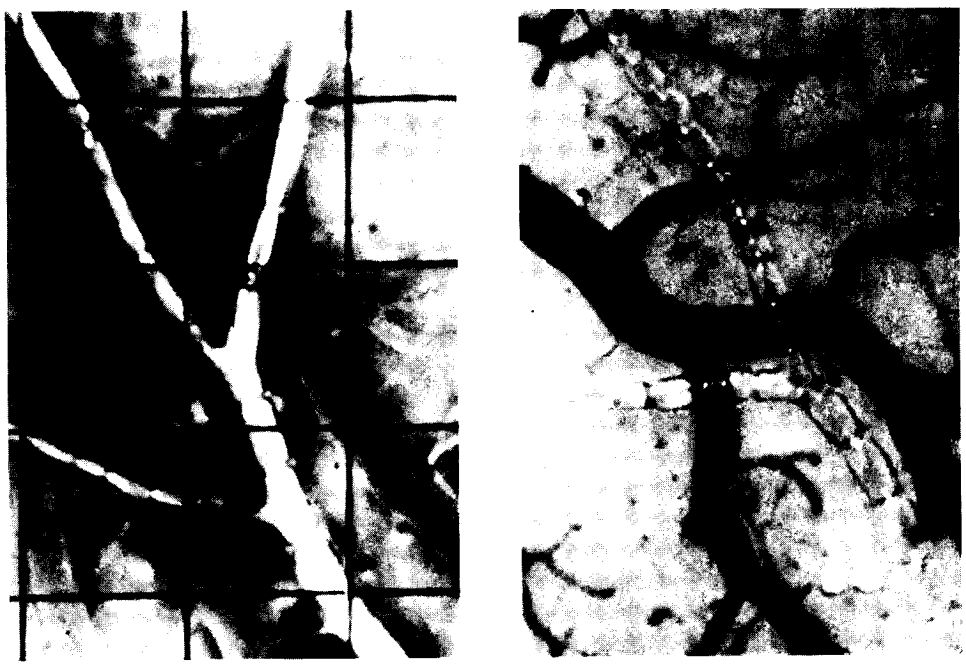

Fig. 4. Photographs demonstrating characteristic 'slugs' of gas formed in a cerebral artery as an intermediate stage in the coalescence of infused microbubbles.

Bounce Compression

A brief "bounce" compression to 6 ATA using air was effective in removing all gas from the surface arteries, whatever its state of coalescence. Although the pial vessels could not be observed during the compression procedure, the vascular bed was examined shortly after the animal was removed from the chamber. In animals with stable vital signs after compression, no gas was observed in either surface arteries or veins. In fact, removal of 80-90% of total brain gas was confirmed by measurements of residual gas volume in the excised brain using a tissue compressibility technique. Venous gas was observed in some animals that expired shortly after being returned to 1-ATA pressure, suggesting clearance of gas through the capillary bed.

Oxygen Microbubbles

Oxygen bubbles entering the window arteries showed diameters approximately 10-15 μm less than those in the infusion stream, indicating some dissolution during transit. Accumulations of long, single-file bubble chains in the pial arteries tended to remain uncoalesced and showed a progressive decrease in size when redistributed into smaller vessels; these eventually disappeared into various penetrating arteries over a period of several minutes. Rapid accumulation of bubbles in arteries larger than the bubble diameter showed typical coalescence to produce the segmented gas column pattern. These gas formations showed dissolution predominantly at the proximal and distal ends where the gas surface was in contact with arterial

blood, and also at points where blood remained in contact with the gas formation via small side branches. Gas plugs contained in vessel segments where blood was entirely displaced showed no apparent loss in volume over periods up to 50 minutes. Coalesced gas formations in general were not dissolved sufficiently to permit redistribution into smaller vessels or restoration of blood flow.

Oxygen Breathing

Oxygen breathing at 1 ATA produced rapid and progressive dissolution of air emboli localized in more distal regions of the middle cerebral artery distribution. Coalesced gas formations were resorbed over 3–7 minutes while they were still contained in the surface vessels. Reduced coalescence between individual bubbles was again observed. More extensive gas formations producing total obstruction to blood flow in the window area showed only limited dissolution. In these cases, circulation was not restored.

Discussion

Demonstration of the typical "bolus response" in the guinea pig (blood pressure rise and apnea) standardized this experimental animal (Fig. 5) with the dog, which has been used in previous studies. The finding of gas in the basilar artery at postmortem following a carotid

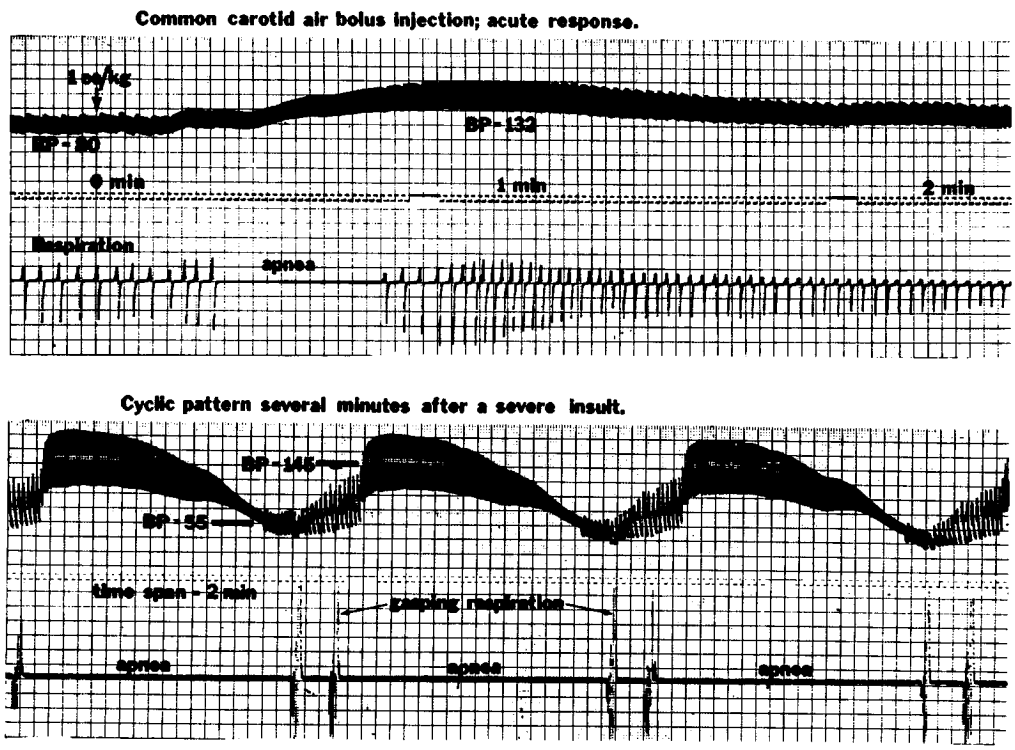

Fig. 5. Blood pressure and respiration after injection of an air bolus.

bolus injection does suggest that the bolus model for cerebral air embolism produces a sudden displacement of blood in the large arteries, thus providing the main routes of supply to the cerebral circulation. When gas is introduced into cerebral blood flow in the form of dispersed microbubbles, acute physiological changes are not observed, even with relatively large cumulative volumes of gas.

Coalescence was identified as a fundamental part of the embolization process which follows the accumulation of several bubbles in a given vessel segment. Coalesced gas formations represent confluent gas volumes which are much greater than the volume of individual infused bubbles. This emphasizes the cumulative nature of the embolization process and points to the *cylindrical gas plug* rather than the spherical bubble as the relevant model in discussing gas embolism. The stability of air emboli in the untreated state which was observed in these studies is consistent with the results of Fries et al. (3), where air columns were found in the pial arteries of dogs killed up to 48 hours after a carotid bolus injection.

The studies using oxygen microbubbles and oxygen breathing during infusion of air microbubbles demonstrated that significant volumes of intravascular gas can be rapidly resolved when a partial pressure gradient for gas diffusion is present. This is perfectly consistent with the proven benefit (6) of recompression upon oxygen as a standard treatment for decompression sickness. However, contact between the gas surface and desaturated blood appears to be a necessary requirement for continued gas dissolution. Conditions of limited surface area for gas transfer and impaired blood flow in the region of embolization are unavoidable with more extensive coalesced gas formations, and would severely reduce the degree of emboli resorption in these cases. The implication is that oxygen breathing alone could not be considered adequate for treatment of any symptomatic cases of cerebral air embolism. However, oxygen breathing would be a valuable therapeutic measure which could be initiated immediately before recompression treatment, or as an adjunct to recompression.

After microbubbles have coalesced in the vessels, the columns of air have much the same appearance as emboli produced by other means. Hence it is not surprising to find that clearance by "bounce" compression essentially follows the same pattern as that found by Waite et al. (8).

A bounce compression to 6 ATA imposes a mechanical volume reduction rather than gas dissolution, since arterial blood would already be equilibrated with air in the lung and the period at pressure would be too brief to allow significant dissolution into the tissues. Any removal of gas would then occur more by clearance of gas through the capillary bed. This hypothesis is largely confirmed by the observation of venous gas after mobilization of gas formations in the pial arteries. Unfortunately, with the equipment available for these studies, it was not possible to observe the pial vessels during the compression treatment.

In conclusion, this work not only adds direct visual evidence to support the use of hyperbaric oxygen to treat cerebral manifestations of decompression sickness, but it would suggest serious consideration of the following practices:

(1) Preceding normal treatment with a very deep "bounce" recompression to somewhere between 200-300 feet, or possibly deeper, to clear arterial gas columns mechanically. Any gas remaining lodged would then, one would hope, be dissolved by the subsequent treatment administered according to present schedules.
(2) Subjects undergoing submarine escape training should take several breaths of oxygen before ascent in the tank, since oxygen emboli are so much easier to resolve.

However, before advocating a deep bounce recompression as standard pretreatment, there is the possibility that it could release gas emboli filtered out by the lungs. Their transfer to the arterial system could then be serious, so this pretreatment would only be recommended for those patients showing Type II symptoms.

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FINE STRUCTURE OF DECOMPRESSION SICKNESS

R. A. Bennet

Many investigations have been made into the origin and effect of bubbles in decompression sickness (1). However, nucleation is a random process, and intracellular bubbles, as well as the more well-known intravascular ones, can form.

The necessary conditions for nucleation include supersaturation of the gas solution at a suitable interface, and movement often activates this process. There is a maze of fluid-fluid and fluid-solid interfaces in the body where bubbles may form under the appropriate circumstances. In particular, the intracellular organelles possess an extremely large surface area within a compact volume.

In this investigation, tissue sections were examined with the electron microscope in an attempt to locate the sites of intracellular bubble formation more exactly.

Method

Four groups of five male white mice (average weight 25 gm) were used in the experiment. Of these, three groups were subjected to high pressure followed by rapid decompression in a small compression chamber. The fourth group, as a control, was not subjected to pressure.

The dive profiles were arranged so that the first two groups would be expected to show symptoms of decompression sickness while the third would not (2).

On completion of the compression-decompression schedule, the mice in Groups 1 and 2 either died rapidly or showed signs of decompression sickness. All animals in Group 3 survived without any apparent distress.

Those animals that survived, including those of Group 4, were killed with intraperitoneal pentabarbitone, after which all animals were treated in the same manner.

The abdominal and thoracic viscera were exposed by a mid-line incision, and the right atrium was cut open so that a 4% solution of buffered gluteraldehyde could be injected through the left ventricle and portal vein without overloading the vascular system. This ensured that the tissues were rapidly fixed with a minimum of mechanical damage.

Sections of tissue 1 mm thick were taken from liver, lung, trapezius muscle, skin, and spinal cord. These were placed in cold gluteraldehyde solution for another hour. After this, 1-mm cubes of tissue were processed by the usual preparation methods for electron microscopy (3).

Results

In the photomicrographs, bubbles were frequently seen in the mitochondria of the animals

of Groups 1 and 2. They were especially observed in the skin, muscle, and spinal cord, although the liver was occasionally affected. The lung mitochondria appeared to escape these lesions. Figure 1 shows this feature, and the extensive damage to the rough endoplasmic reticulum caused by a large amount of intracellular air.

Air was occasionally seen in the tissues of the animals of Group 3, although no major decompression sickness was observed in this group. This air was extracellular, and no intracellular bubbles were present in these mice. Figure 2 compares a section of the spinal cord from such a case with that from a severely affected animal.

Extravascular air can force its way into small vessels, causing vascular damage and thrombosis (Fig. 3). In one section of lung, a small thrombus was found. However, intracellular bubbles were not found in the lungs of animals in Groups 1 and 2, nor were there any significant differences in these lungs compared to the lungs of unaffected animals.

A few bubble lesions were found in liver cells, but again most of the sections showed little difference between normal mice and decompression-sickness mice.

There was considerable variation in the degree of tissue damage in the mice of Groups 1 and 2. In some, lesions were found in cells of the spine, skin, and muscles. In others, the lesions were confined to the tissues of one or two organs.

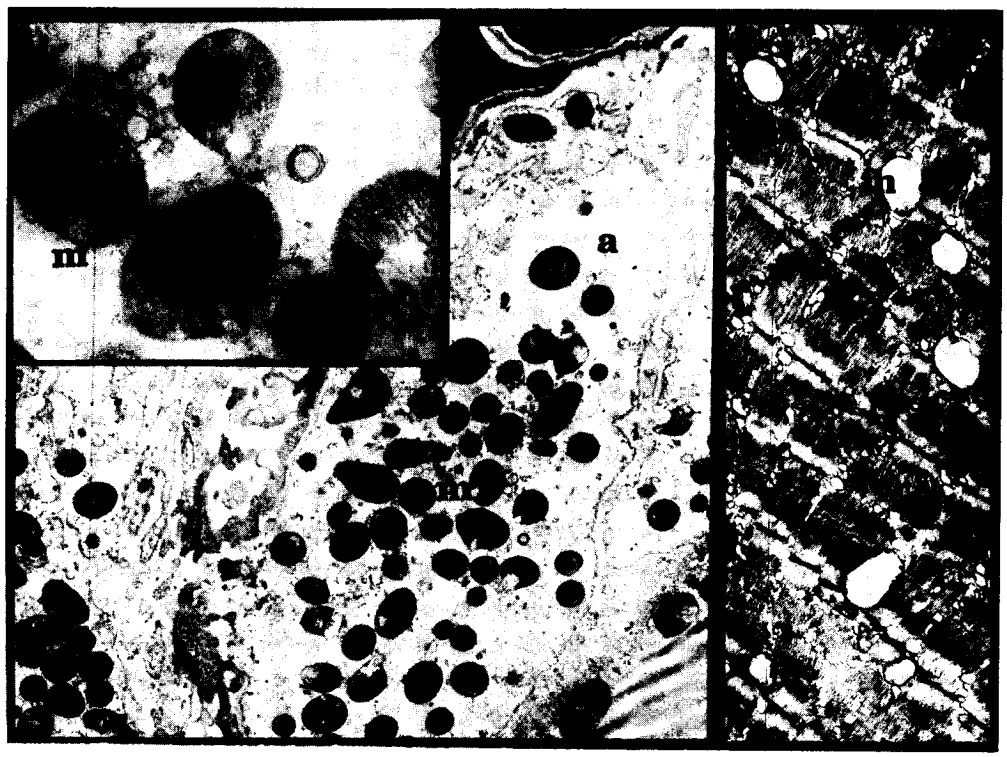

Fig. 1. *Left panel* = skin; bubble formation inside mitochondria (m) and large air spaces (a) disrupting the rough endoplasmic reticulum are shown (×4000; *inset*, ×22000). *Right panel* = muscle; intramitochondrial (m) bubbles are shown (×4000).

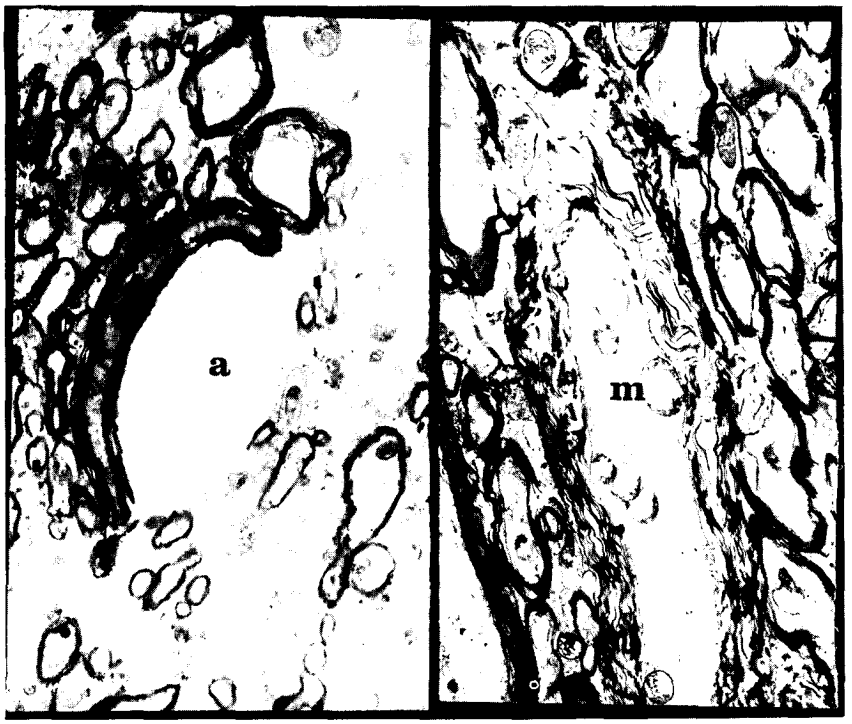

Fig. 2. Spinal cord sections, showing an extracellular air bubble (a) compressing an axon in a mouse not suffering from severe decompression sickness (*left panel*) compared with axons severely damaged by bubbles in the mitochondria (m) and disruption of myelin sheaths in a severely affected animal (*right panel*) (×6000).

Discussion

Although the results demonstrate the random nature of bubble formation, certain features are apparent in the pattern of tissue damage. Mitochondria appear to be very susceptible to bubble formation and subsequent distortion. The liver is apparently protected to a certain extent. No bubbles were found in the lung sections.

The mitochondria are organelles with large surface areas which are liquid-solid interfaces. They are also the sites of energy-rich metabolic reactions within the cell. Of these factors, the first is certainly important in nucleation; the second could provide a source of energy required to overcome the problems of surface tension in very small bubbles.

The lung may be protected from damage by the thin tissue barrier between the blood and the air in the alveoli. High concentrations of gas can diffuse easily from these tissues without building up to dangerous levels. Thrombi found in the capillaries are probably due to metastatic air emboli arising in other tissues.

Tissue damage products such as histamine have not been found in cases of decompression sickness (4) but other material may well be released. In a series of observations on trainee divers during their course of instruction (5), it was found that the divers' serum creatine phosphokinase (CPK) rose during the first two or three weeks, as would be expected in people

Fig. 3. Skin section, showing an extracellular air bubble (a), which has ruptured into a capillary, causing local thrombosis. Air has also extravasated along the tissue capillary interface, causing further tissue damage (×3500).

starting a course of severe muscular exercise. In the majority of cases, the CPK level returned to normal over the following week or so. In one case, however, the level continued to rise. Eventually, this man felt so unwell that he had to withdraw from the course.

CPK is probably a mitochondrial enzyme (6), and since muscle is one of the tissues found to have been damaged in this series of experiments, it is tempting to suggest that there is a connection between the two observations. This, of course, will require much more detailed investigation.

ACKNOWLEDGMENT

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PART X. DECOMPRESSION SICKNESS AND THERAPY

DISCUSSION

R. C. Bornmann, Chairman

Dr. Bornmann: I would like to ask Dr. Elliott if he would consider the suggestion of Dr. Hills. Also, since we do have the capability, if it is desirable, of making saturation helium dives to 300 feet for therapy, would Dr. Elliott give us his opinion whether going to 300 feet would be of benefit to patients suffering from air embolism. It is a serious problem to navies with a submarine force. There have been fatalities in every navy, I think, which carries out submarine escape training.

Dr. Elliott: First, the question of the last breath being oxygen before making the ascent; there would be technical problems in that our escape training uses the method of very rapid compression in which a hood is used during the compression phase. For those of you who are not familiar with this method, compression is to 500 or 600 feet in 20 seconds on compressed air, and in submarine escape training, therefore, the hood is essential. So it would not be possible to take only a last breath; the hood would have to be full of oxygen and thus the trainee might be under pressure, breathing oxygen, for a time that would expose him to a slight risk of oxygen toxicity. If this occurred, it would be possible to have a person making an ascent with a laryngeal spasm, and obviously this would be a bad thing. That is one reason we don't use it, and there are other problems as well.

Second, the question of bounce recompression. The observation that a quick dive to 300 feet gets rid of the bubbles is obviously relevant. In fact, as you will remember, we had the majority of our patients get rid of their bubbles by going just to 165 feet. Our problem is the recurrence and the difference from Dr. Hills' observation is that we are not dealing with injected air, we are dealing with damaged lungs. I would speculate that the reason we get a better rate of non-relapse by staying longer at depth is that we may be allowing the area of lung damage, which caused the primary embolism, time to seal over. Thus, during the subsequent decompression, no further air escapes into the vascular system. So that is an additional factor which doesn't occur in Dr. Hills' model.

Third, the possibility of going deeper. Of course the benefits of reducing bubble size diminish at an exponential rate. One would have to go very much deeper on a saturation dive, but this would impose certain non-biological factors. Our tank at *HMS Dolphin* is over 100 feet high and we have an aluminum chamber at the top. I think that to make the chamber there go to a significantly greater depth would be quite an engineering feat. So, for one reason or another, while I think the comments which have been made are absolutely valid, they are not in fact of operational relevance to us at the moment.

Dr. Lundgren: I was extremely interested to hear Dr. Elliott say that you had some information to the effect that some of your divers may actually have been exhaling too much or had air hunger as they came up. How many gave this information?

Dr. Elliott: Only 8 of the 88 divers. But, after all, we are perhaps dealing with a multiple etiology. We know one or two or them were breath-holding on the way up. As far as the factors that you are considering, airway collapse and air trapping, these may be relevant to about 10% of the instances we've reported.

Dr. Lundgren: As you have already said, this might be an interesting point to make. In theory you might be able to get lung damage by exhaling too forcibly during free ascent, and I wanted to bring the point up here because if people have the chance to observe cases like this and would look out for it, we might get a little more information.

Dr. Bennett: A question for Dr. Hanson, in regard to his comment on the relationship between the exercise on the bicycle ergometer and decompression sickness incidence. I just wanted to point out as was shown early in this meeting by Dr. Fagraeus and myself, in the Duke studies we were using arm exercise, but despite that, the predominant bends incidence was in the legs. Perhaps we should find some way to determine what this peculiar tissue is, perhaps by using radioactive gases.

Dr. Hanson: Yes, I agree.

Dr. K. H. Smith: I would like to comment on the paper of Dr. Beeser. In some less-than-1,000 platelet counts on animals that have been compressed and decompressed, there was absolutely no indication of a 3-day depression in platelet count. In somewhat less than 500 human platelet counts after dives from 700 feet on up, we have seen no reduction in platelet count. In humans we have measured Factors V, VIII, IX, and XIII, and there is no correlation with decompression sickness or decompression problems in any of our studies. The only statistically significant and routine finding that we have had is the reduction in platelet survival where we have seen a suppression to half-normal survival of the platelets when the individuals had been exposed to severe decompressions. May I ask how your platelets were counted?

Dr. Beeser: The platelets were counted in the chamber.

Dr. Smith: In a Coulter counter?

Dr. Beeser: No, direct microscopic count.

Dr. Hennessy: Dr. Hanson and Dr. Vorosmarti, you mentioned that you had greatly increased benefit from using 0.4 bars of oxygen instead of 0.2.

Dr. Vorosmarti: Correct.

Dr. Hennessy: Obviously this doesn't sound very dramatic, lowering the total inert gas tension by 0.2 bar, but you have actually doubled the oxygen window, and the rate of elimination of inert gas is proportional to the oxygen window on these saturation dives. Obviously, doubling the rate of elimination is going to be very beneficial.

Dr. Kawashima: I would like to ask questions of Dr. Elliott and Dr. Hanson. First, Dr. Elliott, I know you reported bone necrosis at an earlier meeting. Did you find any bone necrosis in these divers? Did you find any relation between bone necrosis and bone barotrauma? Next, Dr. Hanson, you showed gas in the joint space of the knee joint in your slides. I would like to know the cause of the pain in the case of bends; does pain come from the irritation of bubbles in the muscle, tendon sheath, bone marrow, or joint capsule?

Dr. Elliott: Bone necrosis; we did some films on the submarine escape training tank instructors. As far as I remember we found two of the instructors had lesions in the shaft, but of course shaft lesions have little clinical significance and we are not pursuing this further.

Dr. Hanson: With regard to the slide I showed, the diver really didn't have much pain at all. In fact all the response afterwards was purely because when I moved his knee joints they made a sucking noise and there was crepitus up and down his thighs. With regard to knowing the exact cause of pain in decompression sickness, I think it is a question we would all like to know the answer to. I know that there has been some correlation in aviator's bends when pain was found where there was gas around the periarticular structures, but no correlation with actual gas in the joint itself.

Dr. Weatherley: Many of my comments directed to Dr. Beeser have been pre-empted by what Kent Smith said, and I would like to support Dr. Smith's findings. In my hematological studies I have also not detected a postdive fall in platelets. In addition, I would like to ask Dr. Beeser whether in his platelet counts, corrections were made for hematocrit?

Dr. Beeser: What?

Dr. Weatherley: You recorded changes in hematocrit following a dive. Is that right?

Dr. Beeser: No, we didn't have reliable changes in the hematocrit.

Dr. Weatherley: Second, do you think your observed maximal platelet depression on the third day and its possible association with the syndrome of intravascular coagulation can really be considered a cause for the symptoms of decompression sickness, which by and large occur within a few hours of a decompression?

Dr. Beeser: The decrease has a'falling tendency over several days, and I do not think it is a sign of disseminated intravascular coagulation tendencies. I think it is only a tendency of the platelets to adhere to the bubbles which are in the circulation.

Dr. Weatherley: Do you think therefore that platelet changes are of any significance?

Dr. Beeser: Yes, I think so. If you do another dive in a short interval you may have potentiation of this status because the platelets are already sensitized by the liquid-gas bubble interfaces. So as a consequence of increasing structural changes you may get a status of a hypercoagulability.

Dr. Beeser: I must point out that we didn't do these investigations to find hypercoagulability status; our intention was to take the absence of hemostatic system activation as a sensitive parameter for the evaluation of our profiles.

Dr. Ackles: This is a comment for Dr. Beeser. In contrast to Dr. Smith, we at DCIEM do see consistent platelet falls following decompression, and we have also seen them following exposure to altitude on breathing normoxic mixtures at 16,000 feet. We would take exception to your interpretation that it is not safe to dive until the platelets come up again, because we have evidence from a statistically analyzed large number of dives that a diver actually has less of a chance of getting a bend within one or two days of a previous dive then he does after four or five days. This exactly corresponds with the time the platelets are normally depressed. We explained this by saying that the platelets that are reacting with the bubbles, and perhaps they are also somehow related to the symptoms, are the stickier platelets. These have been removed from the circulation and therefore next time a diver dives he has less chance of getting the blood-bubble reaction going. In fact we have completed repetitive dives and we had very peculiar results, but we did not get a continuing depression.

Dr. Rudell: I have a question for Dr. Bennet. You speak of a rise of the CPK enzymes over a few days and sometimes longer; we usually fractionate the CPK isoenzymes to the MM, MB, and BB bends. Do you have any particular comments as to which fractions rise first?

Dr. Bennet: I was frightened that someone might ask that; I don't have any particulars myself. I haven't been able to undertake any enzyme studies. It is something that should be gone into in the future.

Dr. D'Aoust: Dr. Hallenbeck, about a year ago at the Undersea Medical Society meeting in Washington, I recall taking you to task regarding the extremeness of the delta P of the decompression necessary to produce these symptoms. I would like at this time just to recount one incident that is probably only of passing interest, but it has changed my thinking quite a bit. We have studied nitrogen elimination in dogs, awake dogs, by pulmonary catheter samples of the pulmonary artery, mixed venous blood, nitrogen content. In one case we had a dog go into decompression sickness so acutely that we no longer wanted to sample. We recompressed rather quickly. The dog was obviously going to die if we didn't do this. On arrival on the bottom, consciousness was regained, though there was residual paralysis in the hind quarters of the animal, which were hanging from the sling which we used for moderate restraint. It is not necessary to restrain the animals much, but because of the catheter we do. We then started some low molecular weight dextran therapy by a rather direct route through the pulmonary artery catheter which we had been using for our samples. The effect of this was quite remarkable. It is only one example, but it was spectacular. I was putting the doses of 20 or 50 cc in as fast as I could because I had limited no-decompression time and had to get out. I had the distinct sensation that the hind quarters of the animal were being jacked up with every slug of this stuff until virtual full recovery of standing ability was attained. I think some of these compressions and decompressions like yours are extreme. I think the merits of this infusion technique really have to be considered; this is standard therapy in a lot of our treatments. I have another comment to Dr. Wells. Do you suspect that the effect of prostaglandin is mediated through synthesis or perhaps through nonabsorption by the lung? If you recall, it has been demonstrated by Dr. Robertson of Seattle that the lung absorbs prostaglandin E2 in one pass through the pulmonary vasculature. We did some studies of arterial and venous levels in decompressed dogs. The results were inconsistent, but we never got the normal arterial venous difference. The question is, are you ready to exclude the possibility that the lung is simply not absorbing prostaglandin E which it normally does, or are you going further to say that it is synthesizing new prostaglandin E?

Dr. Wells: The information we have about indomethacin's pharmacologic activities suggests to me that the effects of this compound on fluid losses of dysbarism must result from inhibition of prostaglandin synthesis.

Dr. D'Aoust: One more comment for Dr. Bennet. It just occurred to me since you mentioned the lung was free, relatively free, of these structures, that the lung is the tissue you could look at if you could somehow use a variable degree of pulmonary edema or atelectasis in the lung. It would have to be a fairly good system to get some idea of what critical supersaturation would do this.

Dr. Bennet: It would, indeed, and I hope some day to do this.

Dr. Lundgren: Dr. Hallenbeck, now that this beautiful work you talked about has helped us understand quite a lot of what is going on in the spinal cord, would you be willing at this stage to speculate about the explanation for the curious biphasic cause of spinal injury that has been described? I'm thinking of the diver who comes up after an insufficient decompression and develops weakness in the legs, and during transportation to the chamber, this weakness disappears and he is able to walk and to move perfectly normally. However, before treatment can be instituted again, he slides into a severe, and this time very difficult to reverse, paralysis.

Dr. Hallenbeck: My answer has to be speculative. First of all the initial insult to the cord would appear to have a vascular basis, would appear to be ischemia. There are many models of central nervous system ischemia from which we can draw inferences. I can think of work by Hossmann and Olsson, for instance, in which they clamped the

brachiocephalic and left subclavian arteries near the aortic arch in cats and caused ischemia of the brain in that way. They observed the pial circulation through a craniotomy. They found that after ischemia which had persisted long enough to damage the tissue there did seem to be biphasic changes in flow. Immediately after the clamps were removed the restored flow in the pial vessels was brisk, but after a period of 20 to 60 minutes the flow slowed and sludging and stasis occurred. There is a term for this which comes from another model in which spinal cord damage is produced by dropping a weight directly on the spinal cord to produce a standardized and reproducible traumatic insult to the cord. The term used is autodestruction. If for some reason flow was restored in the situation you described but the period of ischemia had been long enough to damage the tissue, it is possible that through processes which are not well understood at this point the restored flow could again deteriorate. This phenomenon has been observed.

Dr. Lundgren: Is a therapy postulated for that condition?

Dr. Hallenbeck: The man who coined the term autodestruction is Osterholm and he has come under fire for his work. There is a good deal of controversy about it. But he thought that perhaps the basic mechanism involved a release of norepinephrine in the damaged area and he treated it with alpha methyltyrosine and got some good results. I would say that there isn't a clear therapy.

Prof. Walder: Could Dr. Bennet tell us how he eliminates the possibility of artifacts introduced during the preparation of his material explaining the findings?

Dr. Bennet: I try to move the animals as little as possible; I open them by central incision and then give the gluteraldehyde intravascularly, hoping the tissue will be fixed without moving it. I did some prior experiments with dye and saw that this method would get solutions throughout the animal's body. I then leave the animal for five minutes for fixation, and then take strips of tissue to give them better fixation. Then the small cubes are taken as far from the original incision as possible.

Dr. Chryssanthou: I would like to make a general comment. Dr. Fructus' work has shown that Migristene prevents decompression sickness but it does not prevent aggregation of platelets. This raises some questions as to the relative significance of platelet aggregation in the pathogenesis of the disease. Also, Dr. Wells has attributed the extravasation of fluid primarily to prostaglandins, but again Migristene, which prevents decompression sickness, and many other similar substances that showed dramatic effects in preventing the disease in our laboratories, do not have any antiprostaglandin effect. That raises still another question. What is the relative significance of hypervolemia in the mechanism of decompression sickness? It is evident that decompression sickness is the sum total of effects of a multiplicity of factors which come into play in a chain reaction which is probably triggered by gas bubbles. Up to now our emphasis was, and rightly so, to prevent this chain of reactions at the very proximal links. But this doesn't work all the time. It is therefore necessary to consider the possibility that it may be that we have to administer pharmacologic agents, perhaps a cocktail containing inhibitors and antagonists of many of those factors. We could start, for example, by making our cocktail with Migristene; we could then add some heparin to it and maybe a little bit of indomethacin, and see where we go from there.

Dr. D'Aoust: Dr. Chryssanthou, did you just dispense with my suspicion that SMAF is a prostaglandin? Would you care to comment on that?

Dr. Chryssanthou: This has been debated at length in many other meetings. The chemical and physical properties of SMAF and its pharmacologic effects exclude that possibility, although it has many similarities to prostaglandin. This gives me an opportunity to ask Dr. Wells another question. You said that the loss of fluid was due to prostaglandins. Prostaglandins are known to modulate the effect of other vasoactive substances. Is it possible therefore that the loss of fluid could be due to the action of bradykinin, histamine, or other substances on which prostaglandins have a modulating effect?

Dr. Wells: I would think it highly likely; however, to the best of my knowledge the information necessary to answer this question is still lacking.

Dr. Kitano: I have a question for Dr. Bennet. I was much impressed with your electron microscopic work. Were there any changes in thrombocytes and/or in mitochondria of thrombocytes?

Dr. Bennet: I only saw the one thrombus and apart from the agglutination I haven't seen anything further, just the clumping together. I have not seen anything in their mitochondria.

Dr. Behnke: I would like to make a statement first, and that is that the term as used, disseminated intravascular thrombosis, is I think a misnomer. Decompression sickness is a reversible process. I have never known a diver or a tunnel worker to die of heart failure under pressure. It is a repair process. I would like to ask Dr. Hallenbeck a question. Certainly the study of Drs. Elliott, Hallenbeck and Bove is a classic study. It is rather puzzling that if venous obstruction is the prime cause of spinal cord injury, it is difficult to explain why the gray matter is not affected. The type of injury that occurs affects the white matter primarily, and we get a spastic type of paralysis limited to that part

with poor circulation. Second, in altitude decompression sickness, chokes is not uncommon and we have reason to believe that there is venous obstruction. Yet it is rare that the spinal cord is injured, presumably because the bubbles that form in altitude decompression sickness are large, so large that they do not block the arterial circulation.

Dr. Hallenbeck: What was your first question again?

Dr. Behnke: The question is how do you account for the specific type of lesion which affects the white matter? Why is not the gray matter affected? One would expect to see a flaccid type of paralysis and injury to the gray matter nerve tissue if there were a general obstruction of venous flow.

Dr. Hallenbeck: The pathologic picture of damage to the cord in decompression sickness is that of white matter hemorrhage with gray matter sparing so there certainly is pathologic confirmation of your statement. As to whether the gray matter is affected functionally, the ease with which this could be detected would vary with the level of the lesion. If for instance the thoracic cord was damaged, as it is very frequently, one could miss the signs of neuronal damage in the gray matter of the cord and one would concentrate on spasticity and increased reflexes and all the signs of upper motor neuron damage. It would only be when the process involved either cervical or lumbar enlargement that one would have readily diagnosable lower motor neuron involvement. The white matter hemorrhage and gray matter sparing that occurs pathologically is somewhat puzzling, and I don't know for certain why it occurs except that it is the pathologic picture which is associated with venous obstruction. One could I guess conjecture that with the richer vascular supply in the gray matter there would be more avenues for collateral flow in the event of a limited venous obstruction. The gray matter in the cord might be able to drain enough to avoid the occurrence of hemorrhage. I can't really give a definite answer. The question of chokes in aviators is an interesting one and until the cinephotomicrography studies, we ascribed great importance to the cardiovascular changes that occurred in our model. I think that these changes are definitely facilitatory. If there is increased pulmonary artery pressure and increased central venous pressure, as we have very often observed, it does cause congestion of the epidural veins and probably also interferes with their drainage. But in the cinephotomicrography work there were three dogs which were exposed to dives which were at the threshold for producing spinal cord damage in an anesthetized dog. The exposures were 220 fsw for 25 minutes. In these animals there was no pulmonary artery pressure rise, there was no right ventricular pressure rise, and there was no central venous pressure rise, yet the epidural veins became completely occluded and there was pathologic and clinical evidence of spinal cord damage. We have therefore had to revise our view of the sequence of events that leads to cord damage in decompression sickness and say that the cardiopulmonary changes which we often see in our model are probably facilitatory but are not absolutely essential for the occurrence of the spinal cord lesion. That still doesn't answer your question as to why aviators do not have more spinal cord damage.

Dr. Spencer: I believe that you showed that the gray matter had about 54 or 50 milliliters per minute per 100 grams, and that the white matter had about 17. Is that right?

Dr. Hallenbeck: In the example it was 14, but 14 to 17 ml/100 gm/minute would be normal white matter flows in the spinal cord.

Dr. Spencer: If you compute the half times of tissue very readily from the perfusion rate, at a flow rate of 54 milliliters per minute, it is on the order of seconds, 6 seconds or something like that. It would be almost impossible to produce decompression sickness in any kind of a reasonable decompression procedure in a tissue which is perfused at that rate. The brain as a whole has also that high a flow rate, and perhaps this is the reason that you see the cord lesions so frequently. The ratio of gray matter to white matter I believe is higher in the cord, at least there are nerve fibers running along the outside and not so many nerve bodies. Could this be the explanation for why cord lesions are so frequent? In the axons with a lower flow rate there is the possibility of decompression sickness, whereas in the gray matter and with a higher concentration of cells one would not expect it.

Dr. Akers: One possible answer to Dr. Behnke's question was flashed on the screen in Dr. Bennet's nerve cell slide. If you recall, the Schwann cell was very badly destroyed, and most intracellular damage due to bubbles was only in the mitochondria, and there wasn't much of that. Remember that Schwann cells and myelin sheath are very poorly perfused and don't have much internal cellular flow, while the inside of a nerve cell does; this may be why white matter is hit and gray matter is not.

Dr. Hallenbeck: The group from Japan (Hayashi et al.) that showed those elegant photomicrographs of human decompression sickness material had one slide in which there was disruption of the myelin figures in an area of cord. There was a thrombosed or at least congested vessel in the same region, and this is the other kind of pathologic picture that we have seen. We have seen hemorrhage as the predominant type of lesion but the other type of lesion resembles what they showed. With an H&E stain one can see that congested vessels are present in areas where the myelin is distorted and disrupted. One could postulate that when you interfere with transport of inert gas out of the fat-rich myelin, a secondary factor of tissue bubble nucleation enters and complicates the problem.

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