PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Interactions Between Pressure and Anaesthetics [and Discussion]

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The interactions between pressure and anaesthetics

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Compression of animals causes excitation, which has recently posed a barrier to deeper diving. The broad question addressed here is how far the inert gas breathed modifies the excitatory effects of hydrostatic pressure. By using aquatic animals we first show that helium postpones the onset of pressure-induced paralysis by some 35 atm.† Next we show that in mammals compressed with helium, five anaesthetic gases (nitrogen, argon, nitrous oxide, carbon tetrafluoride, sulphur hexafluoride) all elevated dose-dependently the median pressure of four distinct phases of the high pressure neurological syndrome (h.p.n.s.) (complete spasms, clonic convulsions, tonic convulsions and non-tonic death). All the gases were equally efficacious relative to their anaesthetic potency. However, the sensitivity of each phase of the h.p.n.s. to anaesthetic gases differed. Most notably, the median pressure for tonic convulsions was elevated about three times more by a given partial pressure of anaesthetic gas than were the median pressures for complete spasms or non-tonic death. These observations can be fitted remarkably well by the hypothesis that a given phase of the h.p.n.s. is activated when some hydrophobic region is compressed beyond a certain critical amount by the application of pressure. Absorption of an inert gas in this region will cause it to expand, tending to elevate the median pressure for that phase of the h.p.n.s. Our data and analysis allow the following conclusions relevant to diving practice. All gases protect against the h.p.n.s. but some phases of this complex syndrome are more effectively controlled than others. Although addition of a second inert gas to helium allows substantial increases in the pressure at which h.p.n.s. occurs, the onset of anaesthesia (or inert gas narcosis) will limit the ultimate gain. The composition of therapeutic gas mixtures becomes more narrowly defined as the pressure increases. The optimum mixture may be different for each phase of the h.p.n.s., and the order of presentation of the h.p.n.s. symptoms may be changed by the second inert gas. We may also predict that physiological sites may exist where helium acts like an anaesthetic. If such sites resulted in physiological dysfunction, addition of a second gas would exacerbate the situation.

Introduction

The high pressure neurological syndrome (h.p.n.s.) is a complex syndrome experienced by animals after compression to pressures above 10–20 atm. The neurological basis of the syndrome is barely understood, but it is clear that the origins of different phases of the syndrome are multiple (Brauer *et al.* 1982; Halsey 1982). Thus the immediate probability of finding a 'silver bullet' to overcome this obstacle to deeper diving is small. The object of the present paper is to explore the potentials and limitations of the paradigms available to us today. What can we expect from exploiting existing knowledge, while the long-term search for greater understanding and more selective remedies proceeds?

The strategy to be explored is the conventional one of manipulating the inspired gas mixture

† 1 atm = 101 325 Pa.

to overcome depth barriers to ever deeper diving. Just as the limitations imposed by oxygen toxicity and nitrogen narcosis were overcome by the substitution of the non-anaesthetic helium for the inert diluent gas, as suggested by Hildebrand (1928), so in an ironic twist, the reintroduction of controlled amounts of nitrogen into the helium–oxygen mixture has brought some, albeit partial, amelioration of the h.p.n.s. Although we have called this approach conventional, its optimum application has received little systematic study, and a number of areas remain to be explored (Bennett *et al.* 1974).

First, will a single gas mixture provide protection against all phases of the h.p.n.s. over a wide pressure range? It is known that pressure reversal of anaesthesia and anaesthetic postponement of an early phase of the h.p.n.s. occur at different sites (Miller 1974). It seems probable therefore that the inert gases might oppose each component of the h.p.n.s. complex at a separate site. Thus, even if the same mechanism were operating at each site qualitatively, the exact quantitative balance between excitation and depression would differ. Each end point would then demand a somewhat different gas mixture to prevent symptoms, which, in turn, might account for the only partial success of inert gas mixtures in increasing deep diving thresholds. Thus, a single gas mixture would not prove optimal at all depths and against all degrees of severity of the h.p.n.s.

Secondly, to what extent does helium merely transmit mechanical pressure? Does it, like the other gases, contribute to the amelioration of the h.p.n.s., or is it simply a transmitter of hydrostatic pressure?

A third area of exploration is the use of inert gas mixtures other than helium–nitrogen. There may, or may not, be specific pharmacological benefits in substituting other gases for nitrogen, but there are certainly clear environmental and economic reasons for reducing the helium content of these mixtures. Indeed, the latter factor probably weighs heavily in the commercial exploitation of helium–nitrogen mixtures. Can we use our existing knowledge of helium–nitrogen mixtures to predict optimal mixtures of other gases?

The only quantitative framework that is simple enough to provide clear theoretical guidance is the critical volume hypothesis defined below (Miller 1974). At this point it is sufficient to note that this hypothesis does not allow calculations to be made *ab initio*; that is, it is a null theory. If the pressure at which symptoms occur is known for one gas mixture, the pressures at which they occur in any other gas mixture are completely defined. Since we are working to equal physiological states, the neurological event responsible for arriving at that state may remain undefined, thus allowing us to finesse our undoubted ignorance of the underlying process. Conversely, the equilibrium nature of the approach precludes prediction of temporal adaptation within the physiological system. (The only kinetic features expected are those involved in the uptake and distribution of the gases to their sites of action. These processes occur on a minute time scale and would only be of interest during the most rapid compression.) Nonetheless, provided such adaptation is a function only of time at pressure, not of the gas inspired, the null approach can still be successfully exploited for *comparing* the effect of changing inert gases for a given dive profile.

In this paper, we first describe some simple experiments with aquatic animals designed to reveal the effects of helium against h.p.n.s. Secondly, the ability of five separate anaesthetic–helium mixtures to ameliorate the h.p.n.s. in mice are determined. Analysis of these data for mammals suggests that these anaesthetics ameliorate all phases of the h.p.n.s. by similar mechanisms, but their sites of action for each phase are distinguishable from the general

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

anaesthetic site and, in some cases, are distinguishable from each other. Implications of these findings for the use of inert gas mixtures in deep diving are then discussed.

Methods

Tadpoles were exposed to pressure in small water-filled pressure bombs fitted with a plexiglass window. Compression was achieved either by a hydraulic pump or with compressed helium. The ability of the animals to maintain an upright position when the whole chamber was rotated was used as an end point for high pressure paralysis.

Work with mice (male, CD-1, 20–30 g, Charles River Breeding Laboratories) was performed under closely controlled physiological conditions. (For full details see Miller & Wilson (1978).) In each experiment ten mice could be observed in cages, while two more mice were fitted with rectal thermistor probes and maintained at 36.5–38 °C. The sealed chamber was flushed with oxygen and compressed with anaesthetic gas at 1 atm min^{-1} until the desired partial pressure was attained, whereupon compression was continued with helium at the same rate. The pressure at which each of four physiological symptoms (defined below) were first observed in each mouse was recorded. The onset of tremors proved too variable a symptom to be used as a reliable end point, especially since the anaesthetic gases *per se* produced a similar fasciculation associated with hyperactivity but not related to high pressure. Thus, the first symptom for which we have recorded useful quantitative data was complete body spasms, defined as the rhythmic tensing and relaxing of all muscle groups throughout the body but of insufficient severity to cause a loss of upright posture. The second symptom was clonic convulsions, defined as spasms of sufficient severity to cause the animals to lose their upright posture. This end point is comparable to the generalized motor or type I seizures reported by Brauer (1975) and others. The third end point was the onset of tonic convulsions, where continuous contraction of the muscles occurred and ventilation ceased during the convulsions (type II seizures). Finally, the pressure at which the animals died was recorded. Animals whose death was not associated with tonic convulsions were assumed to die in a manner related to total applied pressure (see results) and were termed accordingly the non-tonic death group.

Cumulative pressure–response curves were constructed showing the fraction of animals exhibiting a given end point at a given pressure for each of the four end points. These curves were analysed by logit methods to determine the median pressures for the appearance of each end point (Waud 1972).

Results

When tadpoles were compressed hydraulically, no paralysis was seen below 120 atm. At 130 atm, 24% of the animals were paralysed and by 160 atm all were paralysed. Compression with helium revealed a protective effect up to 150 atm. Above this pressure, paralysis occurred increasingly until all animals were paralysed at 200 atm. The dose–response curves for hydraulic and helium compression were parallel and the ED_{50} for helium was 35 atm higher.

In the mouse experiments, upon compression in helium, animals exhibited coarse tremors (47 ± 5.6 atm), complete spasms (83 ± 2.5 atm), clonic convulsions (88 ± 1.4 atm), and tonic convulsions (96 ± 4.2 atm); 62% of the animals survived tonic convulsive episodes to die at 126 ± 4.0 atm ($ED_{50} \pm$ s.d.). These data are similar to those reported previously by various workers. For an excellent review see Halsey (1982).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

When the pressure was raised with helium in the presence of an anaesthetic gas, all of the previously defined end points of the h.p.n.s. were generally observed to occur at higher pressures than when helium alone was used. Typical pressure–response curves are shown in figure 1. The anaesthetic gases examined were argon, nitrogen, nitrous oxide, carbon tetrafluoride, and sulphur hexafluoride. The median onset pressure for complete spasms was increased by 27 atm per ED_{50} of anaesthetic gas. The corresponding figures for clonic convulsions, tonic convulsions, and non-tonic death were 54, 90, and 35 atm per ED_{50}, respectively (figure 2). Note that the pressure reversal of anaesthesia causes an increase in the anaesthetic ED_{50} of about 36% per 100 atm (Miller *et al.* 1978). Thus, all the animals in these experiments had intact righting reflexes at the pressures where h.p.n.s. symptoms occurred.

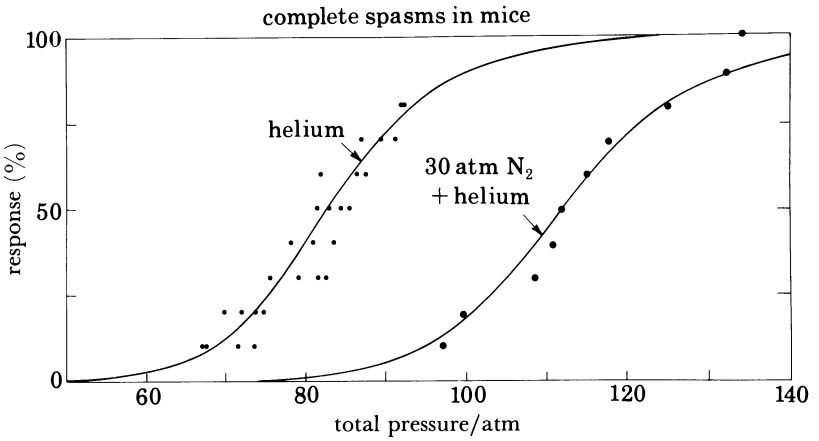

FIGURE 1. Accumulative dose–response curves for complete spasms. The left curve shows data for compressions obtained in four separate control runs. The right curve shows data for a single compression in which the pressure was raised to 30 atm with nitrogen before switching to helium as the compression medium. The left curve has an ED_{50} of 83 ± 2.5 atm and a slope (scale parameter) of 12 ± 3.5 and was obtained with 40 animals; the corresponding figures for the right curve are 112 ± 6.6 atm, 13 ± 9.4, and 10. (See text for details.)

A number of problems complicate a cohort study, such as this one, which compares different gases. Ideally the agents should be administered in a randomized manner throughout the study (Rowland-James *et al.* 1981), but, with gases, all ten animals in each experiment experience a single partial pressure of anaesthetic gas. Thus, each point in figure 2 represents an ED_{50} derived from a single experiment on ten animals. (The order in which experiments were done was randomized.) Attention should therefore be focused on overall trends rather than on specific points. The small pressure differences between h.p.n.s. end points also complicated the derivation of these ED_{50}s. This was particularly true of the clonic convulsion end point. Being sandwiched between two other end points, this end point was often observed with only a low frequency, accounting for the variability in figure 2(*b*). Complete spasms, in contrast, were observed with high frequency (figure 1). Tonic convulsions were observed with high frequency at low anaesthetic partial pressures, but protection was so exceptionally good that at higher doses death occurred before tonic convulsions and few data were obtained. Non-tonic death occurred by definition in all cases, the only exceptions were when insufficient helium pressure was available to complete the pressure profile.

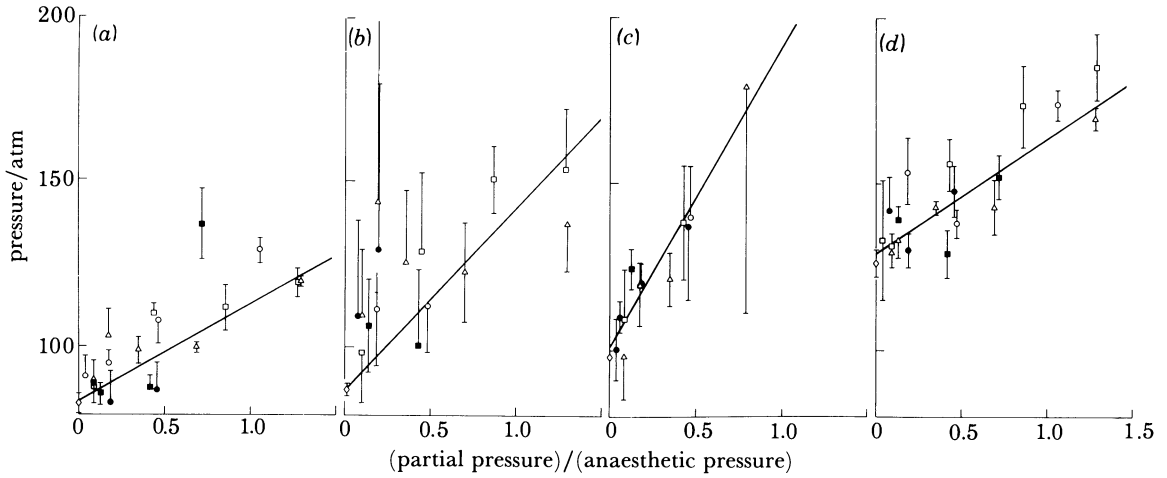

FIGURE 2. Median pressures for the onset of four symptoms of the h.p.n.s. in mice ((*a*) complete spasms; (*b*) clonic convulsions; (*c*) tonic convulsions; (*d*) non-tonic death) are plotted against the fraction of an anaesthetic dose of the second gas in the helium mixture. The anaesthetic dose used was the hypothetical dose at 1 atm. This value was obtained from data for the pressure reversal of anaesthesia in the same species of mouse used herein (Miller *et al.* 1978). The procedure for each gas was to plot its ED_{50} as a function of total pressure and to extrapolate to one atmosphere. The values thus obtained were: N_2, 35.0; Ar, 17.1; N_2O, 1.16; CF_4, 24.1 and SF_6, 5.5 atm. ◇, Helium; □, N_2–He; △, N_2O–He; ○, Ar–He; ■, CF_4–He; ●, SF_6–He. The bars indicate the standard errors (Waud 1972). The lines were fitted by the method of least squares, and each point was weighted by the reciprocal of the variance. For each end point the slope (±s.d.), intercept (±s.d.), and correlation coefficient were respectively: complete spasms, 27 ± 1.5, 86 ± 1.2, 0.91; clonic convulsions, 54 ± 6.5, 89 ± 1.4, 0.94; tonic convulsions, 90 ± 17, 101 ± 2.9, 0.86; and non-tonic death 35 ± 2.7, 129 ± 1.6, 0.92.

THEORETICAL ANALYSIS

Our data can be used to test the critical volume hypothesis, which states that for a given h.p.n.s. end point the symptom occurs when some hydrophobic region is compressed beyond a certain critical amount by the application of pressure. Absorption of the inert gas or gases in the breathing mixture compensates for this compression because gases dissolved in a non-polar medium always cause expansion, thus raising the pressure for appearance of the symptom (Miller 1974; Miller 1977). (This is analogous to the hypothesis applied to anaesthesia, in which a hydrophobic region is expanded beyond a certain critical volume by non-specific absorption of an inert gas; applied pressure opposes the expansion and thus reverses the anaesthesia (Miller *et al.* 1978).) The equation defining the hypothesis is

$$\Delta V_c = \sum_{i=0}^{n} \Delta V_i - \beta P_T, \qquad (1)$$

where ΔV_c is the critical volume change associated with the behavioural end point and ΔV_i is the expansion caused by dissolving the ith inert substance in the hydrophobic region. The term βP_T is the product of the isothermal compressibility of the hydrophobic region, β, and total applied hydrostatic pressure P_T. For the h.p.n.s. to occur the $\Sigma \Delta V_i$ must be outweighed by the βP_T term so that the critical volume change is negative, representing a compression. Under hydrostatic pressure ΔV_c will be achieved at the lowest pressure because $\Delta V_i = 0$. Addition of any inert gas will make ΔV_i positive and thus increase the total pressure, P_T, required to achieve ΔV_c. Figure 3 illustrates the application of (1) for the median pressure for the appearance of

some symptom. Note that helium is included as one of the ith gases. This is justified by the observation that hydrostatic pressure-induced paralysis in newts and tadpoles (see the results) is ameliorated when inert gases, including helium, are present (Furmaniuk *et al.* 1982; Lever 1971). In any case there is no reason *a priori* to ignore helium, since like all the other gases it is soluble in physiological fluids, although in practice its solubility is the lowest of all the gases in lipid.

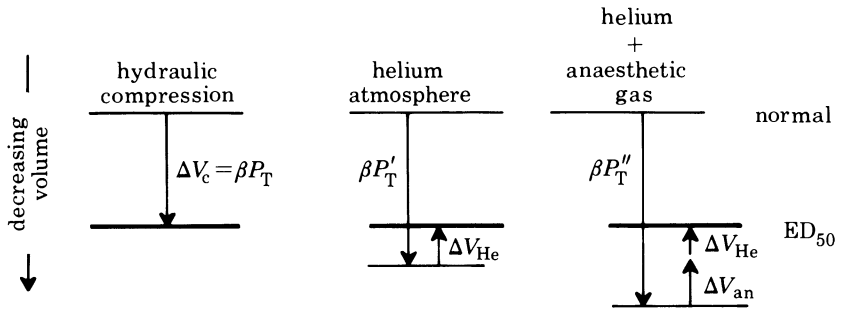

FIGURE 3. The critical volume hypothesis applied to the high pressure neurological syndrome. ΔV_c is the critical volume associated with the appearance of a h.p.n.s. symptom. For hydraulic compression no gases are added, $i = 0$, and $\Delta V_c = \beta P_T$. All gases added contribute to the expansion. With helium alone ($i = 1$; equation (1)), a net compression still occurs, but the ED_{50} pressure increases to P'_T, where $\beta(P'_T - P_T) = \Delta V_{He}$. With helium plus an anaesthetic gas ($i = 2$; equation (1)), even more expansion occurs, and the ED_{50} pressure increases further to P''_T. Helium differs from the anaesthetic gases because of its low solubility, so that $\Delta V_{He} - \beta P_{He}$ is usually negative (but see figure 5), while the analogous expression for an anaesthetic is always positive.

To test exactly the hypothesis stated in (1) it is necessary to know the solubility and partial molar volume of each agent at the site of action, since

$$\Delta V_i = P_i x_i \bar{V}_i / V_m, \tag{2}$$

where ΔV_i is the fractional expansion, P_i is the partial pressure, x_i is the mole fraction solubility and $\bar{V}_i$ is the partial molar volume of the gas, i, in the hydrophobic region, m, of molar volume V_m. The actual site of action is, of course, unknown, but, analogous to the case with general anaesthetics, it behaves much like a simple non-polar solvent. The vest solvent model for a given end point can be chosen objectively when data for fully fluorinated gases are available (Miller *et al.* 1972*a*). We have previously described in detail how this may be done when data are available over a range of pressures (Miller *et al.* 1978). Briefly, the solvents are ranked according to their solubility parameters δ (Hildebrand *et al.* 1970), and the volume expansions caused by equally effective mixtures of gases for a given physiological end point are calculated in each solvent. The procedure is illustrated in figure 4, where the calculated expansion, $\Sigma \Delta V_i$, for each gas mixture is plotted against the pressure at which it causes non-tonic death for two solvents. Figure 4 (*a*) shows that a solvent with a solubility parameter of 10.0 better simulates the site of action of the inert gases than one with a solubility parameter of 8.2 (figure 4 (*b*)). In fact, it is the position of the fluorinated gases, carbon tetrafluoride and sulphur hexafluoride (solid symbols), that forms the basis on which the two solvents may be distinguished. The non-fluorinated gases fit well with whatever solvent is chosen (Miller *et al.* 1978). The solubility parameter that best characterizes each end point can be estimated by plotting the deviations of the fluorinated gases from a line fitted to the other gases. Results of such an analysis are shown in table 1. The sites at which the inert gases exert their anti-h.p.n.s. effects cover a narrow

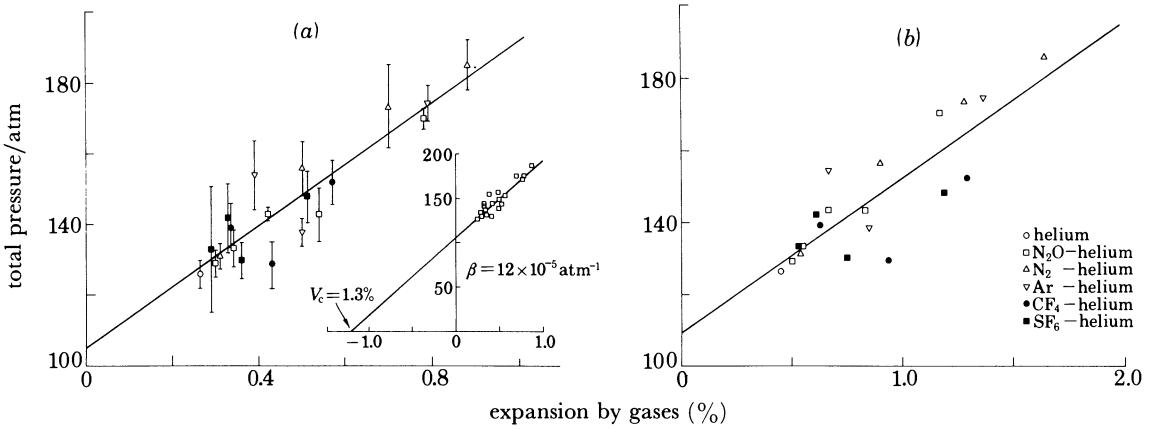

FIGURE 4. The data for non-tonic death from figure 2(*d*) are used to test the predictions of the critical volume hypothesis (1). The expansion caused by each gas mixture at its ED_{50} pressure for non-tonic death is calculated according to (2) (for details of the method, see Miller (1977)). The good fit (*r*, 0.95) obtained when carbon disulphide (*a*) (δ, 10.0) is used as model solvent is contrasted with the worst fit (*r*, 0.86) obtained when cyclohexane (*b*) (δ, 8.2) is used as model solvent. The inset to (*a*) shows the same data scaled to illustrate the extrapolation required to obtain the critical volume change for non-ionic death (ΔV_c in (1)).

TABLE 1. DERIVED PHYSICAL PARAMETERS OF VARIOUS SITES OF ACTION

physiological end point	estimated solubility parameter, $(\delta \pm$ s.d.) $(\text{cal ml}^{-1})^{\frac{1}{2}}$	nearest model solvent (δ)	critical volume change ($\Delta V_c \pm$ s.d.) (%)	compressibility $10^5(\beta \pm$ s.d.) atm^{-1}
complete spasms†	9.8±0.4	CS_2 (10.0)	−0.64±0.17	10.5±1.65
clonic convulsions	9.1±0.4	C_6H_6 (9.2)	−0.96±0.12	13.8±1.61
tonic convulsions	9.4±0.4	C_6H_6 (9.2)	−0.66±0.14	9.6±1.73
non-tonic death	9.8±0.4	CS_2 (10.0)	−1.27±0.11	12.0±0.92
anaesthesia†	10.8±0.1	Octanol (10.3)	+0.49±0.03	2.6±0.31

† From Miller *et al.* (1978).

range of solubility parameters between 9.1 and 9.8, all consistently less than that for the anaesthetic site.

Next, to derive the compressibility of the site of action, we have used a solvent model that matches as closely as possible the solubility parameter of the site of action in each case. The compressibility is then derived from plots like those in figure 4 and the relation in (1). Table 1 shows the four h.p.n.s. end points to have compressibilities in the range 10^{-4}–14×10^{-5} atm^{-1}, all clearly higher than that for the anaesthetic site of 2.6×10^{-5} atm^{-1}. The following pairs of h.p.n.s. end points seem to be statistically distinguishable: clonic convulsions and tonic convulsions ($p \leqslant 0.01$); complete spasms and clonic convulsions ($p < 0.01$).

The percentage critical volume change for all h.p.n.s. end points is negative, as demanded by the hypothesis. The magnitudes range from $-\frac{1}{2}$% to $-\frac{4}{3}$%. The absolute magnitudes of these changes are all larger than that responsible for anaesthesia.

The precision of the analysis is not so great as when it is applied to general anaesthesia. There are several reasons for this. First, the ED_{50} for an anaesthetic can be obtained at greater precision than for an h.p.n.s. end point. Secondly, and of greater importance, the pressure range over

which data may be obtained must be considered. For the pressure reversal of anaesthesia, data have been obtained from ambient pressure up to 100–150 atm. Figure 2 shows that for h.p.n.s. the data start at high pressure and cover a much smaller range of pressure. The inset in figure 4*a* illustrates the long extrapolation that is required to obtain ΔV_c as a consequence.

Discussion

Experimental findings

Our data make two fundamental points. First, helium pressure is not equivalent to hydrostatic pressure and secondly, all the inert gases protected against all four h.p.n.s. end points studied.

Helium pressure is not equivalent to hydrostatic pressure because helium has a weak anaesthetic effect that is related to its lipid solubility (Dodson *et al.* 1982). In tadpoles, the effects of hydrostatic pressure are postponed by 35 atm when compression is done with helium. In liquid-breathing mammals, the h.p.n.s. is also observed at lower pressures than in helium-breathing animals (Lundgren & Ornhagen 1976), but a better test would be to compare liquid-breathing mammals in the presence and absence of helium, to eliminate the physiological differences between liquid- and gas-breathing animals. Should the dream of liquid-breathing human divers ever become a reality, the absence of the anti-h.p.n.s. effect of helium would have to be considered. We can predict (1) that the four h.p.n.s. end points would occur at 20–30 atm higher pressures during helium compression than during hydrostatic compression.

The five inert gases all protected against the four phases of the h.p.n.s. studied. Figure 2 suggests that this protection is roughly proportional to the anaesthetic potency of each gas. In principle, any of these gases could be substituted for nitrogen as an additive to helium for deep diving. These findings are consistent with the more limited data of earlier workers reviewed in Brauer *et al.* (1982) and Halsey (1982). On the other hand, these inert gases protected against some aspects of the h.p.n.s. much better than against other aspects. As an example, the increase in symptom pressure per fraction of an anaesthetic partial pressure added to helium was some three times greater for tonic convulsions than for complete spasms or non-tonic death. This resulted in an inversion of the order of appearance of symptoms, such that at high anaesthetic partial pressures non-tonic death occurred before observation of tonic convulsions (figure 2). The relative insensitivity of the complete spasms phase is notable. This phase is the first we observed after the onset of tremors. Should tremors turn out to be particularly sensitive to addition of anaesthetic gases, then the possibility exists that the spasm phase might be encountered before the tremor phase of the h.p.n.s. This might be unfortunate in manned diving. However, the median onset pressures for these two end points are separated by about 35 atm in mice (see the results) so this possibility appears to be remote.

Theoretical predictions

The interaction between the partial pressure of helium and that of anaesthetic gases has now been examined for five physiological end points. The formalism of the critical volume hypothesis is consistent with the observed data in all cases, although the physical parameters of the sites for several of the end points are different. Are the parameters we have derived physically realistic?

The solubility parameter for all five sites fall in a narrow range, from 9.1 to 10.8. Typical

liquids range in values from about 6 for fluorinated hydrocarbons to 23 for water. Why do we observe such a narrow range? It is not possible to derive solubility parameters for such complex 'solvents' as biological membranes, lipid bilayers, or proteins. However, the available experimental data show that the solubilities of small gases in biomembranes are very insensitive to their composition (Miller *et al.* 1977), and this observation is consistent with our findings here.

The compressibility derived from our theoretical analysis (table 1) is essentially an adjustable parameter. It must fall, however, within physically realistic limits. Values for some common hydrocarbons range from 6×10^{-5} to 17×10^{-5} atm^{-1} (see, for example, Miller *et al.* 1978). There are little data for the compressibility of lipid membranes, but those available lie within this range (Liu & Kay 1977). So our parameters are physically reasonable.

These analyses suggest that the anaesthetic gases exert their effects at different sites of action. The anaesthetic site is clearly distinguishable from the h.p.n.s. sites by its small compressibility and high solubility parameter. The sites at which the gases oppose the different phases of the h.p.n.s. are partially resolvable. In particular, both tonic and clonic convulsions, and complete spasms and clonic convulsions are well resolved. In these same mice a more detailed pharmacological dissection was capable of distinguishing all these sites (Rowland-James *et al.* 1981). Other workers have made more extensive studies in a number of species, with similar conclusions (Brauer *et al.* 1974*a*; Brauer *et al.* 1975; Beaver *et al.* 1977). These dissections, which attempt to elucidate the mechanisms underlying the h.p.n.s., should be interpreted with caution, because one cannot distinguish between those actions that directly counter the generation of the h.p.n.s. at its source, and those that act indirectly on the pathways that are involved in the behavioural expressions of the stimuli emanating from the generating focus or foci.

Implications for diving practice

Our characterization of the physical properties of the action of these inert gases is illustrated in figure 5 to emphasize an important corollary of our approach: that the net effect of helium pressure need not be to compress hydrophobic media. Equations (1) and (2) suggest that if a medium has a low enough compressibility and a low enough solubility parameter (solubility, x, is inversely related to the solubility parameter, δ), helium might cause net expansion. How likely is this to happen in practice? In figure 5 we have indicated the dividing line between sites where expansion or compression would occur. This line is not well separated from our observed sites. So it seems probable that sites could occur where helium causes net expansion and where it will thus interact *additively* with other anaesthetic gases. In fact, additivity between helium and nitrogen has been reported in rats undergoing behavioural tests (Thomas 1975; Thomas & Burch 1975).

If there are important behavioural end points where helium has the net effect of an anaesthetic, the use of helium–inert gas mixtures (Trimix) in deep diving would be seriously undermined. While this remains just a possibility, the ultimate limitations of the Trimix tactic are now quite clear, and they stem quite naturally from the difference between the anaesthetic site and the h.p.n.s. sites. Had these sites had similar properties, both anaesthesia and h.p.n.s. could have been postponed indefinitely by adding the correct quantity of anaesthetic gas (Miller *et al.* 1972*a*). However, the higher compressibility of the h.p.n.s. sites compared to the anaesthetic site aborts this possibility. To illustrate the inherent limitation of the Trimix tactic

we have calculated the percentage of nitrogen that yields a median end point for anaesthesia and for three of the h.p.n.s. end points, as a function of pressure (figure 6). This scheme defines the gas mixtures that prevent these symptoms in mice. Mixtures to the left of each labelled line would be therapeutic. This clearly demonstrates that one cannot avoid all end points at the highest pressure, although one has some control over the form of the incapacitation!

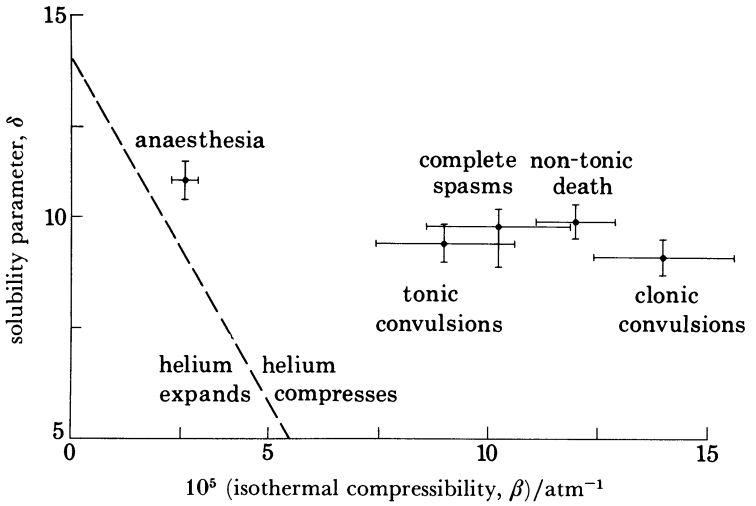

FIGURE 5. The best estimate of the solubility parameter (δ) and of the isothermal compressibility (β) are represented for the four h.p.n.s. end points and for anaesthesia in male CD-1 mice. No correlation between the two parameters is intended. The dashed line delineates the physical properties of solvents for which $\Delta V_{He} = \beta P_{He}$ at 100 atm (i.e. there is no net volume change when exposed to this partial pressure of helium). Note that solubility increases as δ decreases.

Figure 6 shows that significant improvement in attainable pressure may be obtained by adding nitrogen to helium (any other anaesthetic gas would exhibit the same form, but the percentage axis would vary and the line for anaesthesia would shift to the right or left in proportion to the added anaesthetic potency of the gas). Thus, as pressure increases above 83 atm, complete spasms can be avoided by the addition of more and more nitrogen, but at about 160 atm, the percentage of nitrogen required to further postpone complete spasms would cause anaesthesia. Indeed, the spectre of convulsing anaesthetized mice has been observed by two groups of workers (Miller 1977; Beaver *et al.* 1977). Thus, under given circumstances one cannot expect to indefinitely extend the depth limit attained with helium by adding an anaesthetic gas. The degree of obtainable protection will vary with the physical properties of the site involved. For example, very good protection may be obtained from tonic convulsions.

Thus, successful application of the Trimix tactic involves defining those h.p.n.s. end points that one wishes to avoid and characterizing their sensitivity to anaesthetics systematically, while being alert to the fact that this sensitivity may vary dramatically and need not *a priori* be antagonistic.

Our analysis in figure 6 is for CD-1 mice 'diving' at a compression rate of 1 atm/min. The lines accurately represent these data, but other species might behave differently, and slower compressions would cause some shift of the h.p.n.s. lines to the right, but not of the line for anaesthesia. Thus, in no sense does the figure quantitatively represent the situation for man. However, a similar analysis may well be applicable if only systematic data were available.

Examination of the literature does allow some semi-quantitative data to be obtained for the incidence of narcosis in helium–nitrogen mixtures at pressures up to 30 atm (Proctor *et al.* 1976; Bennett *et al.* 1974). Additionally, some data of Smolin, Vigreux, and Zaltsman are not readily available but have been reviewed (Hunter & Bennett 1974). The use of these data to calculate the expansion caused by the helium with nitrogen, as a function of total pressure, yields the estimate that for nitrogen narcosis, the compressibility is 2×10^{-5}–4×10^{-5} atm^{-1} and the critical volume change is 0.04–0.08% when carbon disulphide is arbitrarily chosen as the model solvent. For anaesthesia, in our mice, with the same solvent, the compressibility is 2.1×10^{-5} atm^{-1} and the critical volume is +0.63%. These observations of pressure reversal of nitrogen narcosis in man suggest that nitrogen narcosis is mediated by a site with a compressibility similar to that for anaesthesia in mice, but is experienced at critical volumes some ten times lower. The behaviour of such a site is sketched on figure 6. At depths deeper than 250 m, this limit flattens out at about 15%. To avoid narcosis, lower percentages of nitrogen should be used. This semi-empirical prediction seems to be consistent with practice (Bennett *et al.* 1974).

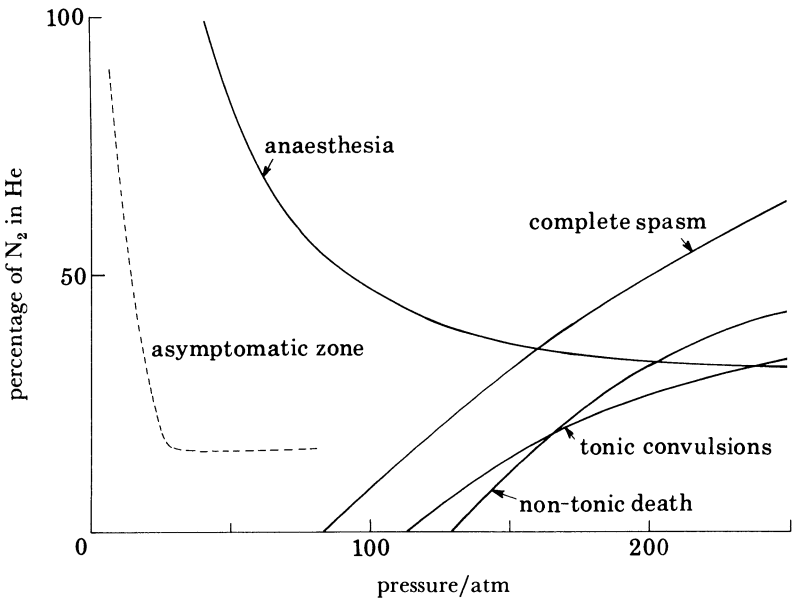

FIGURE 6. The composition–pressure diagram for nitrogen–helium mixtures showing the calculated median conditions under which anaesthesia, tonic convulsions, and non-tonic death occur in 50% of mice when compressed at 1 atm/min. The broken line suggests the mixtures for which nitrogen narcosis would be encountered in men (see discussion).

Overall, our studies show that a remarkably simple semi-empirical null model provides a very useful guide to the application of the Trimix tactic without in any way obscuring the complexity of the h.p.n.s.

The potential uses of hydrogen

As pointed out in the introduction, the use of hydrogen–oxygen instead of helium–oxygen for deep diving would have several practical advantages. Furthermore, in a number of human dives done with hydrogen–oxygen mixtures (for a review, see Fife (1979)), neither inert gas

narcosis nor h.p.n.s. has been reported at the depths so far achieved (about 160 m). The reactivity of hydrogen–oxygen mixtures has been overcome by maintaining the oxygen content below the explosive limit of 4%. Here we examine the small animal data, place them in the context of the critical volume hypothesis, and provide some predictions for mixed gas diving with hydrogen.

Table 2. Effects of high pressures of hydrogen

species	pressure needed for anaesthesia/atm	effects of pressure	reference
mice CD-1	130	weak h.p.n.s.	Brauer *et al.* (1970)
mice	180†	—	Kent *et al.* (1976)
newts	over 204	none	Miller *et al.* (1973)
tadpoles	200	none	Dodson *et al.* (1982)

† Extrapolated from N_2O–H_2 mixtures up to 100 atm.

The anaesthetic potency of hydrogen is weak, but a wide variation has been reported. The data for small animals are summarized in table 2. The values reported range from 130 atm to greater than 200 atm. Compared with other gases, this variability is large. However, looked at in the context of (1) this variability is not puzzling. Equations (1) and (2) show that the higher the anaesthetic partial pressure, the larger the two opposing effects of compression and expansion. Thus the critical volume, ΔV_c, becomes a small difference between two large numbers at high pressure, and small interspecies or interstrain differences in the inert gas–lipid partition coefficient or compressibility, or both, can cause comparatively large fluctuations in the predicted anaesthetic potency.

Much less data are available for the occurrence of the h.p.n.s. in small mammals compressed on hydrogen–oxygen. In one study, tremors followed by convulsions in 10% of the mice, were reported at pressures up to 130 atm (Brauer & Way 1970). In a later study, hydrogen elevated h.p.n.s. thresholds with a potency about one third that of nitrogen when added to helium (Brauer *et al.* 1968; 1974*b*). Studies in primates have also been reported (Brauer *et al.* 1968, 1972; Rostain 1980). Substituting hydrogen for helium produces an effect at very high pressures (above 100 atm), similar to those for certain mixtures of helium and nitrogen; so that both anaesthesia and the h.p.n.s. may be observed simultaneously (figure 6).

Like the other gases we have studied, the anti-h.p.n.s. potency of hydrogen is proportional to its anaesthetic potency, and so it fits into the framework that we have developed. If one were to choose a single gas for deep diving, rather than a mixture, this would be the gas of choice. (We have not examined neon here but its predicted behaviour is closer to that of helium than hydrogen.)

Theoretical calculations of the behaviour of hydrogen are subject to large errors in the predicted pressures for anaesthesia and the h.p.n.s. because the critical volume is achieved as the balance of two large, opposing effects, as discussed above. Within these limits, however, the behaviour of a number of gases and mixtures have been characterized in figure 7, in which the net volume change as a percentage of the critical volumes for anaesthesia and complete spasms have been calculated as a function of pressure. The results are in general agreement with the small animal data summarized above. The relative potency for causing h.p.n.s. is, in descending order, hydrostatic pressure, helium, neon (not shown for clarity), hydrogen. The model also shows that the addition of two and a half times (in percentage terms) more hydrogen

than nitrogen to helium produces equivalent mixtures for the postponement of complete spasms. So, one would predict that, since 5–10% (by volume) nitrogen in helium is a useful mixture for the deepest human dive, 20% (by volume) hydrogen in helium would also be beneficial. Much larger percentages would be acceptable at lesser depths. The advantage of hydrogen–helium mixtures would be a probable further reduction in flammability relative to undiluted hydrogen (Karpov & Severin 1978) and a reduction in respiratory load relative to helium–nitrogen mixtures. We emphasize, finally, that the anti-h.p.n.s. efficacy of hydrogen should be no different from that of nitrogen; it is simply less potent, thereby allowing a greater dilution of helium. Thus the ultimate attainable depth will not be altered unless respiratory load proves limiting in practice.

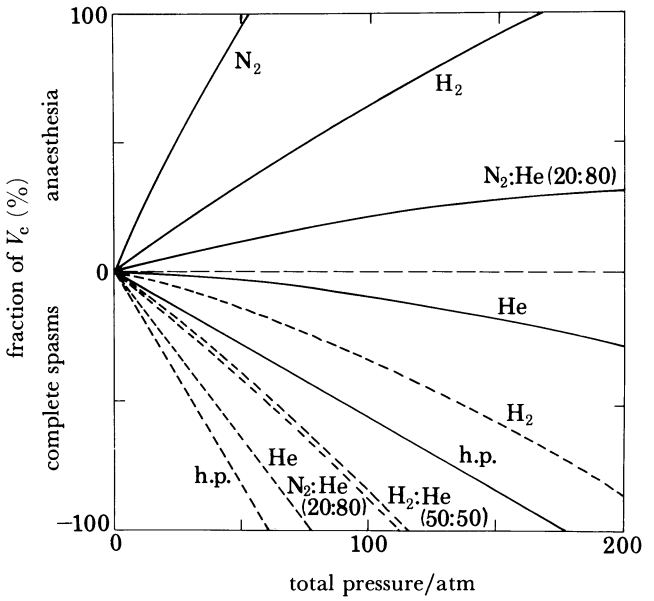

FIGURE 7. The ability of some gases and their mixtures to cause either general anaesthesia (solid lines) or complete spasms (broken lines) has been calculated from (1) and (2) with the corrections described previously (Miller *et al.* 1973; Miller 1977). The expansion (1) has been normalized to a percentage of the respective critical volumes. Parameters for the calculations have been given (Miller *et al.* 1978). Abbreviation: h.p., high pressure.

PERSPECTIVES

In 1970, at the Third International Conference on Hyperbaric and Underwater Physiology, impetus to the use of gas mixtures in deep diving was given by three papers. The first showed that the addition of anaesthetic gases to helium postponed onset of the h.p.n.s. (Brauer *et al.* 1972); the second, that helium pressure reversed the anaesthetic effect of those anaesthetic gases that postponed the h.p.n.s., suggesting a useful trade-off (Miller *et al.* 1972); and the third described the first manned dive to greater than 450 m (Bennett 1972). Since then the use of helium–nitrogen mixtures has been explored both in men and mammals. Our work shows the balance between anaesthesia and four severe phases of the h.p.n.s. and includes systematic data at much higher pressures than previously defined. The increase in median pressure that can be obtained without onset of anaesthesia depends on the phase of the h.p.n.s.; for example, it is very much greater for tonic convulsions than for complete spasms. Therefore, in man,

systematic testing of all relevant performance parameters should be made and optimum trade-offs sought.

An alternative approach under development is to use more selective drugs. This approach may well be beneficial, but again differential action should be expected. In our studies only anaesthetics protected against all phases of the h.p.n.s.; more selective drugs did not (Rowland-James *et al.* 1981). In one case some phases of the h.p.n.s. were ameliorated but others were exacerbated.

Dramatic improvements in the ability to combat the h.p.n.s. are only likely to come from a better understanding of the underlying processes. Even if it proves difficult to extend operational pressures, the removal of the need for slow compression would be a great advantage. In this context, recent studies of Weddell seals fitted with depth recorders showed these mammals reaching depths of up to 500 m at rates of about 100 m/min (Hill *et al.* 1983). Whether the mechanism that permits this involves adaptation in excitable membranes (Janoff & Miller 1982), elevated levels of endogenous compounds such as the β-carbolines, elevated partial pressures of nitrogen in cerebral blood flow, or other undetermined factors remains unknown, but the observation itself poses a clear challenge.

References

Beaver, R. W., Brauer, R. W. & Lasher, S. 1977 Interaction of central nervous system effects of high pressures with barbiturates. *J. appl. Physiol.* **43**, 221–229.

Bennett, P. B. 1972 Some physiological measurements during human saturation diving to 1500 ft. In *Troisième Journées Internat. d'Hyperbarie et de Physiologie Subaquatique*, pp. 35–43. Paris: Doin.

Bennett, P. B., Blenkarn, G. D., Roby, J. & Youngblood, D. 1974 Suppression of the high pressure nervous syndrome in human deep dives by He–N_2–O_2 mixtures. *Undersea biomed. Res.* **1**, 221–250.

Brauer, R. W., Beaver, R. W., Mansfield, W. M., O'Connor, F. & White, L. W. 1975 Rate factors in the development of the high pressure neurological syndrome. *J. appl. Physiol.* **38**, 220–227.

Brauer, R. W., Beaver, R. W., Hogue, C. D., III, Ford, B., Goldman, S. M. & Venters, R. T. 1974*a* Intra- and interspecies variability of vertebrate high pressure neurological syndrome. *J. appl. Physiol.* **37**, 844–851.

Brauer, R. W., Goldman, S. M., Beaver, R. W. & Sheehan, M. E. 1974*b* N_2, H_2 and N_2O antagonism of high pressure neurological syndrome in mice. *Undersea biomed. Res.* **1**, 59–72.

Brauer, R. W., Hogan, P. M., Hugon, M., MacDonald, A. G. & Miller, K. W. 1982 Patterns of interaction of effects of light metabolically inert gases with those of hydrostatic pressure as such – a review. *Undersea biomed. Res.* **9**, 353–396.

Brauer, R. W., Jordan, M. R. & Way, R. O. 1972 The high pressure neurological syndrome in the squirrel monkey. In *Troisième Journées Internat. d'Hyperbarie et de Physiologie Subaquatique*, pp. 23–30. Paris: Doin.

Brauer, R. W. & Way, R. O. 1970 Relative narcotic potencies of hydrogen, helium, nitrogen and their mixtures. *J. appl. Physiol.* **29**, 23–31.

Brauer, R. W., Way, R. O. & Perry, R. A. 1968 Narcotic effect of helium and hydrogen in mice and hyperexcitability phenomena at simulated depths of 1500 to 4000 ft of sea water. In *Toxicity of anaesthetics* (ed. B. R. Fink), pp. 241–255. Baltimore: Williams & Wilkins.

Dodson, B. A., Furmaniuk, Z. W., Jr & Miller, K. W. 1982 The *in vivo* effects of different pressurizing agents. *Undersea biomed. Res.* **9**, 18 (supplement).

Fife, W. P. 1979 The use of non-explosive mixtures of hydrogen and oxygen for diving. *U.S. Govt Res. Rep. Index* **79**(18), 76.

Furmaniuk, Z. W., Jr, Dodson, B. A. & Miller, K. W. 1982 Is helium pressure equivalent to hydrostatic pressure: An *in vivo* test of the critical volume hypothesis. *Fedn Proc. Fedn Am. Socs exp. Biol.* **41**, 1620.

Halsey, M. J. 1982 Effects of high pressure on the central nervous system. *Physiol. Rev.* **62**, 1341–1377.

Hildebrand, J. H., Sayers, R. R. & Yant, W. P. 1928 Helium in deep diving and caisson working. *Nature, Lond.* pp. 3–5.

Hildebrand, J. H., Prausnitz, J. M. & Scott, R. L. 1970 *Regular and related solutions.* New York: Van Nostrand Reinhold.

Hill, R. D., Schneider, D. R., Leggins, G. C., Hochachka, P. V., Schuette, A. H. & Zapol, W. M. 1983 Microprocessor controlled recording of bradycardia during free diving of the Antarctic Weddell seal. *Fedn Proc. Fedn Am. Socs exp. Biol.* **42**, 470.

Hunter, W. L. & Bennett, P. B. 1974 The causes, mechanisms and prevention of the high pressure nervous syndrome. *Undersea biomed. Res.* **1**, 1–28.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Janoff, A. S. & Miller, K. W. 1982 A critical assessment of the lipid theories of general anaesthetic action. In *Biological Membranes* (ed. D. Chapman), vol. 4, pp. 417–476. London: Academic Press.
Karpov, V. P. & Severin, E. S. 1978 Turbulent burning of nearly saturated hydrogen mixtures. *Dokl. Akad. Nauk SSSR. Phys. Chem.* **239**, 123–125.
Lever, M. J., Miller, K. W., Paton, W. D. M. & Smith, E. B. 1971 Pressure reversal of anaesthesia. *Nature, Lond.* **231**, 368–371.
Liu, N.-I. & Kay, R. L. 1977 Redetermination of the pressure dependence of the lipid bilayer phase transition. *Biochemistry, Philad.* **16**, 3484–3486.
Lundgren, C. E. G. & Ornhagen, H. Ch. 1976 Hydrostatic pressure tolerance in liquid-breathing mice. In *Underwater physiology*, part 5 (ed. C. J. Lambertsen), pp. 397–404. Bethesda: FASEB.
Miller, K. W. 1974 Inert gas narcosis, the high pressure neurological syndrome and the critical volume hypothesis. *Science, N.Y.* **185**, 867–869.
Miller, K. W. 1977 The opposing physiological effects of high pressures and inert gases. *Fedn Proc. Fedn Am. Socs exp. Biol.* **36**, 1663–1667.
Miller, K. W., Hammond, L. & Porter, E. G. 1977 The solubility of hydrocarbon gases in lipid bilayers. *Phys. Chem. lipids* **20**, 229–241.
Miller, K. W., Paton, W. D. M. & Smith, E. B. 1972*a* Experiments on animals at ultra-high pressure. In *Troisième Journées Internat. d'Hyperbarie et de Physiologie Subaquatique*, pp. 31–34. Paris: Doin.
Miller, K. W., Paton, W. D. M., Smith, R. A. & Smith, E. B. 1972*b* Physicochemical approaches to the mode of action of general anaesthetics. *Anesthesiology* **36**, 339–351.
Miller, K. W., Paton, W. D. M., Smith, R. A. & Smith, E. B. 1973 The pressure reversal of anaesthesia and the critical volume hypothesis. *Molec. Pharmac.* **9**, 131–143.
Miller, K. W. & Wilson, M. W. 1978 The pressure reversal of a variety of anaesthetic agents in mice. *Anesthesiology* **48**, 104–110.
Miller, K. W., Wilson, M. W. & Smith, R. A. 1978 Pressure resolves two sites of action of inert gases. *Molec. Pharmac.* **14**, 950–959.
Proctor, L. D., Carey, C. R., Lee, R. M., Schaefer, K. E. & van den Ende, H. 1976 Electroencephalographic changes during saturation excursion dives to a simulated sea water depth of 1,000 ft. *Aerospace Med.* **48**, 621–628.
Rostain, J.-C. 1980 Le syndrome nerveux des hautes pressions chez l'homme et le singe *papio papio*. Thesis, Université d'Aix-Marseille, pp. 86–93.
Rowland-James, P., Wilson, M. & Miller, K. W. 1981 Pharmacological evidence for multiple sites of action of pressure in mice. *Undersea biomed. Res.* **8**, 1–11.
Thomas, J. E. 1975 Reversal of nitrogen narcosis in rats by helium pressure. *Undersea biomed. Res.* **3**, 249–260.
Thomas, J. R. & Burch, L. S. 1975 Inert gas narcosis: avoidance behaviour in rats breathing elevated pressure of nitrogen or helium. *Physiol. Psychol.* **3**, 411–416.
Waud, D. R. 1972 On biological assays involving quantal responses. *J. Pharmac. exp. Ther.* **183**, 577–607.

Discussion

T. R. Hennessy (*A.M.T.E. Physiological Laboratory, Gosport, U.K.*). The remarkable diving performance of the Weddell Seal (500 m on a 5 min dive) may provide an opportunity to distinguish between the effects of pressure *per se* and dissolved inert gas, provided, of course, that the partial pressure of nitrogen on the seal's brain does not reach the high levels suggested by Dr Miller. A rough estimate of the nitrogen tension may be made by assuming that the brain can be modelled as a single linear compartment with a half-time for gas uptake of say 5 min. On a 5 min parabolic compression profile passing through 500 m (this allows a full minute between 480 and 500 m), this simple model predicts a peak nitrogen tension of 1.5 MPa (compared to the peak arterial tension of 41 MPa). This value will be even lower if the lung contents are completely absorbed or partially distributed, or both, in tissues other than the brain.

K. W. Miller. Little is known about the respiratory and circulatory dynamics of free diving seals, but certainly the possibility that nitrogen might play the role of an 'endogenous' anti-h.p.n.s. agent has not escaped attention. In support of this idea some recent measurements in dolphins have demonstrated elevated partial pressures of nitrogen in muscle tissue following shallow dives (Ridgway & Howard 1979). Since only a few atmospheres partial pressure of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

nitrogen in the seal's central nervous system would have a considerable protective effect, the use of this mechanism seems quite probable. However, the upper limit of 1.5 MPa that Dr Hennessy calculates would cause severe narcosis if it were attained; this suggests that other mechanisms do limit nitrogen uptake in the seal as they do in the dolphin.

Reference

Ridgway, S. H. & Howard, R. 1979 Dolphin lung collapse and intramuscular circulation during free diving: evidence from nitrogen washout. *Science, N.Y.* **206**, 1182–1183.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES