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THE EIGHTH UNDERSEA MEDICAL SOCIETY WORKSHOP

THE STRATEGY FOR FUTURE DIVING to DEPTHS GREATER THAN 1,000 FEET

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Sea Ranch, California
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THE STRATEGY FOR FUTURE DIVING
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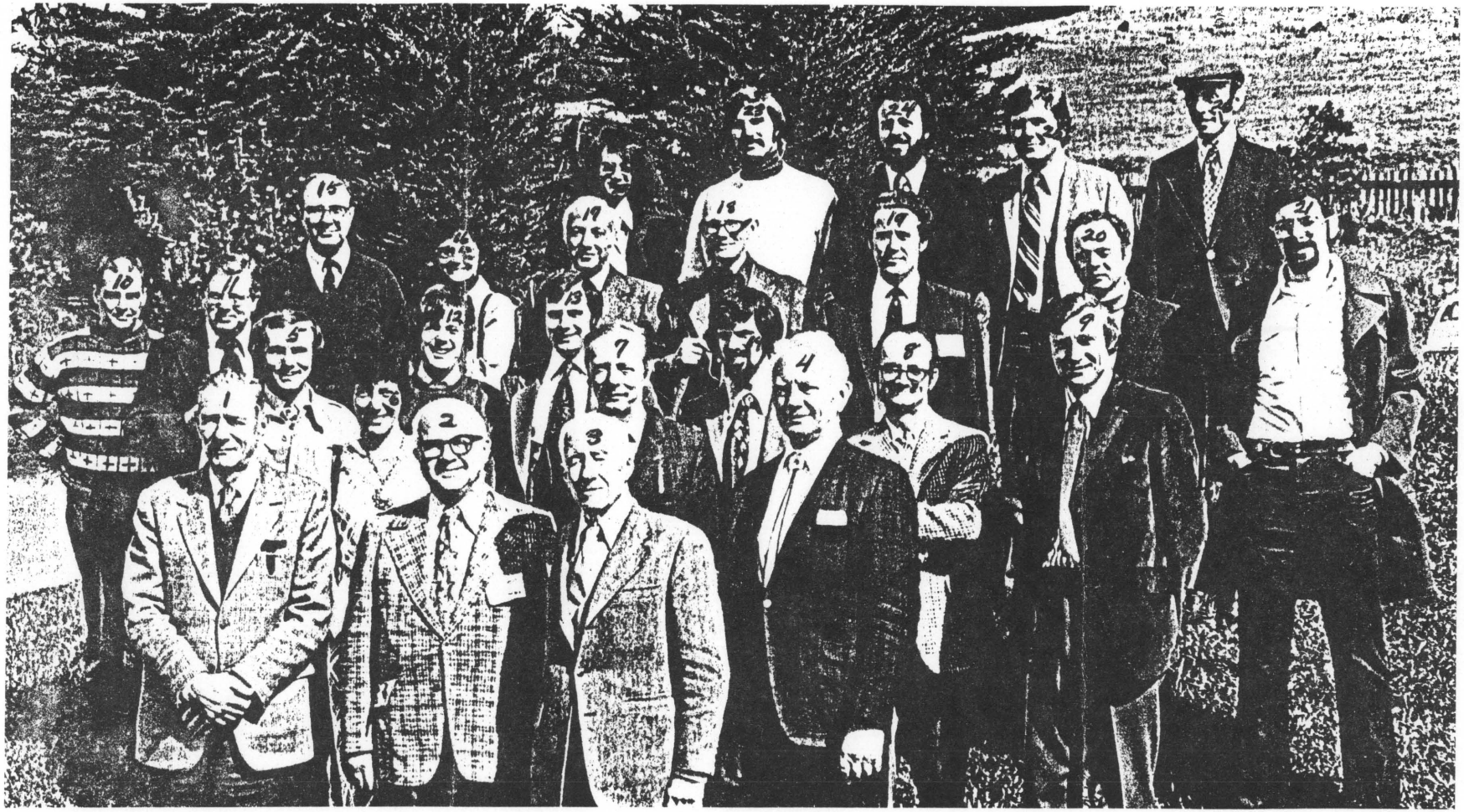

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Chairman's Introduction

This is Bob Featherstone's meeting. He planned it and accomplished much of the organisation before his sudden, tragic death in Europe last year. It was something he felt strongly about. I met him shortly before his death and he was extremely enthusiastic about his plans for this event. Those who have taken over his responsibilities have tried to adhere, as far as possible, to the principles he outlined.

The Workshop will be concerned with the problems divers face below 1000 ft. However, not all diving problems will be tackled - this would be too much for the limited time we have available. Thus the intractable and challenging problems of decompression sickness which have been recently discussed (Workshop on : "Development of Decompression Procedures for Depths in Excess of 400 feet") will only be referred to en passant. Broadly speaking we will be concerned with the problems of the diver when at depth. The meeting will be largely concerned with the effects of narcosis and pressure. The interaction between these is so marked that, at the pressures about which we will be talking, it is impossible to discuss them separately. This is one of the most important lessons that has been learnt from recent research. The first part of the meeting will deal largely with the grosser aspects; the phenomena observed in small animal experiments and the theories, often of a physico-chemical nature, that have been advanced to account for them. The rest of the meeting will be concerned with the more subtle manifestations observed in humans at pressure.

It would be too much to hope that a short meeting of this sort could solve the problems of deep diving. However, I hope we will be able to re-define or at least clarify these problems and to suggest the research that is needed to promote further advances.

E.B. Smith
Sea Ranch, California,
26th February, 1975.

The Report

This report was compiled in the days immediately following the workshop by the rapporteurs, largely from their own notes, together with some written material provided by the contributors. However, it is entirely the responsibility of the rapporteurs and not the original speakers. It has been made as comprehensive as possible although, for reasons of economy, a uniform format had to be adopted. In the recorded discussions it was not always possible to identify individual speakers and it was thought appropriate not to attribute specific comments to individuals. The figures are based on some of the slides shown at the meeting.

Thanks are due to Mr M. Gordon for handling the audio-visual aids, and Miss B.N. Dobson for the complete responsibility of typing this report.

M.J.H.
W.S.
E.B.S.

Summary of General Conclusions
(see also section 20)

1. Experimental diving limits: There now appears to be no "barrier" to deeper diving. Rather, the risks and problems appear to be increasing slowly but steadily with depth. Cautious experimentation may permit men to reach depths significantly greater than the 2000 ft already attained.

2. Anaesthetic additives: It is now accepted generally that the addition of anaesthetic substances can ameliorate many of the adverse effects of high pressure. The only additive so far studied in man is nitrogen and the optimum partial pressure has yet to be defined. It should be recognised that nitrogen is only one possibility, and that a wider pharmacological attack on the problem might prove rewarding. This technique may prove to be a major advance in deep diving technology.

3. Working dives : Routine working dives in the sea have yet to be extended beyond the 600 foot limit. It is essential to gain as much information as possible about technical and equipment difficulties, as well as assessing the limitations of various techniques such as excursion diving. It was recommended that practical data from all diving concerns should be collected, collated and made available to interested parties prior to general publication.

4. Therapy: It is of the greatest importance that attention be paid to the problems of establishing an adequate therapy for decompression sickness encountered at great depth. In particular,

rapid recompression (the basis of normal therapy) may introduce further high pressure neurological syndrome problems. More generally the treatment and/or recovery of men who get into difficulties, or even have a simple illness, at depth must be given further consideration.

5. Environmental hazards: The long term effects of diving to great depths are almost completely unknown. In other specialties, such as anesthesiology, chronic exposure to an abnormal environment is now recognised to produce unexpected and subtle changes in several body functions. It was agreed that it was important to encourage as wide a spectrum as possible of clinical follow-up studies on divers in an attempt to determine the most sensitive indicators of any impending problems.

6. Diver selection: As the limits of normal human tolerance are approached, increasing practical benefit should result from the selection of exceptional individuals who have low susceptibility to high pressure effects. It may prove possible to devise suitable tests for diver selection on the basis of our present knowledge.

7. The multidisciplinary approach: Many research workers in a wide variety of disciplines are currently studying the problems of deep diving. It is evident from the different sections of this report that there are likely to be important developments over the next five years. It was agreed that regular small workshop meetings and widely circulated reports have a vital role in helping to close the gaps between these expanding areas of research.

(There were no formal recommendations from the Workshop, and this section only contains some of the more important conclusions from this Report)

Review of Physico-chemical approaches to animals at high pressures

E.B. Smith

Narcosis

Divers cannot, at pressure, breathe pure oxygen below 60-90 ft as the gas produces convulsions even at those modest pressures. Thus a dilutent gas, traditionally nitrogen but now for deep diving helium, must be used. Nitrogen, like most other inert gases, will, at pressure, induce inert gas narcosis which may be regarded as an aspect of general anaesthesia. All (or almost all) "chemically indifferent substances" will produce loss of consciousness in animals when administered at the appropriate concentration (for example, chloroform: approx 0.01 atm, nitrogen: approx 40 atm). It has been customary to make what has been called the Unitary Hypothesis in seeking to interpret this phenomena. Thus, despite known differences in their effects, we assume that all these substances act in the same way and presumably at the same site. The parallel nature of the dose response curves for different agents (1) provides support for this point of view.

There is good evidence for regarding the synapses as the physiological site of action of general anaesthetics. It has been concluded from studies on in vitro preparations of the olfactory cortex that anaesthetics act either by reducing the output of transmitter from the pre-synaptic terminals or by reducing the sensitivity of the post-synaptic membrane(2). Much discussion has centred on the physico-chemical nature of the site which, if established, could suggest the mechanism by which anaesthetics act.

Ferguson's Hypothesis(3) proposes that the potency of all anaesthetics is the same when they are at a concentration that produces the same thermodynamic activity (defined relative to the pure liquid standard state). As activities in all phases are the same at equilibrium, a consequence of this principle would be that it would be impossible to identify the site of action of anaesthetics from consideration of the relative potencies of anaesthetic substances. Put another way, potency would depend only on the molecular properties of the substance and not on the manner of its interaction. Fortunately the principle does not hold well. If we are to define the site of action we must look to substances with unusual inter-molecular forces, such as SF_6 or flurocarbons, as for many common substances all correlations would prove equally satisfactory, and therefore uninformative.

Much discussion has taken place as to the relative merits of the hydrate theory of Pauling and Miller and the lipid solubility model(1). We may summarize the points for and against these models:

(1) The lipid solubility theory gives a better correlation than the hydrate dissociation pressure plot suggested by the hydrate theory. In particular, SF_6 (and indeed other fluorine compounds to a lesser extent) lie off the hydrate plots.

(ii) Mixtures of gases which form Class I and Class II hydrates e.g. halothane-xenon, do not show expected synergism.

(iii) A number of common anaesthetic substances, e.g. ether, halothane, do not form hydrates.

In favour of aqueous phase theories:

(i) He, and Ne, do not form hydrates: No anaesthesia can be produced by these gases. (The significance of this observation is clouded by the fact that the effects of pressure per se intervene as will be discussed later.)

(ii) If the theory is freed from consideration of hydrate dissociation pressures, it is possible to obtain a good correlation using the entropies of solution of anaesthetics in which even SF_6 is accommodated (but not $CHCl_3$ and ether). (4).

(iii) It provides an attractive mechanistic model.

The molecular nature of the site is an open question. It should not be assumed that because the site is hydrophobic in nature it must be in lipid bilayers. Hydrophobic regions of proteins are equally acceptable.

Pressure

The amphibious Italian Great Newts lose their righting reflexes between 165-245 atm irrespective of whether the pressure is applied using helium, neon or hydrostatically. No anaesthesia occurred with mice exposed to helium and neon but both gases proved lethal at 135-145 atm. Thus it was concluded that the predominant effects of high pressures of helium were due to pressure per se. The results of the experiments carried out on mice with helium indicate the effects of pressure to be four-fold: unco-ordinated tremors, convulsions, respiratory distress, paralysis.(5).

Interaction of Pressure and Narcosis

Perhaps the most intersting and potentially useful effect of pressure is the mutual antagonism between the effect of pressure and general anaesthetics. The first observation in this direction was that of Johnson and Flagler(6), who noted that the swimming activity of tadpoles narcotised with 2 - 5% alcohol could be restored by the application of 200 - 300 atm pressure. This pressure in the absence of the alcohol would induce paralysis in the tadpoles. The antagonism between

pressure and anaesthetic agents was subsequently observed with newts and mice as shown in Figure 1. (The contours indicate areas in which newts have equal ability to right themselves at 20°C in the presence of N_2O (doses recorded as P/P_{50}) and high pressures of helium (P(atm)). In the central shaded area the newts have better than 90% values of the righting reflex. The centre square indicates the region of high performance that would be observed if the pressure and anaesthetics acted entirely independently. The antagonism produces a large extension of this region). With mice the same can be observed. Roughly, the presence of anaesthetics doubles the lethal pressure and we have had them surviving to 300 atm.

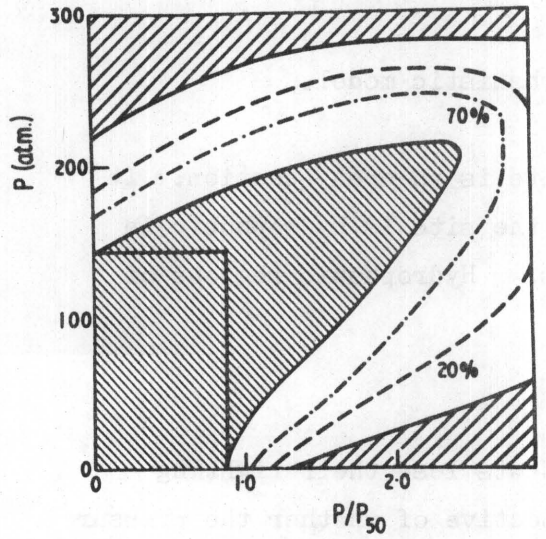

Figure 1

The degree to which this phenomenon of 'pressure reversal' can be used to the advantage of human divers must be an important area for discussion. Barbiturates are equally promising and a wider pharmacological attack on the problem would be valuable.

Constant Volume Hypothesis

I would like to outline our own theory of pressure reversal. This will be described in a more quantitative form later. Basically, it supposes that, if the volume of the hydrophobic 'site of action' of anaesthetics is maintained at a fixed value

by the simultaneous application of anaesthetics (which cause an expansion) and the appropriate pressure (which leads to contraction), the response of the organism will be unchanged. If we assume that the physical nature and the solubility properties of the site are the same in all species, the model (to a first approximation) predicts that a plot of $\frac{\Delta P}{P^*_{50}}$ vs total hydrostatic pressure should give a universal straight line for all species and all anaesthetic agents. In fact, the slope for mice(Δ), newts(O) and luminous bacteria(X) only varies by $\pm$15% as shown in Figure 2: (ΔP is the additional dose of anaesthetics above the P_{50}, and P_{helium} the pressure required to restore the response of the organisms to the 50% level. P^*_{50} is the pressure required to reduce the righting response of mice to the 50% level). How far these physico-chemical models can be of service to those concerned with human diving will be, I hope, one of the areas that we can fruitfully discuss at this meeting.

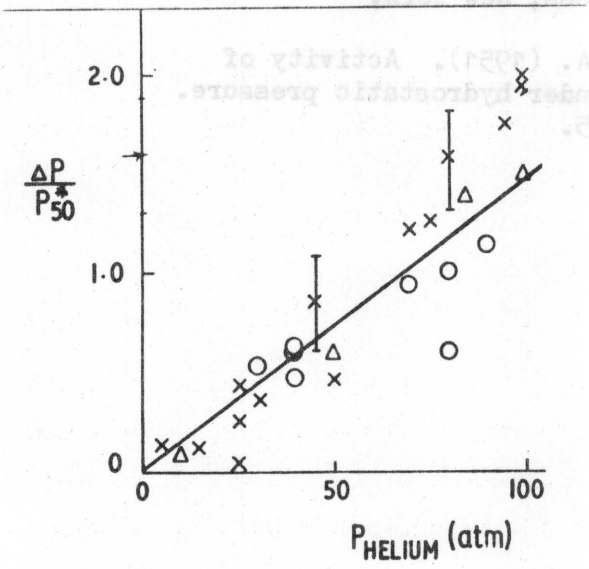

Figure 2

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Structural aspects of the interaction of anaesthetics with proteins and bilayers

B. P. Schoenborn

The first part of this paper is concerned with the direct interaction of some anaesthetics with the "semi-model" biological systems haemoglobin and myoglobin. Anaesthetic solubility in blood is generally higher than that in saline because of the presence of proteins. Using X-ray diffraction techniques we demonstrated that xenon and cyclopropane bind specifically to haemoglobin and myoglobin (1,2,3) and the feedback mechanism associated with carbon monoxide binding is affected.

Since xenon and cyclopropane are uncharged simple molecules we had expected that they would bind on the surface of the protein as suggested by Pauling and Miller's anaesthetic clathrate hypothesis. However, we found that the specific site of myoglobin was located in the interior of the molecule and that the distortion of this binding cavity started a complex "lever mechanism" in which the effect was propagated via hydrogen bonds along the peptide chain to another site near the haem group affecting the carbon monoxide binding. The detailed mechanism of this is currently being developed.

Similar studies have also been carried out for haemoglobin, where we unexpectedly found that the anaesthetic binds in two or three possible sites in different areas of the protein. This approach has led to an insight into the type of interaction between anaesthetics and proteins which is based purely on the physical properties of the molecule without any direct chemical bonding. Recently the studies have been extended to include the interaction of dichloromethane with myoglobin (4) and the X-ray difference analyses show that, as the size of the anaesthetic increases to

that of dichloromethane, there is increasing deformation of the same binding site (Table 1).

TABLE 1

Some physical properties of anesthetics whose myoglobin-binding properties have been studied

Molecule	Formula	Profile	Van der Waal's Radius (Å)	Polarizability ($Å^3$)	Ionization Energy (ev)	Binds to Myoglobin?
Krypton	Kr		1.96	2.5	14.00	no
Nitrous Oxide	N_2O		2.02	3.4	12.89	no
Acetylene	C_2H_2		2.13	3.5	11.40	no
Xenon	Xe		2.13	4.2	12.13	yes
Ethylene	C_2H_4		2.21	3.9	10.50	no
Ethane	C_2H_6		2.29	4.1	11.50	no
Cyclopropane	C_3H_6		2.43	5.5	10.09	yes
Dichloromethane	CH_2Cl_2		2.54	4.1	11.35	yes

The rest of this paper is concerned with the larger scale interactions of anaesthetics with membranes. Initial experiments with high concentrations of cyclopropane, ether, chloroform and xenon demonstrated that the X-ray diffraction pattern of a lipid bilayer disappeared indicating that the anaesthetics were disordering the bilayer. However, the amount of information that can be obtained by X-ray diffraction in such a system is limited by the similarity of the scattering factors for the different components. The alternative technique of neutron diffraction (5) has the advantage that the signal to noise ratio can be considerably improved and that the use of deuterated components substantially increases the sensitivity of the technique. We initially characterised lipid interactions in detail. For example,

we have found that there is a direct interaction between the hydroxyl group of cholesterol and the polar head group of dipalmitoyl lecithin in bilayers (with 15% humidity) which is strongly influenced by an ordered water layer on the surface. We have also studied the interaction of anaesthetics with the bilayers. It appears that chloroform first interacts directly with the head group of the lipids and then penetrates between the head groups into the hydrophobic area of the bilayer. In a dipalmitoyl lecithin bilayer (at 22°C and 15% humidity) the half time of penetration is probably less than the 2 or 3 minutes which are imposed by present limitations of the experimental arrangement. It is hoped to extend such observations to include a wide range of anaesthetics and membrane components in order to understand the molecular changes which underlie the functional changes which will be discussed later at this meeting.

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Functional aspects of the interaction of anaesthetics with proteins

W. Settle

The structural knowledge of the binding of anaesthetic gases to haemoglobin and myoglobin makes these two proteins desirable examples by which we can gain a basic understanding of how such agents can alter protein function. At one atmosphere, xenon can reduce the affinity of myoglobin for carbon monoxide to three-quarters that in the presence of no second gas. With the addition of sodium dithionite, or at pH values slightly above 7, the same concentration of xenon will cause an increase in affinity. Similar results are seen with cyclopropane. Analysis of these data requires the assumption of three sites, two of which produce an increase in affinity (one rather dramatically) and another site which produces a decrease in affinity. These sites and their effect on carbon monoxide affinity can be listed as :

site A: +25%; site B: -75%; site C: + 400 to 900%

Sodium dithionite seems to abolish the site which decreases the affinity. The site producing the dramatic increase in affinity appears to be pH dependent. Thus we can form the tentative picture (Figure 1) of the site near the haem producing a slight increase in affinity, the site between the AB and GH corners also causing a large increase in affinity and a third, as yet unidentified, site which is sensitive to

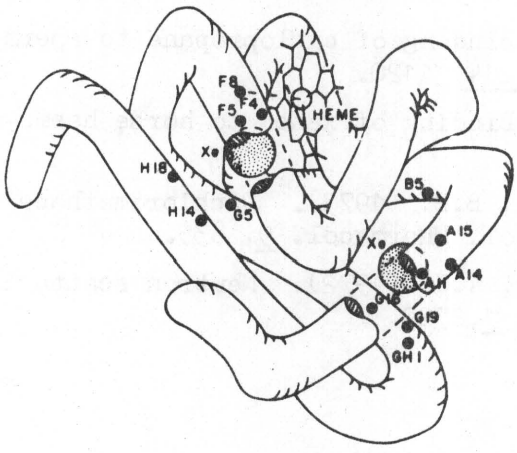

Figure 1

sodium dithionite and decreases the affinity. Figure 1 is a diagrammatic sketch of sperm whale myoglobin(1). The amino acid residues noted are those whose side chains are within van der Waals contact of the xenon atoms.

One would have anticipated that the site nearest the haem would have the greatest effects on haem binding. However, a plausible explanation emerges if one considers the changes in tertiary structure which Perutz has elucidated for haemoglobin(2) Upon ligand binding to the haem , structural changes in the haem result in a movement of about two angstroms of the E helix toward the AB corner. If this movement allowed more van der Waals interactions with the inert gas, stabilization of that form (the oxy or carboxy form) would result. A consequence of this would be that one would expect the dissociation reaction to be altered and the association reaction to be relatively unaffected. Measurement of the reaction rates under conditions leading to the overall increase in affinity confirm this. The dissociation of carboxymyoglobin in the presence of one atmosphere of xenon is nearly one tenth the control value. An important result of this is that it does not require a conformational change to occur upon binding of a molecule for that molecule to have a pronounced effect on protein function.

Figure 2 illustrates the results of the effects of low concentrations of dichloromethane on carbon monoxide binding to haemoglobin. The effects of nitrogen at 50 and 100 atmospheres on oxygen binding to haemoglobin are almost identical to the changes seen with dichloromethane (Figure 3 from Kiesow (3):

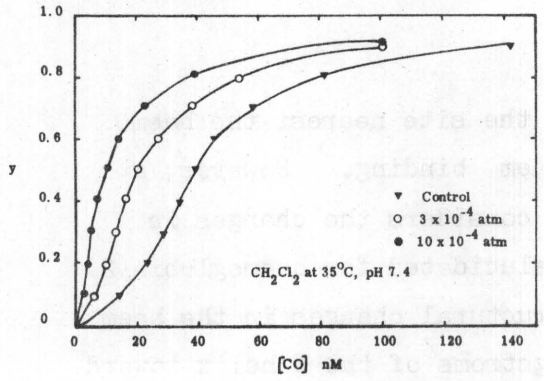

Figure 2

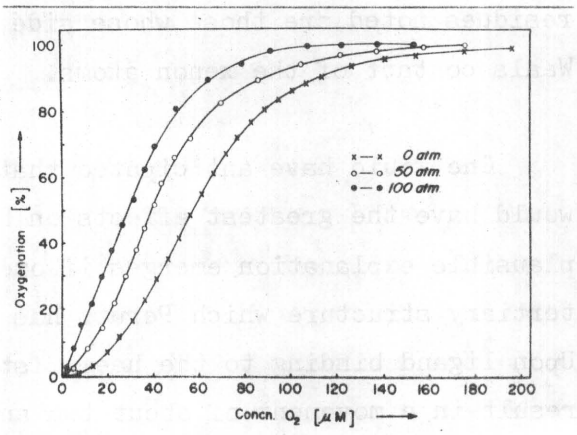

Figure 3

Percentage haemoglobin oxygenation in fresh human blood cells is plotted against the μM concentration of free oxygen. The curve at '0 atm' is the binding curve of red blood cells saturated with nitrogen at ambient pressure). Similar changes are seen with helium (Figure 4 from Kiesow(3): Oxygen binding curve at ambient and at 100 atm partial pressure of helium), though even higher pressures are supposedly required to produce comparable effects.

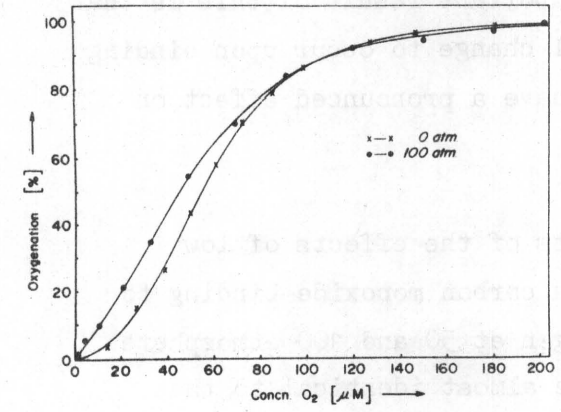

Figure 4

Helium may produce specific alterations in function and the assumption that it can be used to observe hydrostatic effects may not always be valid.

Hydrostatic pressure apparently has little effect on haemoglobin function, because there is very little volume change between the oxy- and deoxy- forms.

On the other hand, haemoglobin must be considered as playing a major role in the transport of these gases. At one atmosphere, around 30% of nitrogen in blood is associated with haemoglobin. Similar values, from 30% to 50%, are found for argon, cyclopropane, xenon and even hydrogen and helium. In comparison lipids dissolve only about 2% of the nitrogen in blood. Thus haemoglobin is a major factor in the transport of these gases. Similarly, myoglobin can also be considered a major storage site. What might happen if a slight shift in pH resulted in myoglobin releasing nearly half of the inert gas bound to it ? - especially if this occurred during decompression ? The variability of functional alterations indicate the possibility of changes in inert gas binding. By learning to optimize binding to haemoglobin, perhaps the protein could be used as an efficient transport system to remove gas from the body.

Summary:

1. Effects on all these agents, ranging from dichloromethane to helium, on haem-protein function are basically identical. There is nearly a million fold range of pressures with dichloromethane requiring the lowest pressure and helium requiring the highest.

2. A major part of the functional alterations appearsto be directly related to specific site binding of the agent.

3. Slight shifts in conditions, many of which are physiologically compatible, may significantly alter the binding of the agent and the functional changes produced by the agent.

4. A better understanding of the nature of the interaction of these agents with the haem-proteins not only gives us a basic concept of the behaviour of these agents, but also could be useful in managing the distribution of inert gases within and transport out of the body.

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Review of the effects of pressure and anaesthetics on membranes

K. W. Miller

The fluid mosaic model of membrane structure envisages proteins "floating" on, or in, a lipid bilayer (1). At the moment we know little about the nature of the membrane proteins. However, anaesthetics may interact with the lipids, producing a perturbation which in turn affects protein function. This hypothesis implies that the anaesthetics do not have specific sites of interaction but instead dissolve in the fluid bilayer in an analgous manner to dissolving in a bulk fluid. The membrane increases in volume when anaesthetics dissolve in it, and decreases in volume when pressure is applied. Evidence for this has been obtained from experiments with monomolecular layers of lipids at a water-gas interface in which the film pressure at constant area (related to the area at constant film pressure) increases in the presence of argon and nitrogen but decreases in the presence of helium (which has a minimal anaesthetic effect)(2). Similar changes in surface area with other drugs have also been shown in red blood cells (3). The increase in volume is not the same in all directions and it has been demonstrated that anaesthetics increase the surface area while at the same time decrease the thickness of a bilayer (4).

These volume changes can be related to the type of functional changes which may be important in neural function, such as the movement of ions across the membrane. One suitable model system is a lipid bilayer with an ionophore which acts as an ion carrier across the membrane. When the anaesthetic is added the ion flux increases as the membrane fluidity increases and the anaesthetic

concentration causing this effect can be correlated with that blocking conduction in the frog sciatic nerve. Pressure alone reduces the ion permeability and it is possible to balance the effects of anaesthetics and pressure, with the permeability returning to the control value. This effect is not simply the displacement of the anaesthetic from the membrane under pressure, since it does not matter at which stage the anaesthetic is added. It is possible to calculate the increase in volume of the membrane caused by the anaesthetics and the compressibility of the system when pressure is applied. The calculated compressibility (2×10^{-5} atm^{-1}) is consistent with that expected for hydrocarbon compressibility (5).

When anaesthetics dissolve in the lipid bilayer the volume increases by about 0.25%. The effect of this volume change may be magnified by several factors. First, the proportional increase in surface area of the bilayer may be up to ten times greater than the volume increase. Second, the volume change may alter phase transitions in membranes. One phase transition is when the arrangement of the lipids changes from a more solid gel phase to the more liquid fluid phase. An analogy can be made with a melting point which is usually associated with an expansion of a substance. However, unlike a melting point the phase transition occurs over a temperature range up to $5^{o}C$. Interaction of anaesthetics with the membrane tends to shift the bilayer towards increased fluidity, while increased pressures have the opposite effect. Studies with model systems have examined the effects of pressure at different temperatures on either side of the phase transition. The changes observed for the same pressure increase have been greater in the gel phase than the fluid phase and very much greater in the intermediate range of the phase transition(6).

It has been postulated that such phase transitions are important in modifying protein function and it may be that this is one of the ways in which volume changes can have a profound effect on functional changes.

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Performance during mouse saturation dives to 100 ATA

H. Rahn

This report describes the performance of 8 mouse colonies during 6 dives at 100 ATA. Animals lived at this pressure for 2 or 3 days and were then successfully decompressed.

The selection of these animals and their preparation for these dives should be noted.

1. To exclude the possibility of genetic predisposition to functional characteristics which might jeopardise their performance under stress, we chose a strain of wild mice - Peromyscus.

2. To avoid placing these animals into a new and strange cage environment prior to pressurisation, we elected the following procedures :

a. Colonies of 5 animals (mixed sexes) were reared in habitats consisting of 3 chambers connected by tunnels and a climbing tower. An activity wheel allowed for their daily exercise. They were subjected to a 12-hour dark/light cycle (see Figure 1).

b. Under these conditions they thrive, reproduce and each animal runs about 2-3 km per day. We monitored the running activity of each colony before, during and after a dive to evaluate their performance.

c. Two days before a dive the habitat is transferred to the pressure chamber for control studies. Once compression has started we believe that any observed behavioural changes are only due to the effects of pressurisation.

Effects of N_2, Ar and N_2O

Figure 2 illustrates spontaneous running activity on day 2 of exposure (probit scale), when colonies were exposed to various

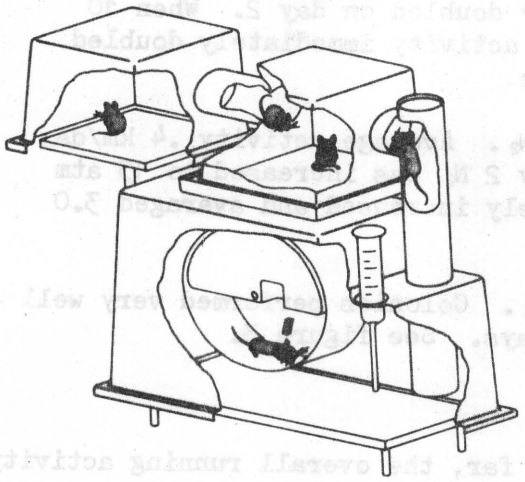

Figure 1

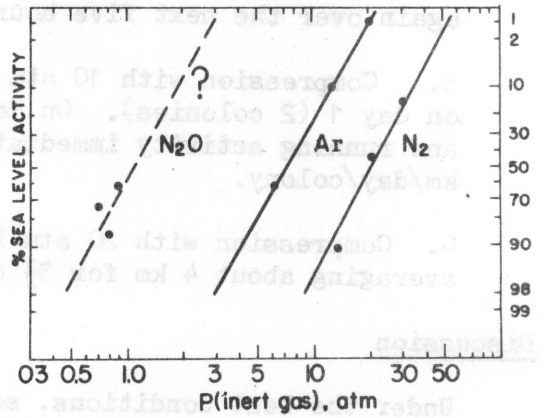

Figure 2

inert gases. Note that at 12 atm N_2, 90% of running activity is maintained and reduced to 50% at 21 atm N_2.

Exposure to 100 ATA

a. Compression Rate 0.25 atm per minute

1. If normoxic mixtures (O_2-He) are used, we observe typical signs of HPNS above 50 atmospheres.

2. When compressed with 10 or 20 atm N_2 and He, no such signs are noted.

3. Throughout the compression the animals are active on the activity wheel.

b. Behaviour at 100 ATA

After reaching 100 ATA animals were under continuous observation for 48 to 72 hours.

Animals reached 100 ATA about 6 P.M., went to sleep at 9 P.M. (light cycle) and started their activity the following morning at 9 A.M. (dark cycle).

1. Observations of activity during the first day are as follows :

No N_2 added (4 colonies) averaged .3 km/day
10 atm N_2 + He (4 colonies) " 1.0 km/day
20 atm N_2 + He (1 colony) " 5.0 km/day

2. Performance usually improved on second day in absence of N_2 (2 colonies). Activity doubled on day 2. When 10 atm N_2 were added on 3rd day, activity immediately doubled again over the next five hours.

3. Compression with 10 atm N_2. Average activity .4 km/day on day 1 (2 colonies). On day 2 N_2 was increased to 15 atm and running activity immediately increased and averaged 3.0 km/day/colony.

4. Compression with 20 atm N_2. Colonies performed very well - averaging about 4 km for $3\frac{1}{2}$ days. See figure 3.

Discussion

Under the best conditions, so far, the overall running activity is reduced to about one-half of their ground level activity. However, to achieve this level it appears necessary to add N_2. 10 atm N_2 abolishes clinical signs of HPNS and 20 atm N_2 provides for even better performance.

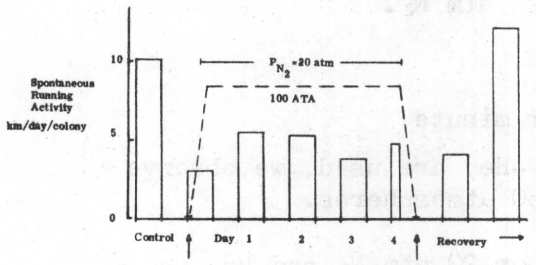

Figure 3

The relative gas density (compared to air) at 100 ATA with He is 14.0 and with 20:80 N_2-He it is more than doubled.

Previous experiments have shown that the effective narcotic dose of N_2, ED_{50}, for running activity of mice is 21 atm.

After successful decompression after $3\frac{1}{2}$ days at 100 ATA (decompression at 0.5 atm/min), normal running activity returns within the next 2 days (see figure 3).

Hydrostatic pressure effects in liquid breathing mice

C.E.G. Lundgren

We have been interested in the hydrostatic pressure tolerance of oxygenated flurocarbon liquid breathing mice (NMRI) and studied them at reduced body temperatures to keep the metabolic demands level with the animals' ventilatory capacity(1). We recorded EKG, EMG and rectal temperature and observed the trembling and convulsions which are part of the High Pressure Nervous Syndrome in mice. Maximum tolerated pressure (MTP) was defined as the pressure at which breathing and/or cardiac activity ceased, and the pressure at which convulsions started was called convulsion onset pressure (COP). The lower MTP at low body temperature (Table 1) might be due to the fact that high pressure convulsions last longer and consequently are more exhausting. Presumably the relatively low MTP at 27°C can be attributed to the increase in metabolic strain that the higher body temperature induces in liquid breathing animals.

Table 1

Rectal Tem. °C	MTP (atm)	COP (atm)	Convulsion Duration
17	149 ± 29		1-3 min
21	216 ± 15	85 ± 15	1-3 min
27	216 ± 35		10 sec
31	120 ± 23		

(Compression rate 2 atm/min; Pressure figures are mean ± 1 SD; n = 6)

The relatively low MTP at the slowest compression rate (Table 2) may have been due to the long time spent at increased pressure; at the highest rate on the other hand, the high convulsive activity may have curtailed MTP. The changes leading to convulsions while being initiated at a certain pressure may require a certain period of time to develop. During this time the higher rate of pressure increase

would result in a higher pressure reading at the onset of convulsions than would the slower rate of compression.

Table 2

Compression rate (atm/min)	MTP (atm)	COP (atm)
0.5	140 ± 17	no convuls.
1	165 ± 32	76 ± 20
2	216 ± 15	80 ± 16
4	243 ± 20	90 ± 4
6	190 ± 30	100 ± 10

(Pressure figures are mean ± 1 SD; n = 4)

One observation of particular interest to us was that above pressures of about 200 atm, convulsions were usually not seen. This could not be ascribed to exhaustion because, in animals which were decompressed and subsequently compressed again, convulsions reappeared and the COP could actually be reproduced several times within ± 10 atm. It is worth noting that it was only when entering the pressure zone of convulsions by compression that convulsions were seen. Thus, during decompression the organism appears to be in a similar situation as when kept at stable pressure within the "convulsion zone" - mice are then known to gradually escape convulsions by some process of adaptation. Pressure possibly acts by causing compression, i.e. volume reduction in some target structure. If so, most experimental results, including observations in humans, can be inferred to indicate that the development of HPNS is dependent on a minimal volume reduction per unit time (dV/dt). Could it be then that the absence of convulsions in our mice at pressures above 200 atm is due to a reduction in compressibility of the target structures at these high pressures ? The

compression rates applied may not then have yielded the dV/dt required for convulsions. We do not have enough data yet to decide if the convulsion-free high pressure zone begins at lower pressure when low compression rates are used than when high rates are used.

The sex difference in hydrostatic pressure tolerance in NMRI mice is such that while the MTP was 237 atm for females it was 85 atm for males. Örnhagen (2) also studied adult mice that had been castrated and the difference was still there, with an MTP of 212 atm for females and 148 atm for males. However, in adult mice that had been castrated at 2 weeks of age no significant difference in hydrostatic pressure tolerance was detected (MTP for females 147 atm; for males 155 atm). The theoretical significance of these observations is not yet clear but the practical one should be considered in experimental work.

Our studies of heart rate depression(3) by high hydrostatic pressures are still in progress. In liquid breathing mice (colonic temperature 23°C) the initial heart rate of 185 beats/min fell to 95 beats/min when they were compressed to 175 atm at a rate of 4 atm/min. Subsequent decompression brought the heart rate back to 155 beats/min. We have observed a time dependent reduction in heart rate in liquid breathing mice kept at stable, low pressure, and the final heart rate in the mice that had been at high pressures was higher than expected had they been subjected to the same tendency to time dependent bradycardia as the non-compressed animals. In other words, had it not been for the time dependent bradycardia, the decompressed mice might have returned to low pressure with a heart rate being higher than at the beginning of compression. This again brings up the question of a time dependent adaptational mechanism which counteracts the effects of compression on nerve and pace-maker cells.

The question of whether the pressure bradycardia is due to direct pressure effects on the heart or to changes in autonomic control was tackled by autonomic blockade. With atropine injections (15 mg/kg s.c.) the pressure bradycardia seems to be somewhat less pronounced than in non-medicated animals. This might indicate that increased vagal tone is responsible for part of the bradycardia in the latter. This notion also gains support from the observation that mice whose vagi were functioning while their sympathetic system had been blocked (propranolol 30 mg/kg s.c.) had a somewhat more pronounced bradycardia than mice which had obtained both atropine and propranolol. We have seen no evidence that changes in sympathetic tone play a role in high pressure bradycardia. The main part of the high pressure bradycardia in hypothermic, liquid breathing mice appears to be attributable to a direct action of pressure on the impulse generating mechanisms of the heart.

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Sustained dives with mice up to depths of 8000 ft.

M. J. Halsey

We have studied the interaction of high pressures and inhaled general anaesthetics, using mice in a series of experiments which determined both the lethal limiting pressures and the conditions under which the righting reflex is lost.

The mice (20 - 30 g male Swiss Webster) were put in a large rotating cage, which was divided into 8 separate cylindrical compartments. The cage could be rotated in either direction at 4 rev/min. In addition, three other mice had rectal thermistor probes inserted, and were put in separate fixed cages. Some experiments were also carried out using electrical stimulation via electrodes on the tails of six mice.

We first confirmed previous reports that with helium/oxygen gas mixtures, mice have tremors, convulsions, display respiratory distress, and die at pressures around 100 ATA of helium(1). We then determined the nitrous oxide partial pressure which produced a loss of righting reflex in 50% of the mice (ED_{50}) at different pressures of helium. We found that the ED_{50} increased in proportion to the total pressure up to 150 ATA (Figure 1). At higher pressures we began to observe a decrease in narcotic requirement, and animals began to die above 200 ATA.

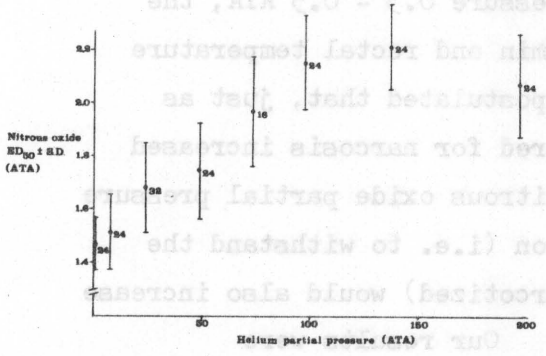

Figure 1

We then investigated the factors influencing the death of these mice. First we tested if the exposure time needed to achieve the higher pressures was an important factor. We therefore investigated the time dependence of the nitrous oxide potency at 100 ATA for a period of three days. We determined the nitrous oxide potency immediately after the initial compression; maintained the animals at 100 ATA, continuously removing carbon dioxide and ammonia from the chamber gases, and monitoring for carbon monoxide and methane (no build-up of the concentrations of any of these four gases was detected). We repeated the potency measurements each day, and found that there was no significant change with time in the anaesthetic requirement of 24 mice at 100 ATA. We were unable to detect any diurnal variations in the requirement as has been found in some other experiments(2). After four to five days, the mice generally deteriorated, but this was probably due to inadequate conditions inside the chamber.

In 50 mice we tested whether variations in nitrous oxide and oxygen partial pressures and compression rates could increase the number of survivors at higher pressures. The best survival rate was obtained when the nitrous oxide partial pressure was 0.5 - 0.8 ATA, the oxygen partial pressure 0.3 - 0.5 ATA, the compression rate less than 0.68 ATA/min and rectal temperature maintained between 36° - 37°C. We postulated that, just as nitrous oxide partial pressure required for narcosis increased with total pressure, similarly the nitrous oxide partial pressure required to maintain "normal" function (i.e. to withstand the effects of pressure but not to be narcotized) would also increase in proportion to the total pressure. Our results were consistent with this hypothesis at pressures up to 200 ATA. However, using the conditions for maximum survival rate, although

some mice died above 200 ATA, others were pressurized up to 270 ATA over a prolonged period. It is interesting to note that with the most favourable compression conditions, no convulsions or respiratory distress were observed below 200 ATA, and tremors were always very slight.

Our very high pressure studies agree with the predictions and experiments of other investigators in demonstrating that anaesthetics can prevent some of the C.N.S. and lethal effects of high pressures (3,4). However, there appears to be a finite limit to the protective effect of nitrous oxide and therefore our current experiments have used nitrogen. The preliminary results of the anaesthetic potency of nitrogen on its own and in the presence of increasing pressures of helium are shown in Figure 2.

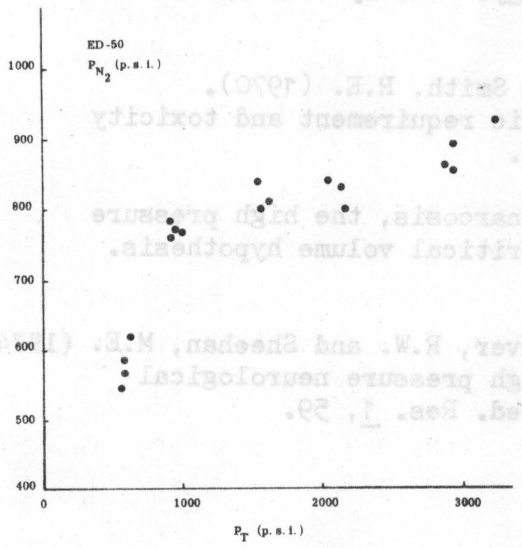

Figure 2

We expected that the pressure changes in nitrogen potency would be quantitatively similar to those found with nitrous oxide. However, the results are very surprising for two reasons: a) Above 100 ATA the increased nitrogen anaesthetic pressures started to plateau but there is no evidence of a maximum in the effect below 200 ATA.
b) The increase in nitrogen anaesthetic potencies below 100 ATA is definitely curved rather than linear. This effect has now been confirmed in two species of mice using different experimental arrangements in two institutions: University of California,

San Francisco and the Clinical Research Centre, Harrow.

We interpret all these results as implying there must be at least two critical molecular sites for the lethal effects of high pressure. If the non-linearity of the interaction between anaesthesia and pressure below 100 ATA is confirmed for other agents besides nitrogen, some of the currently accepted hypotheses on the pressure reversal of anaesthesia will have to be modified.

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Application of the critical volume hypothesis to problems of deep diving

K.W. Miller

The critical volume hypothesis offers a description of the interactions of pressure and inert gases which produce anaesthesia and convulsions. It accounts for the pressure reversal of anaesthesia and anaesthetic antagonism of pressure induced convulsions (HPNS). The hypothesis may be stated as follows: anaesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the absorption of an inert substance; an applied pressure opposes the expansion and reverses the anaesthesia. Conversely, convulsions occur when some hydrophobic region is compressed beyond a certain critical amount by application of pressure; absorption of an inert gas will compensate for such compression and raise the convulsion threshold pressure. Studies on the pressure reversal of anaesthesia(1) and the convulsion threshold in mice (2) provide a basis for testing the hypothesis.

The percentage expansion, Eg. caused by a dissolved gas, is given by :

$$Eg = \overline{V}_2 \cdot x_2 \cdot Pa/Vm$$

Where $\overline{V}_2$ is the partial molar volume of the gas in the solvent of molar volume, Vm, x_2 its mole fraction solubility and Pa the applied partial pressure. In a gas mixture each gas contributes additively and at elevated pressures compression must be included to give the net volume change E_T.

$$E_T = \left[\sum_i \bar{V}i \cdot xi \cdot Pai/Vm\right] - \beta P_T$$

or $$E_T = Eg - \beta P_T$$

where β is compressibility and P_T total pressure. A set of isonarcotic (or isoconvulsive) data at different pressures, together with $\bar{V}_2$, x_2 and Vm data from a model solvent, allow Eg to be calculated at each P_T. A plot of Eg . vs P_T then yields the compressibility (slope) and critical percentage expansion (intercept). Figure 1 shows such a test. β for the convulsive site is greater than for the anaesthetic site, whilst critical volumes for these two sites are of the same magnitude but opposite in sign.

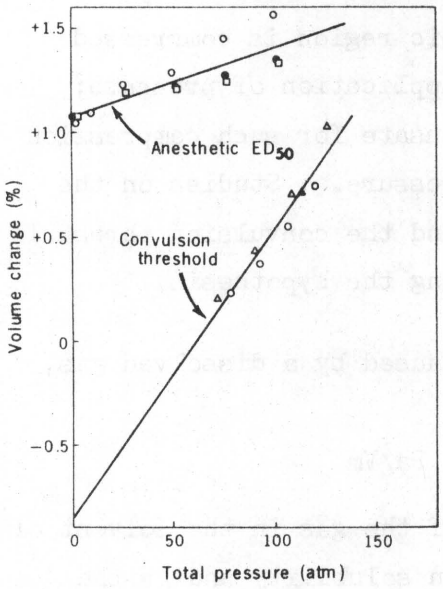

Figure 1

Knowing these physical parameters of the sites of action we can calculate the pressure at which convulsions or anaesthesia will result for any given gas or gas mixture. (See Figure 2). Carrying out these calculations for mixtures enables one to construct a graph or map which predicts safe mixtures at various pressures (Figure 3).

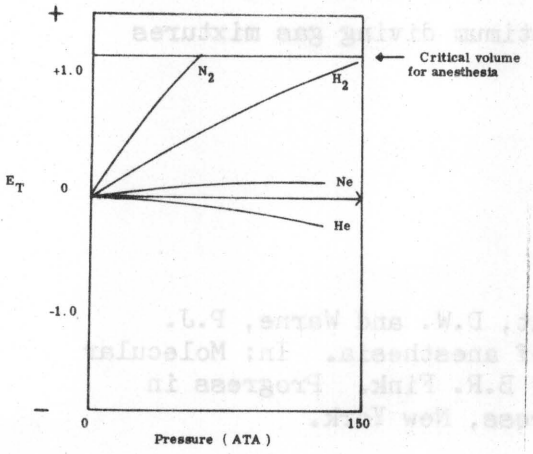

Figure 2

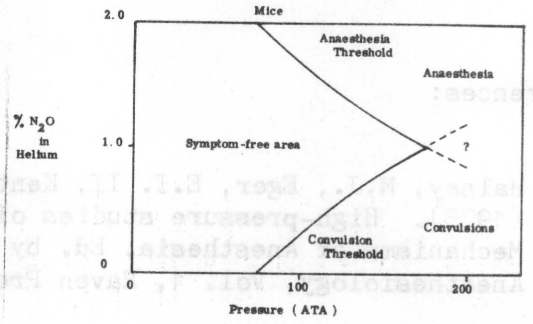

Figure 3

Preliminary application to manned diving was made for He - N_2 mixtures. Deriving the critical volume and β for narcotic and convulsive sites in man is difficult because of the paucity of quantitative data. However, tentative predictions suggest that inclusion of <u>not more</u> than 15% N_2 should avoid narcosis at all pressures up to the convulsion threshold. Considering the current uncertainties in the calculations, $10 \pm 5\%$ N_2 may be regarded as the best working mixture. The convulsive threshold itself is significantly raised by use of such mixtures compared to values for pure helium. The theory allows ready extrapolation to other gas mixtures. The immediate need is for more data at high pressures on the narcotic threshold of gas mixtures. Ideally the similar data for convulsion threshold is required, but this may be obtained from experiments on primates to a good approximation.

The critical volume hypothesis provides a semi-empirical theoretical approach which enables optimum diving gas mixtures to be predicted.

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2. Brauer, R.W., Goldman, S.M., Beaver, R.W. and Sheehan, M.E. (1974). N_2, H_2 and N_2O antagonism of high pressure neurological syndrome in mice. Undersea Biomedical Research, 1, 59.

Pressure effects on the fluidity of lipid bilayers

J. R. Trudell

A research group at Stanford, including Drs. Kendig, Cohen and myself, have been studying the effect of inhalation anaesthetics on the biophysics of synthetic phospholipid bilayers (1,2). We have observed an increase in fluidity in the bilayer due to the action of anaesthetics which was reversed by high pressure. We further demonstrated that the effect of high pressure was not to exclude the small anaesthetic molecule from the bilayer, but rather to reorder the bilayer around it.

Studies by Fox on bacterial mutants and McConnel on phospholipid bilayer systems have shown the importance of phase transitions in the membrane lipids to the functioning of membrane-solvated proteins. There appear to be some fluid and some solid lipid phases present at all times and such a phase separation allows for maximum lateral compressibility of the surrounding bilayer matrix which is essential to some protein functions.

If application of high pressure raises the phase transition temperature of all of the phospholipids in the bilayer, then at the same temperature more of the lipids in a membrane will be below their phase transition temperature. That is, more or all will be in the gel phase and the essential fluid-gel equilibrium will no longer exist.

Figure 1 is a diagram of the phase transition of a pure

dipalmitoyl-phosphatidylcholine bilayer. The internal fluidity(f) is plotted against temperature. Both the pre-transition point in the region of 32°(which is thought to involve the rearrangement of the phospholipid head groups) and the major transition at 43°, (which represents the fatty acid chains of the phospholipids going from a fluid to a gel state) are influenced by the application of pressure. Thus, if an isothermal line is drawn vertically at 43° through the centre of the phase transition for the 68 atmosphere curve, it can be seen that at atmospheric pressure the bilayer is nearly all in the fluid phase (high f), whereas at 136 atmospheres it is nearly all in the gel phase. We have also demonstrated this effect for mixed systems of dipalmitoyl and dimiyristoyl-phosphatidylcholine.(1)

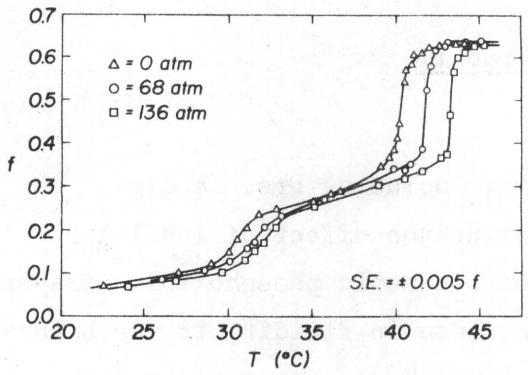

Figure 1

Another point of interest is the effect of the solubility of high pressure gases on the internal fluidity of the bilayers. Figure 2 illustrates the fluidity expressed as ΔS vs. time for the application of different gases at 102 atmospheres of pressure. It is seen that initially the membrane is put in a much less fluid-like stage by the application of high pressure. Then, as the gas molecules

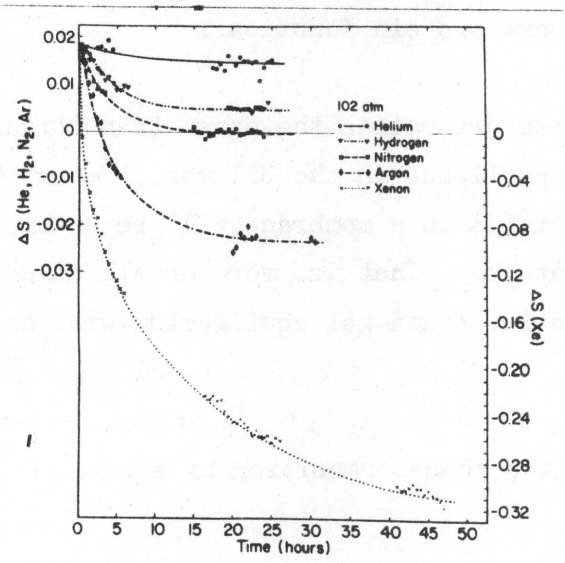

Figure 2

have time to diffuse into the membrane suspension, the internal fluidity of the bilayer is gradually increased. It is seen that the more lipid soluble gases have the greatest effect but take the longest to reach equilibrium. There is a good correlation between their oil solubility and the increase in the internal fluidity of these layers. (2)

Those gases, such as helium, which are capable of transmitting high pressure but which have a low solubility in a lipid region and therefore a very small anaesthetic effect, tend to increase the order of the membrane. On the other hand, soluble gases, such as nitrogen and xenon, briefly make the membrane more gel-like and then rapidly decrease the internal polarity. The long equilibrium time is due to the fact that we used a 1 cm deep suspension. Calculations based on the 100 angstrom depth of a nerve membrane suggest that equilibrium would be obtained in about 10 microseconds.

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Mechanics of Bilayers

S. Simon

As it is generally accepted that the anaesthetic, as well as the HPNS 'site', is either a biological membrane (a lipoprotein complex) or a lipid bilayer, it is necessary to ascertain how such systems will react to hydrostatic pressures as well as normal stresses. The hydrostatic effects may be generated directly by hydrostatic pressure or by the application of helium gas. The normal stresses may be generated by applying a voltage across the membrane.

From a mechanic's viewpoint, biological membranes, such as red blood cells, are 2-dimensional hyperelastic materials(1) whereas lipid bilayers (above their transition temperature) are two-dimensional liquids. The difference in these two systems is that biological membranes have a shear modulus which simply means that the structure will support a shear stress. That is, if a 1 cm^2 piece of biological membrane is deformed by a shear stress into a rhombus, the material will return back to its original shape once the stress is relieved. Liquids do not possess a shear modulus. Although biological membranes and lipid bilayers react differently to shear stresses, they will react similarly to hydrostatic or normal stresses.

In a lipid bilayer, transport measurements as well as mechanical ones tell us that the properties in the plane are different from those in a direction perpendicular to it. Mechanically we are unable to characterize the above system as a three dimensional isotropic liquid such as benzene or carbon disulfide, which has only one elastic constant, i.e. a bulk modulus.

Let us now consider a unit area of bilayer under no external stresses. The attractive forces are due primarily to the fact that the hydrocarbon chains of these molecules want to avoid water. These are balanced by the compression resistance of the molecules in the surface. That is, the interfacial tension is acting to minimise the area (no hydrocarbon associated with water) whereas the surface pressure is attempting to expand the surface. The resistance to the energy necessary to expand the surface is given by the surface elastic constant K_a (this assumes the bilayer is isotropic in the membrane plane). It is noted that the resistance to compression may be very high whereas the resistance to dilation may be much smaller.

As an example, let us consider billiard balls(that are not sticky) as models for the polar groups of phospholipid molecules that are held together by a rubber band (representing surface tension). It is very difficult to push the balls together, but if we stretch the rubber band then the billiard balls will roll apart. The rubber band may be stretched by, say, trying to fit marbles, (representing inert molecules) in between the billiard balls. Quite naturally we cannot stretch the rubber band too far, otherwise there will be little attractive interactions between the molecules and the membrane will break.

Now there is no <u>a priori</u> reason to suspect that when we push on a bilayer equally in all directions (hydrostatic pressure), the change in area (ΔA) relative to the initial area (Ao) where :

$$\frac{\Delta A}{Ao} = \frac{A - Ao}{Ao} \qquad \text{(the area dilution)}$$

will be the same as the decrease in thickness of the membrane in the direction perpendicular to the membrane plane (Z - direction) $\frac{\Delta Z}{Z_o}$. In fact, a theoretical analysis of bilayers shows that three elastic constants are necessary to specify the mechanical equation of state of bilayers. They are K_a : the elastic modulus in the membrane plane, K_z : the elastic modulus perpendicular to the plane and K_{az} the elastic modulus that couples stresses perpendicular to the membrane plane with those in the plane.

Insofar as compressibility measurements have been used to test certain theories of anesthesia (2), it is clear that, at least mechanically, a lipid bilayer (or a biological membrane) does not resemble a bulk three dimensional isotropic liquid.

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Discussion on Fundamentals of Narcosis and Pressure

I. The Concept of a Site of Action for Anaesthetics and Pressure

Anaesthetic materials, whether clinical agents or the diving gases, become widely distributed throughout the body. They can affect a wide range of physiological processes and thus have a number of 'sites of action'. However, many of these effects are not related to the problems of diving. It is therefore important to define the particular end-points that are of direct concern. These would include, narcosis, loss of consciousness, and the amelioration of tremors and convulsions. Even if we confine ourselves to these end-points (or indeed any one of them) there may be more than one physiological site involved. Thus the observed effects of an anaesthetic substance may result from a combination of presynaptic and postsynaptic perturbations. It was recognised that single site models are almost certainly a gross over-simplification. The considerable predictive value of these models may be due to similarities in the physical nature of the sites involved. The unitary hypothesis of anaesthetic action was felt by many to be a reasonable, and indeed at the present time necessary, assumption in the development of models of anaesthetic action.

The criticial sites at which pressure (and in diving practice this meant helium) acts may not be the same as those involved in anaesthesia, despite the strong antagonism observed between the two.

II Physical Interactions of Narcotic Agents

Many workers have sought to identify the physical nature of the site (or sites) of action of anaesthetics by correlation of the potency of anaesthetic substances with their physico-chemical properties (see Section 3). It was argued that if one such correlation is observed then many others are likely to prove equally successful (for example, olive oil solubility, Van der Waals a and b constants, polarizability etc.). The counter argument was advanced that lack of correlation can be used to rule out a number of unsatisfactory hypotheses and that if particular attention was paid to substances of unusual physico-chemical properties (e.g. SF_6, fluorocarbons) the olive oil solubility correlation showed an absence of exceptions which was not observed for other models. It was noted that early experiments with alcohols indicated that there were differences in the solubility properties of the membranes of synapses and axons. The binding of Xenon and cyclopropane to myoglobin could not be explained in terms of a single property (see Section 4). Both polarizability and size effects were invoked and the belief that a single physical property determined anaesthetic potency was thought by some to be improbable.

Many who accepted that evidence pointed to a hydrophobic site of action, noted that this did not necessarily involve lipids but could indicate that anaesthetics act at a hydrophobic region within a protein.

III Evidence for the involvement of lipids and proteins

The lipid/protein question was not a simple either/or problem. Clearly both could be involved. It might be reasonable to ask if anaesthetics caused a general disruption of all membranes affecting all membrane proteins or exerted their effect through

binding to a single protein (which need not be membrane bound). Progress was difficult as almost all systems investigated to date are model systems (red blood cell membranes, myoglobin, luciferase). Further uncertainty is introduced as there is not yet complete agreement as to the relationship between lipids and proteins in membranes.(see Section 6).

The paucity of evidence for the effects of anaesthetics and pressure on bio-membranes of relevance was raised. The evidence that was produced in discussion (see also Section 15) indicated that 200 atm of helium reduced Na^+ and K^+ activation in membranes, and depressed transmission at nicotinic synapses in the mammalian sympathetic ganglia. (It was noted that such nicotinic synapses are also present in the mammalian central nervous system). One observation of interest was that ganglionic tissues from polychaetes recovered from depths of 7,000 m had enlarged synaptic vesicles. It was hoped that in the near future membranes from crustacea recovered alive from 1000m would be available for study.

When considering the role of proteins it was noted that there was clear evidence for the binding of simple gases with proteins. The binding to myoglobin and haemoglobin was reviewed earlier (Section 4). It was stated that the enzyme luciferase from luminous bacteria also appeared to have its function modified in elevated pressures of simple gases. These organisms when treated with anaesthetics exhibit comparable sensitivity and potency ratios to those observed with mammals. They also show such complex responses as hyperactivity at low doses and pressure reversal. The <u>in vitro</u> enzyme in the presence of diethylether showed the same features and it was suggested that two sites were involved. At one, which was hydrophobic in nature, the anaesthetic appears to replace a long chain aldehyde co-factor.

Though the proteins discussed can be regarded only as model systems and do not, themselves, have any direct relevance to effects occurring in nervous tissue, it was pointed out that there was likely to be little difference between the 'interior' regions of proteins, whether soluble or lipid-bound.

IV Pressure Reversal

The reversal by high pressures of the action of anaesthetics and the corresponding amelioration of the effects of high pressure by the addition of anaesthetic substance appears to extend to most species and even model systems that have been investigated. Therefore a quite general mechanism is indicated. The only species known not to show this phenomenon are marine and freshwater shrimps. It was suggested that this may be because shrimps have glutamate as the transmitter in motor control and do not show a twitch response having graded synaptic transmission. It is still not certain that the pressure acts at the same site (or on the same process) that is affected by anaesthetics. It was suggested that pressure might stimulate transmitter release whereas anaesthetics might depress post-synaptic functions. Also in axonal transmission anaesthetics appear to inhibit the sodium ion flux whereas pressure inhibits the potassium ion countercurrent.

The effects of the volume change which might be produced by pressure, and which have been invoked in various theories of pressure reversal, were discussed. Little enough is known about the behaviour of membranes under pressure. Membranes are modified by pressures up to about 100 atm. Pressures of this magnitude appear to stabilize the gel form and if higher pressures are applied no further changes in structure appear (see Section 11). As membranes can undergo phase transitions it was recognised that the volume changes brought about by the application of pressure

or the addition of an anaesthetic agent could be orders of magnitude greater than those observed for simple liquids. No marine species that has been thoroughly investigated appears to have a depth range greater than 2000m. The pressure range ($\approx$200 atm) may prove to have a more general significance.

The volume changes induced in proteins by pressure were said to vary over a wide range. In general it was felt that the phenomenon of pressure reversal was now well established though we have little understanding of the mechanism. This might have to await a deeper knowledge of the mode of action of general anaesthetics.

V Transient Effects of Pressure

No theories have yet been advanced which take into account those effects of pressure which appear to vary or remit with time. In at least six species the convulsion threshold in oxy-helium mixtures exhibits no dependence on the compression rate. In fourteen other species a marked dependence is observed. With men the rate of compression affects the intensity of tremors more than the the threshold pressure. A number of possible mechanisms were proposed to explain effects of the rate of compression and of the adaption of animals to pressure.

(i) Membrane lipids could exchange with the cellular environment. A half-time of about 2 hours was suggested. In this way the effects of pressure on membranes could be reduced.

(ii) Cells might possess 'space' proteins that become active at high pressures.

(iii) Changes in the osmotic pressure of protein solutions exposed to high pressures have been observed.

Hypoxia resulting from changes in the haemoglobin-oxygen affinity curve at high pressures (see Section 5) was not considered relevant

in the conditions that prevail in deep diving.

VI Additional Mechanisms

The relevance of the effects of anaesthetics on the active transport of ions was discussed. The evidence clearly suggested that this was not relevant to anaesthesia. Passive transport (in particular the changes in sodium flux produced by anaesthetics) was generally agreed to be of more importance.

It was stated that the experiments on large axons did not rule out the inhibition of axonal conduction as a potential mechanism of anaesthesia. In small fibres, doses as low as 150% of a normal anaesthetic dose could effect the potentials.

The close relationship between certain agents that produce convulsions and anaesthetic substances was noted. The view that all anaesthetics produce convulsions just below the lethal dose and that pressure lowers the dose at which convulsions occur was reported.

The effects of anaesthetics on microtubules were discussed but it was felt that this was unlikely to be the primary mechanism of anaesthesia. However, the effects of anaesthetics on microfilaments or the contractile proteins associated with neurotransmitter release may be more relevant.

The importance of the central role of calcium, particularly as a control mechanism for many intracellular and membrane processes was noted. It may be that an effect on calcium fluxes rather than a direct effect on the more obvious aspects of neural function will prove to be the basic mechanism of the action of anaesthetics and pressure.

Review of Physiological Factors and Limits to Deep Diving

C. J. Lambertsen

(Presented by R.C. Bornmann, Suzanne Kronheim and P.G. Linaweaver)

There are a number of functions which are affected specifically by high hydrostatic or gas pressures which may limit the depth to which man will eventually be able to dive. These include:

Heat loss: The body surface tolerable temperature range narrows drastically with increased pressure. In addition there is a progressively increasing respiratory heat loss with increasing depth. A naval diving suit is designed to prevent heat loss by providing insulation (with warm air or water) rather than to heat the diver. One cumbersome but effective method is to use a free flooding supply of hot water (100°F at 4 gal/min) mixed when necessary with sea water (less than 40°F) which heats both the diver's skin and gas supply. Thus thermal balance is an equipment and technological problem rather than a physiological limitation.

Tissue inert gas exchange: The rate of elimination will lead to a barrier which now may be approaching.

Muscular and cardiac function: There is probably no severe limitation down to 5,000 f.s.w.

Neural function: This is affected by both high pressures and rates of compression but is compensated by adaptation. Fine

muscle function, coarse function of balance and probably respiration are disrupted. Some data on this subject was obtained during the U.S. Navy 1600 ft dive but the information available at the moment is inadequate.

Mental function : May be affected by pressure and helium but suitable tests have not yet been devised to measure any impairment. An arithmetic index used in the University of Pennsylvania's 1200 ft dive demonstrated decrements of ability when breathing nitrous oxide (up to 0.5 ATA) and nitrogen (between 10-13 ATA) but not with helium or neon (up to 35.5 ATA).

Pulmonary gas exchange: The limitation of breathing gas density was investigated with four trained athletes during the University of Pennsylvania's 1200 ft dive in a dry hyperbaric chamber (1). At high pressures the subjects breathed three different normoxic gas mixtures of either helium, nitrogen or crude neon (75% neon/ 25% helium) in an attempt to simulate the breathing gas densities of helium to depths equivalent to 5000 f.s.w. Figure 1 illustrates the effect on maximum voluntary ventilation, which

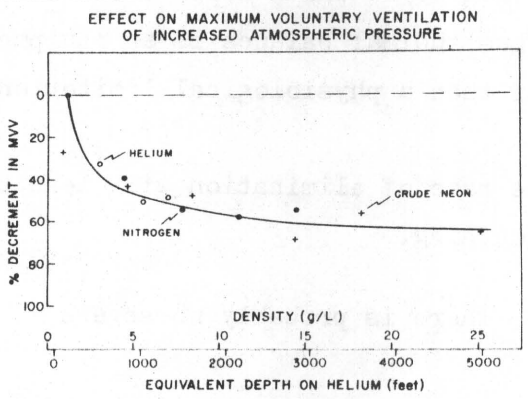

Figure 1

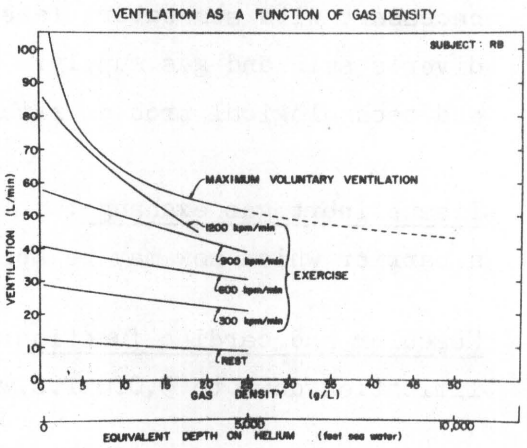

Figure 2

appears to approach a plateau of 55-60% decrement at "helium depths" of 3000 - 5000 f.s.w. Figure 2 illustrates the effect of

gas density on ventilation during exercise for one subject. The effect of density became more pronounced as the work load of the bicycle ergometer increased and the subject was unable to complete the most strenuous work of 1200 kpm/min at 1200 f.s.w. while breathing neon/oxygen. Figure 3 illustrates the carbon dioxide production at different work loads of the subject. On this basis the tolerable and maximum work loads at different densities have been predicted. The implication of this study is that, even at 5000 f.s.w. a diver can carry out moderate levels of work while breathing helium, although other factors such as impairment due to hydrostatic pressure per se have not been considered.

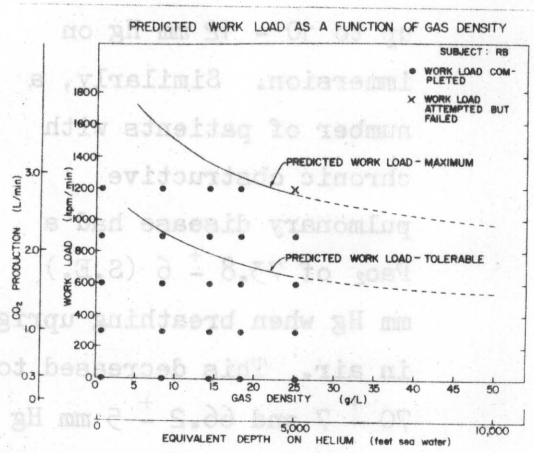

Figure 3

Physiological effects of immersion: A number of additional factors have to be considered when a diver is in the water at depth. First, the external breathing equipment will increase respiratory resistance which may become increasingly important as the total gas density increases. Second, cumbersome and restrictive diving suits will add directly to the compliance changes in the chest and respiratory system. Apart from these equipment problems, there is also an effect from immersion (2). For example, experiments with dogs have demonstrated that the functional residual capacity decreases on immersion (Figure 4) and this can only be restored by unacceptably

high levels of positive pressure breathing. There are also changes in ventilation/perfusion distribution. It has been demonstrated that arterial-alveolar gradient of normal divers increases up to 10 - 12 mm Hg on immersion. Similarly, a number of patients with chronic obstructive pulmonary disease had a Pa_{O_2} of 73.8 $\pm$ 6 (S.E.) mm Hg when breathing upright in air. This decreased to 70 $\pm$ 7 and 66.2 $\pm$ 5 mm Hg when immersed to the nipples and thoracic inlet respectively. Thus, when these effects of decreased alveolar-arterial gas exchange and increased respiratory resistance are coupled with the density effects demonstrated in the dry chamber work, there may be a physiological limit to man's ability to dive to great depths.

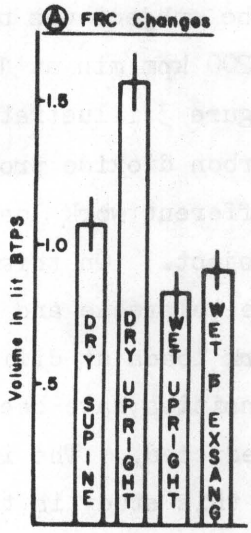

Figure 4

Conclusion: The major problems of physiology now relate to rates of onset of effects, rates of adaptation, and rates of decompensation. These physiological adaptations and deteriorations may prove to be prompt in some instances, but to have extremely long time constants in others. There is also a great likelihood that during the course of adaptation to one form of physiological stress, development of failure due to another may occur, to be followed by further adaptations or

deteriorations in each, and each at different rates. With these probabilities in mind, it becomes necessary to effect continuous measurement on a broad physiological front, over long periods of exposure.

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Review of synaptic physiology and some effects of pressure

Joan Kendig

The activities of nerve cells include two different processes, namely conduction of the action potential along the electrically excitable axonal membrane, and transmission of information from one cell to another at a specialised point of contact - the synapse. At most synaptic junctions there is no one to one transmission of impulses (so-called relay junctions), which would be a waste of metabolic work. Instead there is a transformation of information carried by a train of impulses in one cell to a different type of information in the subsequent cell. This integration of the signal can be either temporal (e.g. transmission of information only if the frequency or pattern of impulses in the presynaptic neuron is appropriate) or spatial (e.g. some crustacean second order interneurons will only fire if two hair cells on the periphery are stimulated in a particular order).

The transformation of the information is carried out by the release of a chemical transmitter from vesicles contained in the presynaptic cell which then interact with the specialised post-synaptic membrane of the second cell. Since there are technical difficulties with studying synaptic transmission in the higher centres of the nervous system, most of our concepts are derived from studies in model systems such as the neuromuscular junction in mammals and the large cells in both molluscs and crustacea. However, it is believed that the release of transmitter from pre-synaptic terminals has similar features in all types of synapses. The common features include :

a. Dependence on the depolarization of the membrane produced by the arriving action potential. If the level of the resting potential of the cell is changed so that the height of the action potential is altered, the amount of transmitter released is also affected. This may be part of the mechanism both of presynaptic inhibition and of post tetanic potentiation.

b. Dependence on calcium ion which must be present at the extracellular surface of the presynaptic membrane.

c. The amount of transmitter released is always in small packets or quanta which appear to correspond to the entire contents of a vesicle. The availability of the transmitter vesicles for release can be altered by the frequency of impulses arriving at the presynaptic terminal.

There are many chemicals which are potential transmitters, although they have not all been demonstrated to fulfil every criterion for classification as true neurotransmitters. The two most firmly established neurotransmitters are acetylcholine and noradrenaline. Other "putative" transmitters include adrenaline, dopamine and serotonin as well as glutamic acid, glycine and gamma aminobutyric acid. Whether a particular synapse is excitatory or inhibitory does not depend on characteristics of the transmitter itself but on the characteristics of the receptor sites and on their coupling to the membrane response of the postsynaptic membrane. An example of this is the action of acetylcholine in molluscan ganglion cells which can be either excitatory or inhibitory at different junctions.

The pharmacology of receptors is currently in a state of flux. The two well-known types of cholinergic receptors - nicotinic and muscarinic - are defined on the basis of interaction with different drugs. However, nicotinic synapses differ within themselves. Similarly the noradrenaline, adrenaline, dopamine

series of receptors have slightly different pharmacological characteristics. The post-junctional membrane is not electrically excitable and hence will not conduct an action potential. However, it will respond to the interaction of transmitter with the receptor site by a change in permeability to selected ions leading to a change in membrane potential. Until recently it was thought that all synapses functioned by increasing specific ion conductances. However, recent suggestions for certain synapses include a decrease in the potassium conductance which would lead to excitation, and an increase in the sodium pump or other active ion transport which would lead to hyperpolarization.

All these various phenomena, conduction as well as the different steps involved in synaptic transmission, are potential sites at which pressure, and also anaesthetics, could intervene to distort nerve cell function. There have been several studies on the effects of pressure and the interaction between anesthetics and pressure on conduction in peripheral nerves(1-3). Recently we have examined the interactions between pressure and anaesthetics on both conduction and synaptic transmission, using the rat superior cervical sympathetic ganglion(4). Helium pressure (35 - 103 ATA) partially restores the amplitude of the action potential in the directly stimulated preganglionic nerve treated with halothane (0.5-1 mM) but has little effect on conduction in the normal nerve. In contrast with its effects on conduction, helium pressure depressed both fast (nicotinic) and slow (muscarinic) excitatory transmission in the unanaesthetised ganglion in a reversible pressure-dependent manner. Instead of antagonising the effects of anaesthetics, pressure added to the depressant effects of halothane (0.25 - 0.5 mM) and methoxyflurane

(0.24 mM) on both types of excitatory transmission. It is only when we know the effects of pressure and anaesthetics on several different synapses that we can assess the relevance of these results to *in vivo* pressure effects such as HPNS and pressure reversal of anaesthesia.

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High pressure neurological syndrome

R. Brauer

Rate dependency of convulsions

The effect of compression rate is both of practical importance in deep sea diving problems and of theoretical interest in understanding the thermodynamic and kinetic basis of the high pressure neurological syndrome. We have studied the dependence of the convulsion threshold (as defined by locomotor convulsions) on the compression rate (varying from 1 to 1000 atm/hour). In general terms the results can be expressed as a regression line with the formula: convulsion threshold = basal convulsion threshold - k log compression rate. However, in the 17 different animal models (varying from the newt to the rhesus monkey) which we have studied, it has not been possible to produce any simple phylogenetic order on the basis of the slopes of these regression lines. For example, a number of species (including baby mice < 13 days old) show compression rate independence, while the hamster appears to have a reverse compression rate dependence (i.e. its convulsion threshold pressure increases with higher compression rates). However, the order of the convulsion thresholds for different species at high compression rates (1000 atm/hour) is: amphibians > reptiles > birds > rodents > carnivores > primates, with but minor exceptions.

Reserpine

The convulsion threshold in mice (Pc) can be reduced by reserpine which has an initial dose-response dependency - the maximum effective dose being 5 - 10 mg/kg (Figure 1). The time course of the reserpine effect (Fig. 2) matches that expected for

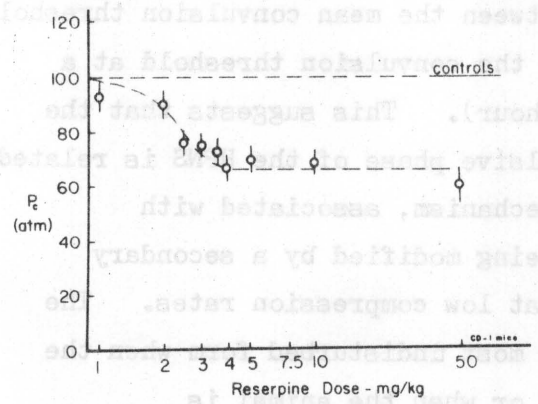


Figure 1

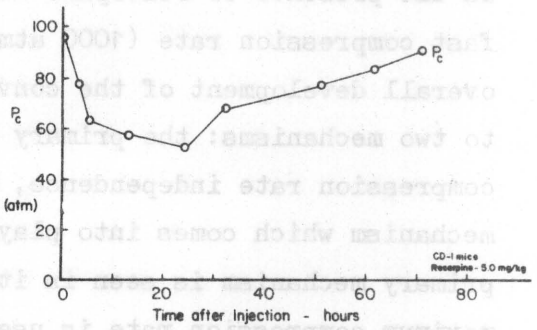


Figure 2

the synaptosome monoamine release or storage rather than alterations in the total monoamine stores in the central nervous system or a direct effect of reserpine replacing monoamine. This has been tested by studying the interaction of reserpine with Tranylcypromine - a monoamine oxidase inhibitor - given before the reserpine which builds up local stores of monoamines and blocks the reserpine effect (Figure 3). Similarly, amphetamine given after the reserpine enhances the effect of any monoamines still available. Additional experiments with small doses of reserpine injected directly into the lateral ventricles emphasise that the phenomenon is a central effect upon central release of monoamines. At the moment it is not possible to distinguish between serotonin, nor-adrenaline and dopamine as the possible monoamines involved.

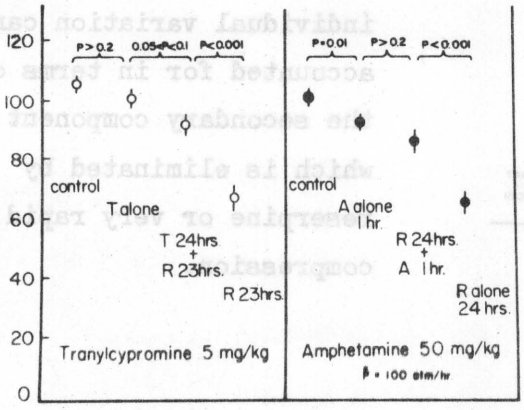


Figure 3

In most species showing compression rate dependence, reserpine lowers convulsion thresholds at slow but not at rapid compression

rates and thus abolishes compression rate dependence. There is a good correlation ($r = 0.93$) between the mean convulsion threshold in the presence of reserpine and the convulsion threshold at a fast compression rate (1000 atm/hour). This suggests that the overall development of the convulsive phase of the HPNS is related to two mechanisms: the primary mechanism, associated with compression rate independence, being modified by a secondary mechanism which comes into play at low compression rates. The primary mechanism is seen in its most undisturbed form when the maximum compression rate is used or when the animal is reserpinised.

The wide range of susceptibility of individuals to the HPNS may also have two components. First, the susceptibility to the primary effect. Second, the individual variation in the ability to temper the primary effect by the convulsion delaying effects of monoamine release. We only have limited data to test this hypothesis but a plot of the standard deviations of the convulsion threshold (σ_{p_c}) against log compression rate (Figure 4) indicates that up to 50% of the total individual variation can be accounted for in terms of the secondary component which is eliminated by reserpine or very rapid compression.

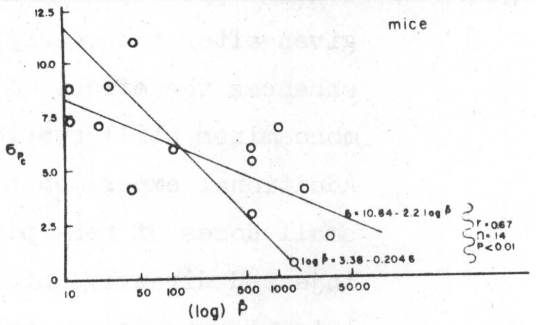


Figure 4

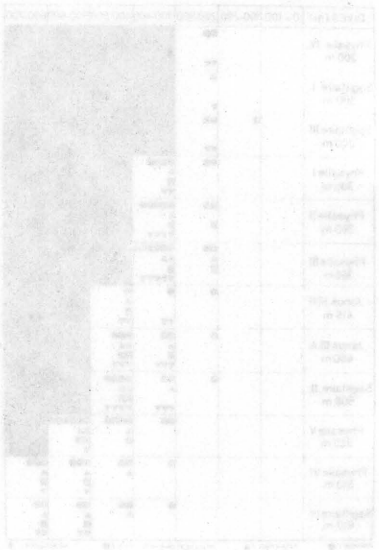
Anatomy

I believe that it may now prove possible to separate the HPNS anatomically. By stereotactic microinjection of drugs which selectively destroy certain monaminergic pathways we hope to

obtain evidence concerning the pathways involved in the secondary convulsion delaying effect. The evidence from some EEG tracings with electrodes planted in different areas of the central nervous system of the squirrel monkey and the rat indicate that the primary paroxysmal discharge starts somewhere in the reticular formation or lower mid-brain, followed by a generalisation of this paroxysmal discharge.

Conclusions

The practical implications of these results include the possibility of using pharmacological means of preparing a diver to protect him, to some degree, against the disastrous consequences of severe HPNS episodes. However, these studies have been concerned with only one end point of the HPNS - locomotor convulsions. There are also fine tremors and the preconvulsive coarse tremors (observed in squirrel monkeys but not unlike some of the human tremors) - both of which have different pharmacology and compression rate dependencies. This emphasises the enormous complexity of the high pressure neurological syndrome.



High Pressure Nervous Syndrome: Clinical and Electrophysiological studies in man

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

This data was all obtained in a series of experimental chamber dives at COMEX Hyperbaric Research Centre in 1968, using 24 subjects exposed to pressures corresponding to different depths between 1,000 - 2,000 ft (Table 1). We have characterised the clinical signs of high pressure neurological syndrome as tremor, dysmetria, fasciculation, myoclonus and drowsiness (microsleep) (Table 2 : 24 subjects; heliox dives). The major E.E.G.modifications are augmentation of theta waves (slow activity), diminution of alpha waves (fast activity) and microsleep (Table 3: 24 subjects; heliox dives). Our

Table 1

Number of times our men have reached 300 meters depth and more
(24 subjects)

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

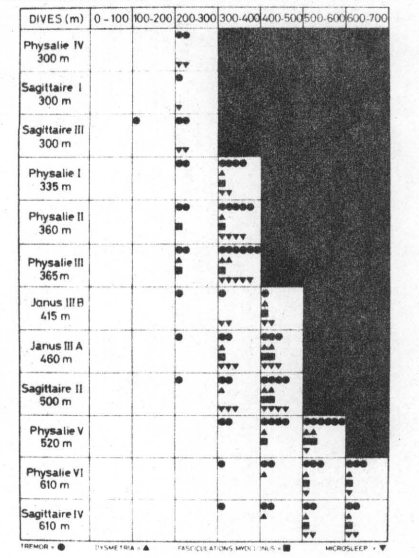


Table 2

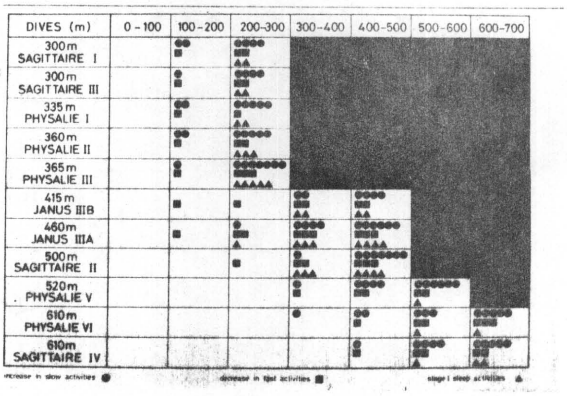


Table 3

experience is in agreement with other workers (1,2), except that we did not observe any disorientation, nausea or dizziness, which are probably dependent on the rate of compression. Tables 2 and 3 also illustrate the influence of the mode and rate of compression on the appearance of different symptoms. For example, the onset of tremors in the early dives occurred around 200-300 m, whereas in the later dives tremors did not appear until 300-400 m. Similarly, microsleep was observed at 200-300m in the first dive but not until 500-600m in the last dive.

The effect of changing the compression rate can also be seen in particular dives. In Sagittaire II the compression rate was relatively fast without any rest stops and at 500m the degree of tremor reached 400%. However, in Physalie VI the compression curve was interrupted by a series of stops and the degree of tremor was considerably reduced (maximum 250%). It was noted that both the men had more tremor in the morning than in the evening but we do not have a satisfactory explanation. Finally, in Sagittaire IV the compression rate was much slower with a series of stops and a depth of 610m was reached in 11 days. However, during the 50 hours at this depth the tremor was not an important problem and only reached 200% in one subject. The E.E.G. modifications are very variable between different subjects. There is usually an augmentation of theta waves together with microsleep. However, in some subjects there is little increase in theta activity and there may or may not be microsleep. Indeed, one particular subject appeared to be perfectly normal at 2,000 ft. Microsleep can be characterized as a decrease in the ability to maintain a normal level of vigilance at rest. However, if roused the subject can adequately perform different tasks. At the

moment we do not have an adequate explanation for this phenomena, which increases both at depth and with an increasing rate of compression.

I would now like to report on some very recent experiments using 9-10% nitrogen/oxygen-helium (with the inspired Po_2 approximately 0.4 atm). The compression schedule for this "Coraz I" experiment is illustrated in Figure 1, and no tremors were observed after compression to 300m in four hours. However, there was a serious problem of euphoria over a period of 2-3 hours, which meant that the men were unable to work or perform any task in the water. In all three subjects the E.E.G. theta rhythm was important but one of the subjects showed randomly a completely unexpected paroxysmal discharge, which first appeared at a depth of 240 and became more pronounced at 300m (Figure 2). This particular subject had previously been to 600m without any abnormality in his E.E.G. response. Twenty four hours after the arrival at depth, E.E.G. paroxysmal activities had disappeared and theta waves were less important in the three subjects. At the moment we do not know if these effects are due

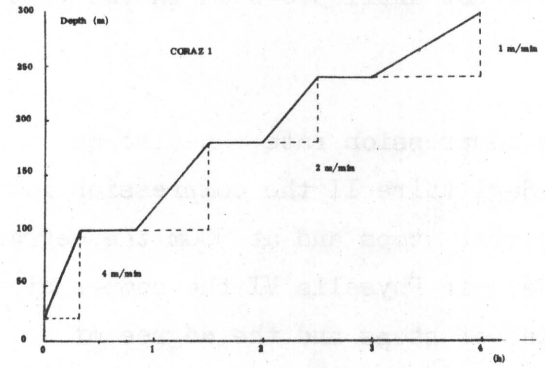


Figure 1

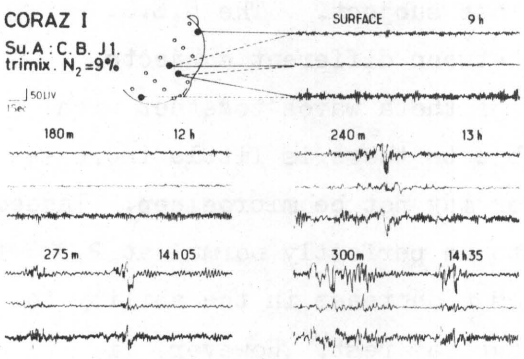


Figure 2

primarily to the speed of compression or the gas mixture but our preliminary results suggest that there are a number of problems, including variation in individual susceptibility which we do not yet understand.

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Practical limits to the use of man under pressure

H. V. Hempleman

The first underwater physiology symposium proceedings (1) contained no reference whatsoever to the problem of osteo-necrosis, whereas in the proceedings of the fourth symposium on underwater physiology (2) there is very evident concern with this problem. It has since become clear that, although there is a problem to be overcome, it is not of major proportions, but it could quite well have been the case that the incidence of this disorder was of sufficient magnitude that deep diving and compressed air work could have been stopped or severely curtailed. One cannot rely upon nature endlessly obliging with problems that are relatively easily overcome when they are eventually recognised and investigated. It will be convenient to divide the ensuing discussion into the four separate phases of diving activity, namely, descending to depth, at depth, decompression and post-dive.

Descending to depth

Descent to depth is accompanied by the usual phenomena, such as ear clearing, temperature changes, density changes, voice changes etc., but these I will ignore because many years of usage at normal depths have not yet revealed any serious problems. The new problems that arise on compression to depths greater than about 150 msw (500 fsw) are two-fold. First, there is the problem of pressure arthralgia. Bradley and Vorosmarti (3) conclude that gas induced osmosis causes dehydration of the articular cartilage in the joint, leading to a lack of adequate joint lubrication and a pre-disposition to cavitation phenomena. If one situation is affected, then all tissue situations will be affected to a greater or lesser degree, depending upon the ability

of the tissue to sustain a concentration gradient of inert gas of sufficient magnitude for a sufficient period of time to cause a significant movement of water. Slow compression appears to eliminate this problem but it must be remembered that up to the present time we only tend to study the obvious, which means that unless the subject feels a pain or there is some dysfunction occurring to a readily observed body process, e.g. balance, it is considered that no problem exists.

The second well-known problem of compression is reflected in the hand trembling and the EEG changes seen at depths in excess of about 150 msw. It is well established that by allowing the body time to adapt at different pressures, the more obvious signs and symptoms of the compression syndrome are avoided but we do not know the underlying aetiology.

At depth

On arrival at pressure there are several measurable body changes which represent a challenge to the homeostatic mechanisms of the body. The slightly disturbing fact that seems to be emerging over the last year or two of human experimentation is that the longer one stays at great depths the more the basic body functions seem to change. For example, at pressures of 250 msw small but significant changes in the ventilation/perfusion ratio are being observed after 1 week at pressure (4). It may well be that such changes are of an adaptive nature and of no real significance but a good deal of further work is necessary before such statements can be made with confidence. Most people require relatively long periods of sleep or some similar form of relaxation, both mental and physical, otherwise they become very disturbed. Both the difficulties of respiration and the unpredictable muscular twitches and trembling which take place

at pressures greater than 400 msw mitigate against peaceful sleep, or an equivalent relaxation period. Extending the period under great pressure to a week, or perhaps a month, may lead to difficult problems and prolonged sojourn at depths of 500 msw or greater will need to be approached with the greatest circumspection.

Decompression

The aetiology of decompression sickness, even at modest depths breathing air, is far from properly understood but, at least for these lesser depths, effective treatments exist for dealing with any problems that arise. However, as one proceeds deeper on helium gas it is observed that the nature of the signs and symptoms of decompression sickness alter. Instead of initially encountering mainly limb bend pains, one observes various forms of "eighth nerve disturbance" (END). These forms of END involving, as they do, partial or complete deafness in one or both ears or vestibular complications resulting in vomiting, nausea, nystagmus etc., present a new challenge. Similar problems have been found in animal experiments. For example, if goats breathing raised pressures of argon-oxygen mixtures exhibited decompression sickness, it was nearly always an extremely serious form, involving paralysis of two, three or four limbs simultaneously. It did not seem possible to provoke as a first presenting sign the usual sustained hoof-lifting to show the animal had a normal bend pain. Thus a change of gas can cause a corresponding change in the presenting forms of decompression sickness. However, with deep diving decompression sickness, we are also concerned with marked changes in the absolute pressure.

In the case of END occurring at pressures greater than about 100 to 120 msw whilst breathing helium gas, rather urgent recompression seems to be required for, unless recompression takes place

soon after the presentation of the first signs of END, some form of irreversible damage seems to occur and recompression therapy loses its efficacy. We have had the doubtful privilege of treating a case of END which occurred at a depth of about 400 msw (1300 fsw) (5), and it is at these depths that one realizes the difficulty of applying the hitherto well established method of therapy for decompression sickness cases, namely, rapid recompression. It is not practically possible, at present, to recompress rapidly at these depths because of the effects of the high pressure nervous syndrome. Thus the therapeutic armoury is robbed of its principal weapon. Furthermore, it is not really feasible to increase the oxygen partial pressure to attempt some form of therapy based upon increased tissue oxygenation, because there is a marked increase in oxygen toxicity in the presence of large partial pressures of helium gas. Thus we are attempting to define the decompression problem from depths of this order without the assurance that we can guarantee effective therapeutic procedures whenever incidents of decompression sickness arise.

Post-dive

Normal uneventful dives can cause a noticeable drop in platelet count some two or three days later. In Tappan and Heyder (6), we find the statement "The present findings support earlier observations that an extended period is required for biochemical equilibria to become re-established following exposure to pressure/decompression. Tentative evidence is provided here that recovery times of 7 to 9 days may follow shallow or medium depth dives". With statements like this being made as a result of experiences with 100 ft diving, it is clear that we are faced with a much more formidable problem at the depths we are considering.

It cannot yet be confidently stated that repeated exposures to great depths will be a sensible activity for fit young men.

In a discussion that took place regarding the possibility of a conventional type illness at depth, the point arose as to whether any work had been done on the immune responses of the body under pressure. I was unable to discover a single relevant report. This brings one full circle back to the non-existence of bone necrosis as a problem in 1955. Are we neglecting certain fundamental body functions, and are we entitled to encourage diving concerns to suppose that 4, 5 or 600 metre diving is just around the corner ?

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A strategy for future diving

P. B. Bennett

The depths beyond 1,000 ft impose considerable limitations on the diver, especially due to the High Pressure Nervous Syndrome (HPNS), but there are a number of methods we can use to mitigate the effects of these very high pressures on the diver (1).

1. Adaptation

HPNS primarily affects psychomotor performance and induces tremors, nausea, dizziness, somnolence, changes in the electroencephalogram. Intellectual decrement, which is only present at excessive compression rates, usually results from the nausea and dizziness causing a disinterest in performance of the task rather than a true intellectual decrement as with compressed air intoxication. There is usually a considerable improvement after the first hour. This is less noticeable at the very great depths, where compression with stages may require many hours anyway. However, for dives to 1,000 ft and maybe to 1,500 ft it is advantageous to permit the divers to rest in the Personnel Transfer Capsule before entering the water to work.

2. Suitable Rate of Compression

The tremors may be regarded as one of the best indicators of susceptibility to HPNS (2). Usually the faster the rate of compression the worse the tremors and HPNS and the lower the onset pressure. However, theories as to the cause of the rate effects of the HPNS are highly speculative and for the present it is best to only suggest that compression should be largely exponential so

that the greater the depth the slower the rate of compression.

3. Stages in Compression

The increase in theta activity in the electroencephalogram, which is initiated during compression requires some 20 hours before it returns to lower values. Once initiated even stopping the compression will not stop the increase continuing for 6 hours followed by a 12 hour fall.

The best compression profile to ameliorate the HPNS is to use an exponential rate with stages incorporated at various points. The choice of duration at the stage is arbitrary but in very deep saturation dives should be many hours at the deep stages.

4. Excursion Diving

As in decompression, where considerable excursions can be made from a saturation depth without the need for decompression stops, so excursions are useful in ameliorating signs and symptoms of the HPNS. Thus Buhlmann et al.(3) compressed men in 1 hour to 1,000 ft with some transitory HPNS resulting. Three excursions at a compression rate of 17 ft/min were then made on different days with the diver working underwater at depths to 1,150 ft without the return of HPNS. However, a similar rate of compression in the Royal Naval Physiological Laboratory 1,500 ft dive from 1,000 ft to 1,100 ft and 1,300 ft to 1,400 ft did cause an increase in tremor and EEG changes. At Duke University Medical Center 6 men were compressed to 870 ft over 2 days and excursions were made at 16.7 ft/min, 50 ft/min and 100 ft/min for 2 hours each, without any evidence of HPNS (Table 1).

TEST		Predive Test 4	870 ft. (Pre-excursion)	Arrival at 1000 ft. 100 ft/min	1 3/4 hrs at 1000 ft 100 ft/min
Ball Bearing	Mean	15.67	19.00	18.33	18.33
	S.D.	±3.79	±2.08	±2.08	±2.08
Peg Board	Mean	28.67	32.67	31.33	30.00
	S.D.	±3.26	±4.73	±4.51	±1.00
Visual Analogy	Mean	47.00	49.33	51.00	48.33
	S.D.	±6.25	±5.51	±7.00	±2.31
Arith Correct	Mean	11.00	8.00	9.00	13.33
	S.D.	±5.57	±8.89	±7.81	±8.08

Table 1

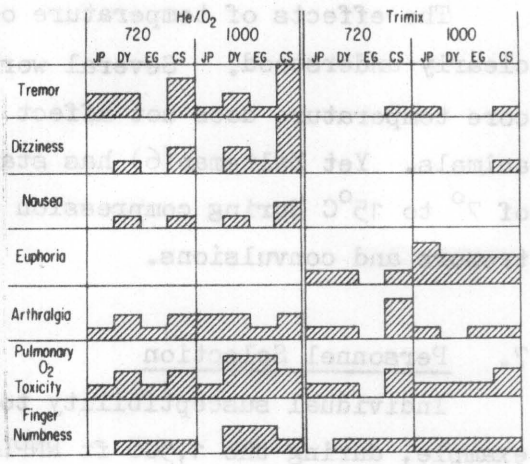

Figure 1

5. Use of Narcotic Agents

Initial studies to 1,000 ft at Duke University Medical Center with 18% nitrogen (5.6 ats abs) added to helium-oxygen proved very effective in prevention of the tremors, nausea and dizziness of HPNS and also seemed of value in reduction of compression arthralgia. Similarly, 25% nitrogen (5.6 ats abs) at 720 ft was equally effective (Figure 1). However, there appeared to be too much nitrogen in the mixture.

A further experiment was carried out in which only 10% nitrogen was utilized for exposure to 1,000 ft with a compression time of only 33 mins, a bottom time of two hours and decompression of only 4 days, with no tremors or HPNS and no nitrogen narcosis(4). At great depths the density of the mixture could affect ventilation,and hydrogen or narcotic by mouth, such as a low dose of barbiturate, may be considered as alternatives.

6. Temperature

The effects of temperature on the HPNS are presently not clearly understood. Several workers (5) maintain that changing core temperature does not affect tremor or convulsions in animals. Yet Zaltsman(6) has stated that increased temperatures of 7° to 15°C during compression increase susceptibility to both tremors and convulsions.

7. Personnel Selection

Individual susceptibility to HPNS varies widely. For example, during the 1,500 ft RNPL deep dive, one of the subjects showed a marked tremor on each compression phase, the other showed no significant tremor(Figure 2). Similarly, subjective signs and symptoms (Figure 1) and performance tests indicate the wide variation in susceptibility.

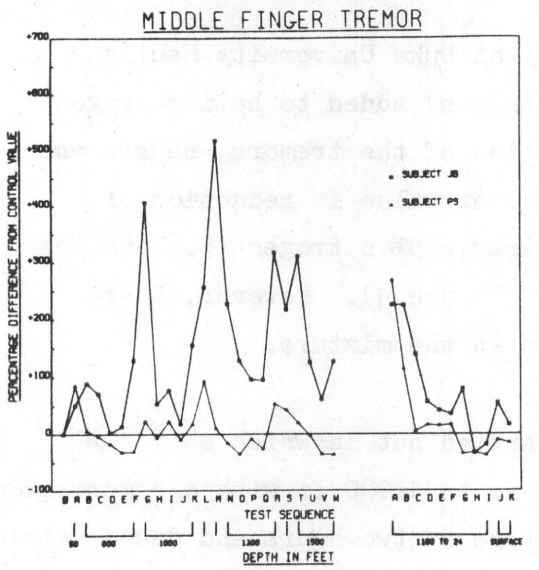

Figure 2

Commercial companies may wish to institute a tremor test of selection in which new divers are compressed at 100 ft/min to 600 ft for 15 mins and the degree of tremor present measured by transducer. Those with the lowest tremor scores should be selected for the deepest dives.

8. Optimal Decompression

Unless saturation diving is necessary for a long task to be performed underwater, the decompression should be as short as

possible. Most commercial tasks require only a 30 min bottom time. If it is possible to compress rapidly and control the HPNS by nitrogen or other narcotic additives then perhaps slightly longer bottom times are feasible.

At Duke University Medical Center decompressions from 1000 ft with a 2 hour bottom time have been carried out satisfactorily using a modified Buhlmann decompression table (Table 2). This

Table 2 Decompression using Duke Modified Buhlmann Table

At 1000 ft:	Trimix (10% nitrogen)
1000 to 850 ft :	Change to He/O_2 with O_2 = 0.8 ATA during travel to 850 ft or at 850 ft. 2.5 ft/min (60 mins)
850 - 300 ft:	12 ft/hour (46 hours)
300 - 100 ft:	8 ft/hour (26 hours)
100 - 60 ft :	4 ft/hour
60 ft - surface:	Change to air (25 hours) 4 ft/hour
Total Dive Times:	At Depth 120 mins Decompression - 97 hours

has caused some pulmonary oxygen toxicity during the first day of decompression and we are therefore attempting to determine whether this decompression procedure will work effectively with only 0.6 ATA oxygen.

9. Engineering Solutions

Engineering solutions include the one atmosphere diving suit and "underwater house" surrounding the well head, but many of

these systems require considerable capital expenditure and are as yet not proven. For the immediate future divers will need to work at depths of 1,500 feet or deeper and it is likely that even with the engineering systems, human diving will still be very necessary.

10. Summary

The procedure for the safest and most efficient deep dives should involve: selection of the divers; computation of a suitable exponential rate of compression with stages; utilization of a mixture of 10 or 11% nitrogen 0.5 ats abs oxygen with the remainder helium; keeping the diver cool, and resting for whatever time is permissable before starting work. For a longer work time, saturation techniques need to be utilized with a similar procedure. Alternatively, excursions of as much as 400 ft to the work depth would save considerable time during the decompression.

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Discussion on strategies for future diving

At this meeting certain areas of common ground were accepted as a base from which to move forward. Although many of the facts had been known already, it is only recently that they have been widely accepted as established concepts upon which the future strategy of deep diving can be planned.

1. Experimental diving limits

At previous meetings the hypothesis of different types of barriers to deep diving had been proposed (e.g. the air diving narcosis barrier; the helium/oxygen pressure barrier) and efforts were made to conceive of new approaches and technology which would enable these barriers to be pushed deeper. However, in contrast, we now feel that there are no obvious insuperable near limits to experimental diving(i.e. dry chamber trials without imposed exercise limitations). Instead of a limiting step function to deep diving it now seems likely that there are, or will be a gradually increasing series of problems and dangers which will have to be overcome. Indeed, although men have now been exposed to pressures equivalent to 2001 feet of sea water, it would be extremely dangerous to proceed further without recognizing that we may have to face even more serious problems. Thus, the steps taken now at depth must be very cautious for a number of reasons, including the difficulty of therapy and recovery if anything untoward occurs, and the many potential environmental dangers which are at present ill-defined (see section 18). There may be some respiratory problems associated with maximum workloads, and the longer term hazards of working at such depths are at present almost totally unknown. As such it was not thought profitable to speculate about the ultimate depths to which man could dive,

although it seems likely that with the aid of a pharmacological attack, depths of the order of 3000 f.s.w. are feasible. Beyond this the problems appear to be moving from central nervous system effects to contractile effects and these may become limiting around 6000 f.s.w. It should be emphasised, however, that such figures are entirely speculative and perhaps the most important area of interest is in consolidating our knowledge about depths in the region of 1-2000 f.s.w.

2. Pathway dependency

It is accepted that there is a time factor which is a subtle variable at every depth. This factor may not only aid the reconciliation of some of the apparently discordant data observed by different workers but also leads to the fundamental concept of pressure effects being dependent on the path, in depth and time, taken to achieve a particular pressure. This concept suggests new modifications to our current models for understanding the effects of pressure. A practical application is the value of slow compression with helium and oxygen to avoid a potentially hazardous situation.

3. Anaesthetic additives

The use of anaesthetics to ameliorate some of the effects of high pressure has been investigated in animals (sections 7,9,10) and recently in man (sections 17,19). The technique has now become accepted but the experiments emphasise a number of potential difficulties which have not yet been resolved.

a. The gas mixtures required to achieve a balance between narcosis and pressure have been predicted, using several different model calculations related to the animal results, as 10 $\pm$ 5% nitrogen in an oxy-helium mixture. It was found with men that 18% nitrogen was too high a concentration at 1000 f.s.w. to maintain adequate

performance. The results using 9-10% nitrogen appear to be conflicting, since one group of divers had significant euphoria while the other group had relatively mild symptoms (sections 17,19). Thus, although the probable area of useful gas concentrations for diving to 1000 - 2000 ft is now known, it would be wrong to base any specific recommendations on these preliminary experiments in man (see future basic research work).

b. In man we so far have data only for nitrogen, which has the potential problem that the density of the gas mixture may become an important factor at increasing pressure. For this and other reasons, a number of different agents were suggested including hydrogen, nitrous oxide, the clinical volatile anaesthetics, the new steroid anaesthetics, barbiturates, and alcohols. On the basis of theoretical calculations, animal experiments at depth, and human exposures at sea level, a number of these agents could be investigated further. Although the concentration required would vary approximately with the anaesthetic potency, there would still be problems with decompression. Furthermore, little is known about the long-term exposure to such agents although there is an increasing awareness of this problem in anaesthesiology.

4. Therapy

We know very little either about decompression sickness occurring at depth or about new problems associated with high pressures. No practical therapy has yet been tested on men working at great depths. There are obvious new problems such as the total isolation when it would be impossible to send a relief diver down to 1000 ft or deeper very quickly. Again, the classic treatment for decompression sickness is rapid recompression, but it might be expected that this treatment at great depths would lead to the onset of further problems associated with high pressure nervous syndrome. It is difficult to extrapolate therapy data obtained in small rodents to man and it seems likely, therefore, that future work in this area will be on an empirical basis. A number of very long-term solutions were discussed including the use of artificial blood substitutes and liquid breathing techniques

5. Selection of the diver

Animal experiments on the effects of high pressure suggest that there is a large variability in any one species and there is no evidence of decrease in this variability in the case of primates. In statistical terms the standard deviations of the results are large and the tails of the distributions are significant. Unfortunately, at present, the number of human subjects are small (only 4 people have been exposed to pressures equivalent to 2000 f.s.w.) and we have no idea as to what the pattern of distribution of susceptibility in a population would be expected to be. Until we have more data on more men it is impossible to estimate any concept of the degree of safety of a particular protocol. This is in conflict with the need to obtain data under a greater variety of conditions for subsequent interpolation of the optimum procedure. However, an important implication is that it may be possible to pick out individual characteristics which particularly reduce the susceptibility to high pressure effects and thus reduce the overall risks by more rigorous diver selection.

FUTURE BASIC RESEARCH WORK

The various topics discussed throughout this report are expanding areas of research and it would not be profitable to list the obvious extensions. However, there are some areas which were not covered by specific speakers which were nevertheless thought to be important in planning the future strategy of diving.

1. Routine working dives

A number of experimental chamber dives have been made at pressures equivalent to 1000 f.s.w. or more, but routine working dives in the sea have yet to be extended beyond the 600 f.s.w. limit.

The challenge of sea dives to 1000-1500 f.s.w. can be divided into two areas: a) technical and equipment limitations and, b) biological and physiological limitations.

a. The technical limitations have already been investigated in a series of chamber dives using a "wet pot" which have allowed the divers to work under water in a controlled environment for a variable length of time. For example, there have been a number of French chamber dives to 400m in which men worked under water for two hour periods without any technical difficulties. On the other hand, the US Navy 1600 foot dive demonstrated that a particular piece of equipment (MK10 MOD4 underwater breathing apparatus) was inadequate for routine work in the sea at such depths. However, as a result of the experinece gained in chamber dives, there is a new generation of equipment which has been developed and the French company COMEX are prepared to test this in the sea in a dive planned to take place off Norway down to 12-1400 f.s.w.

b. The biological limitations to man working both at pressure and under water have not yet been adequately studied. It is vital to consolidate our knowledge about respiratory changes with mild work loads at depth by studying the effects of high work loads over longer periods of time, which may be more relevant to practical diving. The evidence from the chamber and equipment studies is suggestive that changes in respiratory physiology should not become a major limiting factor. Unfortunately, little is known about changes in lung mechanics on immersion and the importance of posture under such conditions (see section 14). For this reason one area of future research is a detailed investigation into the functional and mechanical changes of the lung on immersion at different depths.

2. Anaesthetic additives

The technique of adding anaesthetics to diving gas mixtures now needs to be properly investigated in man. In particular, we need to know the effects of different partial pressures of a number of agents over a complete range of depths. This involves doing dose-response curves using different end-points appropriate to man's performance at depth. Although at first sight this might seem a difficult and expensive area of research, the technique of switching gases and

using an enclosed diving suit to overcome the problems of counter-diffusion, etc. should make it feasible.

The ethics of human experimentation under hazardous conditions are recognized as a problem. The onus for responsibility clearly lies with the individual investigators and their respective committees on human research. It was suggested that a national committee might have a role in advising on the hazards of any particular proposal. However, in deciding the proportion of risk to benefit, it has to be recognized that commercial diving can be very hazardous and that research in this area is both urgent and important.

3. Basic research

a. Time factors

It was agreed that the critical pathway for optimum performance at depth should be investigated using model, animal, and human experiments. The contribution of this factor obviously depends on the phenomenon being observed and, for example, would appear to be a major factor in decompression but a lesser factor in some of the neurophysiology-pressure interactions. The recognition of distinctions between critical pathways, transient and equilibrium conditions may considerably further our concepts of the physiological effects of pressure.

b. Interdisciplinary problems

The gap between the animal studies and the molecular models is difficult to bridge but at this meeting it was clear that there are many people from different disciplines who are working towards an understanding of the effects of pressure. One of the major problems is to understand why pressure seems to affect so little of the overall function, in spite of the considerable number of specific neurophysiological, cellular, or molecular events which must be perturbed by high pressure. It now appears possible to close the interdisciplinary gap in both directions, i.e. from the molecular and cellular approach by detailed investigation of membrane and neural function, as well as from the neurophysiological end by both

identifying anatomically the sites particularly vulnerable to pressure and extending the specific neuropharmacology studies on selected synapses.

SPECIFIC TOPICS

Detailed discussions have been incorporated into the reports on appropriate papers but a number of specific topics which were discussed at length include :

1. Excursion dives

A number of excursion dives have been made at depths around 1000 ft (see appendix and section 19). The technique has proved to be of practical value at shallower depths and it seems likely that this value will be maintained at the greater depths and allow men to work from a fixed base. The limits on excursion dives have been derived empirically. Haldane proposed that the ratio of the two pressures between which a man may safely move should not exceed two. It is known that the ratio decreases with increasing depth but the evidence available suggests that the limit of excursion dives in absolute terms does not vary significantly with increasing depth. Unfortunately, there is at present no adequate physico-chemical approach to this problem and so excursion diving remains a useful but empirical technique.

2. E.E.G. modifications and tremors

It was noted that changes in the E.E.G. rhythms at high pressures have been observed by many workers. The most consistent finding is an increasing percentage of theta waves (see section 17) which appear in the frontal region. However, it is still not knownwhat is the origin and significance of these and other changes. The observation of paroxysmal discharges in one subject (section 17) has further complicated the interpretation of such data. On a

practical level it is still difficult to relate these E.E.G. modifications to the subjects' functional abilities as divers.

Problems with tremors are of great practical importance when considering working in the water at depth.

3. Euphoria

This is a particularly important phenomenon when considering experimental diving gas mixtures which deliberately include a small concentration of anaesthetic agents. The results of adding 9-10% nitrogen in human dives appear at the moment to be conflicting. The Coraz I experimental dive (see section 17) reported marked euphoria which was severe enough to prevent the divers working safely in the water . On the other hand the Duke University dive (see section 19) found relatively little euphoria. Three aspects of this problem were discussed.

a. Euphoria has been assessed on the basis of a subjective check list assessment of signs and symptoms. It is difficult to quantitate in man and impossible in animals. However, the end point is of great practical importance since unpredictable actions of a diver in the water could be extremely dangerous.

b. The situation is complicated by psychological factors such as apprehension or boredom, both of which are known to affect the degree of euphoria. Thus, the degree of motivation of the diver is extremely important, both in the testing of different gas mixtures and in the practical implications of the data obtained.

c. The time course of the onset of euphoria appears to be slow relative to that expected for anaesthesia. The possibility of this being related to the uptake and distribution of the different gases was considered. However, it is known that pressure has an immediate effect followed by remission of some of the symptoms. If the degree of pressure antagonism to narcosis has the same time scale, the subjective measurement of euphoria may have a time course determined by this remission of pressure symptoms rather than by the onset of narcosis.

4. Counterdiffusion

This surprising phenomenon is relevant to the problems associated with breathing one gas while being surrounded by a different gas and is the currently accepted explanation for inert gas "urticaria" and vertigo. It was not discussed in detail, partly because much has been written in the literature and it is more properly related to decompression/gas transport research which was covered at an earlier meeting (see Workshop on: Development of Decompression Procedures for Depths in excess of 400 feet.). However, two relevant topics were considered. First, the problems associated with switching gases in order to determine dose-response curves for anaesthetic additives in man (see section 19). Second, the practical issue of using a submarine vessel which would collect people, saturated on air, from a damaged submarine using compressed air to keep the water at bay and then transferring them to a deep diving chamber which has to be pressurized with helium/oxygen. Under such conditions it is possible that isobaric decompression sickness would occur and unfortunately the small animal data available is inadequate for extrapolation to man.

5. Chronic animal experiments

There have been a number of animal experiments maintaining animals around 100 ATA for periods of three to five days (see sections 7, 9). However, the likely compression schedule for man will require more prolonged periods at depth (the Physalie VI experimental dive had two men at depths greater than 1000 ft for over nine days).

Little is known about the rate of acclimatization of animals to pressure. A further difficulty is that it is difficult to extrapolate the results of animals to man because of the lack of data on the different time constants for different species. It was argued that, because the rate of metabolism per gram of tissue was greater in the mouse than in man, the results obtained from short term exposures with animals might be equivalent to longer dives with men. However, in any case it was agreed that we badly need data on both the long term and short term effects of chronic exposures to pressure. Ultimately a study of the physiological, cellular and molecular changes associated with adaptation to high pressures could be studied using a breeding colony of primates.

Dr J.H. Hildebrand attended as Guest of Honour

He agreed to read his original letter (reproduced below) to Dr C.S. Lind of the Bureau of Mines, Washington, dated January 29th, 1924, proposing the use of helium in deep diving, and to summarise his current research into the diffusion of gases in liquids.

Dr A.R. Benke paid tribute to the contribution of Hildebrand, and referred to the first practical use of helium in diving to rescue the survivors from the submarine "Squalus" in 1938.

"My dear Lind:

You have probably heard of caisson disease and the theory that it is caused by the release of dissolved gases in the blood as pressure is removed on coming out of the caisson, or, in the case of a diver, in coming up from a considerable depth of water. There is considerable physiological evidence in favor of this theory. This is the trouble which limits the depth at which diving operations are practicable.

Inasmuch as helium is only about one-half as soluble in water as is nitrogen, it has occurred to me that a diver breathing a mixture of oxygen and helium should be able to work at greater pressures or to come up from a greater depth more rapidly. I suppose that any oxygen dissolved as such in the blood could be readily disposed of by muscular effort and would not be a source of trouble. If helium is of service in this connection, it would obviously be of great economic importance in salvaging operations.

I had hoped to try a few experiments with mice, releasing them suddenly from a pressure of several atmospheres, using in one case air and in the other case a mixture in which helium replaced the nitrogen of the air. However, my time is so much taken up that there seems to be no very early prospect of trying such experiments. It has occurred to me, therefore, that this

is a problem which the Bureau of Mines might like to investigate. The results, if successful, would doubtless be patentable, so that work should be done with due regard for protection. I am not desirous of making any money personally from such a venture, but I would hate to have any one else do so, and would want any profits used for scientific purposes. I will be glad to hear what you think of the prospect.

With best regards, I am,

Sincerely yours "

(Signed) J.H. Hildebrand

APPENDIX ON EXCURSION DIVING AT 1000 FEET

Excursion diving has been defined as a dive to a shallower or deeper depth from a saturation point, and is distinguished from a bounce dive which is from the surface (or atmospheric pressure) to depth and back almost at once. Excursion diving is a useful but empirical technique (see section 20), although, so far, there have been relatively few studies in the region of 1000 ft of sea water. Some data at these pressures are available either from specific studies or from observations during the intermediate stages of deeper dives associated with relatively rapid compression rates. In the list below no attempt has been made to summarize the conclusions of each study, since there are so many individual variations in both the prior conditions of saturation and the data recorded.

Saturation ⟶ Excursion Depth	Compression Rate (ft/min)	Reference
600 ⟶ 1000	100	5
800 ⟶ 1000	2 - 3.5	3
800 ⟶ 1100	3.5	6
850 ⟶ 1025	-	10
870 ⟶ 1000	16.7 - 100	1,4
1000 ⟶ 1100	16.7	9
1000 ⟶ 1150	17	2
1100 ⟶ 1200	16.7	9
1148 ⟶ 1312	3.28	7,8
1200 ⟶ 1300	16.7	9
1509 ⟶ 1608	3.23	7,8

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