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The Biological Effects of High Pressures: Underlying Principles [and Discussion]

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The biological effects of high pressures: underlying principles

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Physico-chemical principles set constraints on the response of biological systems to high-pressure gases and to pressure *per se*. They also indicate the mechanisms that may be involved. Classical thermodynamics, intermolecular forces and the theory of solutions and many other areas of physical chemistry have contributed to our understanding of the problems faced by divers breathing gases at high pressure, in particular the high pressure neurological syndrome, inert gas narcosis and decompression sickness. The value and the limitations of physico-chemical arguments when applied to the problems of underwater physiology are analysed.

Introduction

Underwater physiology is a subject in which physical principles have guided research more effectively than is usual in biology.

When divers go deep they are exposed to gases at high pressure which may cause adverse effects in two ways. First, pressure by itself may perturb the many physical and chemical processes on which their health depends. The symptoms caused by pressure alone are usually referred to as the high pressure neurological syndrome (h.p.n.s.). Secondly, and more seriously for the *average* diver, the high partial pressure of the gases breathed leads to increased concentrations of dissolved gases within the body. These simple molecules have surprising pharmacological properties that cause inert gas narcosis (which is simply a manifestation of general anaesthesia). Inert gas narcosis sets severe limits on compressed air diving.

The very simplicity of the molecules involved and their lack of common structural features suggest that quite general mechanisms may be involved. Since the intermolecular forces of such molecules are relatively well characterized, inert gas narcosis provides a valuable opportunity to apply physical principles to elucidate pharmacological activity (Miller & Smith 1974).

H.p.n.s. and inert gas narcosis cannot be treated entirely independently because, most remarkably, pressure and anaesthetic gases are mutually antagonistic. General anaesthesia can be reversed by the application of high pressures and the effects of h.p.n.s. ameliorated by anaesthetics (Johnson & Flagler 1951; Lever *et al.* 1971; Miller *et al.* 1973). (In mammals the effects of pressure can be investigated independently of narcosis because helium is found not to lead to narcosis for reasons that will be discussed later (Miller *et al.* 1967).)

A further consequence of the high concentration of gases dissolved in the body is decompression sickness. This, of course, does not directly limit the depths that divers can reach but, by limiting the rate at which they can return to the surface, places practical constraints on diving practice.

In each of these areas the basic principles of physical chemistry have suggested theoretical models and have provided constraints that have been used to eliminate the many theories that have been proposed that are inconsistent with these principles. The value of such reasoning will be illustrated by the examination of specific models.

Physical principles

The thermodynamic principles that have been invoked in the study of high pressure physiology involve, either directly or indirectly, the behaviour of the chemical potential μ_i. The basic equation for the chemical potential of substance i in a mixture is

$$\mu_i = \mu_i^{\ominus} + RT \ln x_i, \tag{1}$$

where x is the mole fraction. In a binary mixture we usually refer to the component that predominates as the solvent. For mixtures in which the components are very similar and for the solvents in most dilute solutions we find that the Raoult law is followed $p_i = p_i^0 x_i$, where p_i^0 is the vapour pressure of pure solvent. So $\mu_i^{\ominus}$ can be identified as the chemical potential of pure component i. For the *solute* in most dilute solutions $p_i = p_i^* x_i$, where p_i^* is an arbitrary constant (as in this case is $\mu^{\ominus}$). This solute is then said to obey the Henry law. This difference in behaviour arises because in dilute solution the solvent molecules are surrounded, for the most part by similar (i.e. other solvent) molecules, a situation similar to the pure liquid. However, the solute molecules are surrounded mainly by molecules of the other species (solvent molecules); a limiting situation that corresponds to neither of the pure components, but depends on the intermolecular forces between the two different species.

The chemical potential of a component in solution may be modified by changes in temperature and pressure,

$$(\partial \mu_i / \partial T)_P = -S_i \quad \text{and} \quad (\partial \mu_i / \partial P)_T = V_i,$$

where S_i and V_i are the partial molar entropy and volume. If we consider a physical or chemical process, the application of the above equations leads to expressions that relate the change of the equilibrium constant (K) to changes in temperature and pressure by

$$\mathrm{d}(\ln K)/\mathrm{d}T = \Delta H / RT^2 \tag{2}$$

and

$$\mathrm{d}(\ln K)/\mathrm{d}P = \Delta V / RT, \tag{3}$$

where ΔH and ΔV are the enthalpy change and volume change accompanying the process.

These equations are of considerable value in the interpretation of physiological data and can often be of value in guiding us to the molecular interactions involved. However, it is important to note that they are merely a way of transforming our original data. The application of (3) to almost any set of properties that characterize an equilibrium will lead to a value of the volume change ΔV. It is only if another source of information about the magnitude of ΔV exists that a theory can be developed. Professor Max McGlashan (1966) in his published inaugural lecture asked the rhetorical question, 'What then is a thermodynamic theory?'; the answer, 'there is no such thing'. This important truth is one that is easily forgotten when analysing the data that arise in underwater physiology.

Theories of inert gas narcosis (general anaesthesia)

There are scientific as well as historical reasons for treating this subject first. Much effort has been expended in trying to identify the physical character of the site at which anaesthetics act. From the end of the nineteenth century, scientists have attempted to interpret anaesthetic

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potency in relation to the molecular properties and solubility of the agents involved. Since then, a large number of theories of this type have been advanced. Recently, Franks & Lieb (1982) and Janoff & Miller (1982) have re-opened the discussion of the mechanisms that might be involved.

(i) *The Ferguson principle* (*Ferguson* 1939)

The most general of these approaches is that of Ferguson, who sought to correlate anaesthetic potency with thermodynamic activity, a, defined on the basis of a Raoult law standard state ($a = P/P^0$, where P is the partial pressure and P^0 is the saturated vapour pressure of the anaesthetic substance at the appropriate temperature). For most common anaesthetics the activity at an anaesthetic dose was found to be approximately 2×10^{-2}. However, when, in order to provide a wider critical test, molecules with anomalous molecular forces were investigated, in particular compounds containing fluorine, the activities were found to differ by as much as a factor of ten (Miller *et al.* 1967). The failure of the Ferguson principle may be regarded as fortunate. The thermodynamic activity defined in this way depends only on the properties of the pure anaesthetic substance and not on any interactions with other molecules. If the principle were adhered to it would not be possible to deduce anything of the character of the site of action of anaesthetic molecules from their relative potencies.

(ii) *Aqueous phase models*

In 1961 Pauling (1961) and Miller (1961) independently proposed that the site of action of general anaesthetics lies in the aqueous phases of the central nervous system. Both theories, though differing in detail, sought to relate potency to the stability of gas hydrates that many but not all anaesthetics may form in aqueous solution. Tests with fluorine compounds, which did not fit the correlation (figure 1), the fact that many anaesthetics including ether and halothane do not form hydrates, and the observed linearity of potency in mixtures all suggested that the model was not well supported by physico-chemical evidence (Miller *et al.* 1972; Eger *et al.* 1969). This conclusion has considerably narrowed the search for a molecular mechanism of anaesthetic action.

(iii) *Meyer–Overton theory*

The oldest theory of anaesthetic action, with its roots in the 19th century, sought to relate potency to solubility in fatty substances. The model was described by Meyer (1937). 'Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell. This concentration depends on the nature of the animal or cell but is independent of the narcotic.' So anaesthetic potency should be directly proportional to solubility in a solvent selected to match the properties of the site of action. This model has been tested in many solvents, olive oil and octanol being most favoured. The site of action has been characterized more systematically by the use of solubility parameter (δ) theory, which relates solubility to the strength of intermolecular forces and is defined for fluids by $\delta = (-E/V)^{\frac{1}{2}}$, where E is the energy of vaporization of the solvent and V its molar volume (Hildebrand & Scott 1962). This indicated that the value of δ consistent with the anaesthetic data was 9 ± 1 (cal cm^{-3})$^{\frac{1}{2}}$†. It was again noted that only the fluorine compounds investigated provided

† One thermochemical calorie = 4.184 J.

sufficient discrimination to characterize the site (Miller *et al.* 1967). The lipid solubility model enables anaesthetic potency to be predicted to within $\pm 20\%$ over a range of potency of factor 10,000. It has been called one of the most remarkable correlations in science (figure 2).

The model implies that the number density of molecules at the site is the crucial factor in anaesthesia and it would be a natural extension of the theory to propose that pressure reverses

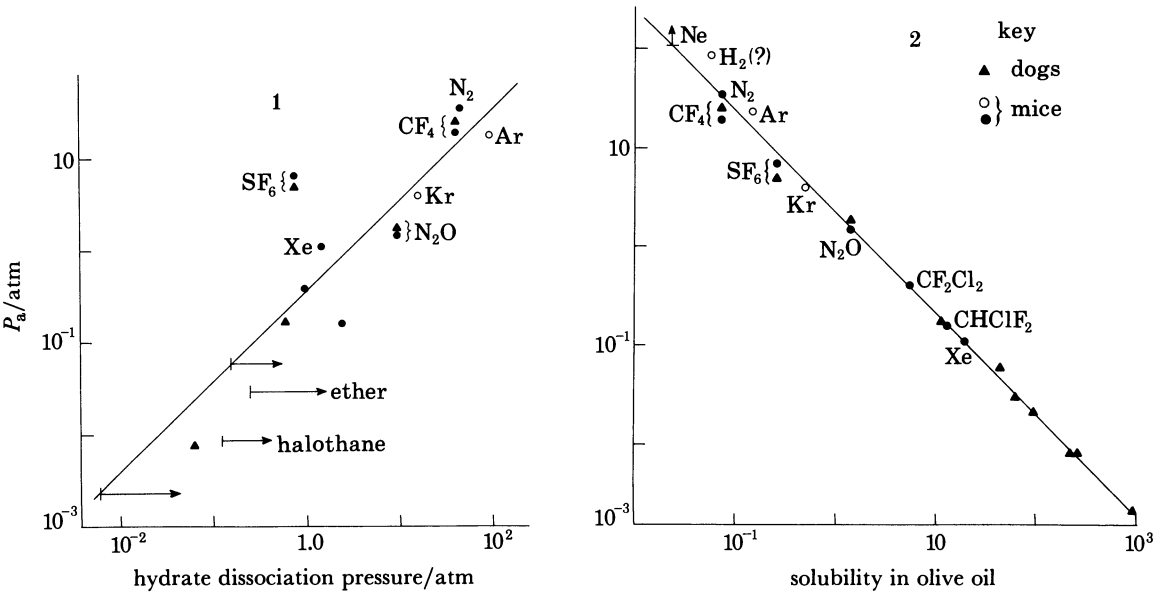

FIGURE 1. Correlation of anaesthetic presssures with hydrate dissociation pressures at 0 °C. The lines indicate anaesthetics that do not form hydrates at their saturated vapour pressures.

FIGURE 2. Correlation of anaesthetic pressures with solubility in olive oil.

anaesthesia by 'squeezing out' the dissolved molecules. This will occur and the extent of the change in the concentration of molecules at the anaesthetic site C_a will be given by

$$\mathrm{d}(\ln C_a)/\mathrm{d}P = V_a/RT, \tag{4}$$

where V_a is the partial molar volume of the anaesthetic at the site. For hydrophobic materials $V_a = 50$–100 cm^3 mol^{-1}. These values, substituted in (4), predict that pressures of approximately 300 atm would be required to significantly reduce the anaesthetic effects of a simple gas. The equation, when modified to account for the fact that the *difference* in partial molar volumes is the relevant parameter for anaesthetics administered in aqueous solution, predicts that under these circumstances pressures of the order of 1000 atm could be required. These values are high in contrast with the experimental value of approximately 100 atm and the 'squeezing out' effect is usually regarded as an inadequate explanation of pressure reversal (Miller *et al.* 1973).

(iv) *Colligative models*

A more recent example of general principles guiding us is what I will call the colligative model, expounded most completely by Hill (1974). This theory supposes that anaesthetics act by lowering the chemical potential of the site material in which it dissolves; they could be said to be acting by 'diluting' the site material. This model makes predictions in keeping with the

Meyer–Overton theory (see below), but it makes a strong additional prediction on the effect of pressure on anaesthetic. If μ_s is the chemical potential of the site material it will be lowered by the presence of anaesthetic molecules at a mole fraction x_a and raised by the application of pressure ΔP.

$$\Delta\mu_s = RT \ln x_s + V_s \Delta P, \tag{5}$$

where x_s is the mole fraction of the site material and V_s its molar volume. Equation (5) can be simplified in dilute solutions for which $x_a \ll x_s$ to give

$$\Delta\mu_s = RT x_a + V_s \Delta P.$$

If the pressure applied is such as to neutralize the effects of anaesthetic then $\Delta\mu_s = 0$ and

$$\Delta P = RT x_a / V_s \approx RT C_a,$$

where C_a is expressed in moles per unit volume. The hydrophobic theories of anaesthesia (Johnson *et al.* 1963) indicate a value of 0.05 M for C_a, which leads to a value of ΔP (the pressure required to reverse its effects) of approximately 1 atm. This is in considerable disagreement with the experimental value of approximately 100 atm. This result can direct our search for a stronger model. I believe it does not support the view that the general perturbations of a hydrophobic region, such as the cell membrane, are associated with general anaesthesia. The changes in membrane fluidity induced by clinical doses of anaesthetics could be achieved by temperature changes of a fraction of a degree or pressure changes of, at most, a few atmospheres (Lieb *et al.* 1982; Pang *et al.* 1980). The model suggests that phase changes in membranes perturbed by clinical doses of anaesthetics could require somewhat larger changes in temperature and pressure to reverse the effects. But the calculations suggest that they will still be too small to provide the basis for a theory of general anaesthesia (Mountcastle *et al.* 1978).

(v) *Site-interaction model*

Franks & Leib (1982) suggest that anaesthetic potencies both at normal and elevated pressures are consistent with a model that supposes that anaesthetic molecules (A) bind at a site (S) and inactivate it,

$$S + A + SA$$

$$K = [SA]/[S][A].$$

Anaesthetic activity is related to the fraction of sites bound. Application of (3) to the model indicated that a single volume parameter $\Delta V = 190$ cm^3 could be obtained from the data for newts and one of $\Delta V = 65$ cm^3 for mice. In each case the volume change on binding appeared independent of the molecular properties of the anaesthetic despite the fact that the partial molar volumes in hydrophobic solvents of the gases employed varied from 45 cm^3 mol^{-1} for argon to approximately 100 cm^3 mol^{-1} for SF_6.

(vi) *Expansion models*

Mullins (1954) was the first to suggest that the molar volume of a substance could play a part in determining its anaesthetic potency. He believed that anaesthetics could act by occluding free space in membranes and suggested that this effect might be *potentiated* by pressure. More recent theories, however, suggest that it is the expansion caused by the anaesthetic at

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its site of action that is the important factor (Miller *et al.* 1973). When this exceeds a certain critical volume, anaesthesia will occur. Like the Meyer–Overton theory, it supposes that this critical volume will be independent of the anaesthetic substance but will be different for different animals. We can express this

$$P_{a50}\, V_a S_a = K, \tag{6}$$

where P_{a50} is the partial pressure required to induce anaesthesia (at normal pressures), V_a is the partial molar volume of the anaesthetic and S_a its solubility coefficient. K is a constant.

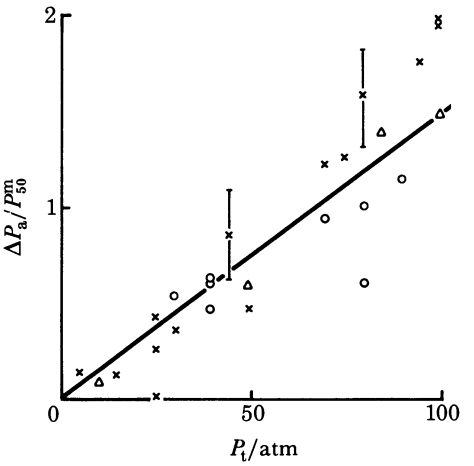

FIGURE 3. ΔP_a is the additional dose of anaesthetics above a reference dose. P_t is the total pressure required to restore the activity to the level accompanying the reference dose. The data is for the effects of the gases nitrogen, argon, nitrous oxide and sulphur hexafluoride on mice (△), newts (○) and luminous bacteria (×). P_{50}^{pm} is the pressure required to induce anaesthesia in 50% of the mice.

Pressure reversal is explained by the effect of pressure compressing the site of action. Thus†

$$\Delta P_a\, V_a S_a = \beta P_t, \tag{7}$$

where P_t is the total pressure required to restore the level of activity to that prevailing before the change of the partial pressure of the anaesthetic by ΔP_a (Miller *et al.* 1973). The term β is the coefficient of isothermal compressibility of the site. If we assume that the properties of the hydrophobic site can be represented by a model solvent (benzene, olive oil and octanol have been proposed), then for each anaesthetic V_a and S_a can be determined. If we further assume that the compressibility and the solvent properties of the site are the same in all species, then $\Delta P_a V_a S_a$ should be a linear function of P_t with slope β, for all species (S. M. King & E. B. Smith, unpublished results).

The equation can be applied by determining V_a and S_a by identifying a suitable solvent. Alternatively, we can apply (6) in the form

$$V_a S_a = K^\alpha / P_{a50}^\alpha,$$

where α refers to any selected species. Then by substitution in (7)

$$\Delta P_a / P_{a50}^\alpha = (\beta / K^\alpha)\, P_t.$$

Again ΔP_a, P_t data from a variety of species are related by a linear dependence (figure 3). The

† A number of smaller terms arising from the solubility of helium, the pressurizing gas, gas imperfection etc. are omitted here.

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choice of the reference species α is not material. It should be noted that this formulation differs from earlier expositions of the model in that the site characteristics, in particular the compressibility, are assumed to be the same in all species. The various species differ in that different P_{50} are observed reflecting, in this version of the model, a different degree of expansion at the critical site. So to predict the potency of an anaesthetic, we require a knowledge of its solubility and partial molar volume in the reference fluid and a knowledge of the anaesthetic potency of *one* substance for the species under investigation. Prediction of the effects of pressure on anaesthetic potency requires in, addition, β, the isothermal compressibility. A test of this minimum hypothesis is given in figure 2 where data for newts, mice and luminous bacteria are included. The agreement is impressive, considering the difficulties involved in the measurements. This represents a highly predictive version of the hydrophobic theories.

It should be noted that the effects of temperature on anaesthetic potency are not in keeping with the predictions of the model, which would suggest diminished potency at low temperatures (Miller & Smith 1973). The opposite is observed but strong hypothermic effects occur, which may be observed in the absence of anaesthetics, so that it is not possible to identify separately the role of changing anaesthetic potency and the direct response of the animal to changes of temperature. In model systems the predictions are often in keeping with the theory.

Mechanisms of inert gas narcosis

Physical principles not only provide criteria that can be used to establish the general nature of the site at which anaesthetics act, but may also provide clues as to possible mechanisms. Most commonly proposed mechanisms suggest that anaesthetics perturb the nervous system in one of two ways. The first suggests that the ion transport mechanisms associated with axonal conduction are modified by the presence of anaesthetics in the cell membrane. The second proposes that synaptic transmission is the relevant process. There is evidence that in many preparations axonal conduction is relatively insensitive to anaesthetics at clinical doses (Larrabee & Posternak 1952).

As remarked above, the physico-chemical evidence may be interpreted as indicating that general perturbation of the cell membrane is not a plausible model. Taken in conjunction with experiments on ion transport in membranes we concluded ten years ago (Hale *et al.* 1972), 'the results give no indication that the general perturbation of membrane processes is an essential feature of the mode of action of general anaesthetics at clinical concentrations'. There seems no reason to reach a different conclusion now.

Considerable attention has been paid to the possibility that anaesthetics may act at a hydrophobic region with a protein. Specific binding sites for Xe or cyclopropane have been observed in myoglobin (Schoenborn 1965). The interaction of anaesthetics with BSA has been related to their solubility in octanol (Helmer *et al.* 1968). For most proteins investigated no functional changes appear to arise from the binding of anaesthetics. However, the inhibition of the light output of luminous bacteria by anaesthetics near clinical concentrations appears to arise from an interaction with bacterial luciferase, the enzymes responsible for the light emission (White & Dundas 1970; Halsey & Smith 1970; Middleton & Smith 1876). Similar observations have been made with firefly luciferase (Udea & Hamcuya 1973). The postulation of a hydrophobic region within a macromolecule as the critical site does not conflict with the Meyer–Overton expansion models discussed above. The interaction appears to be closely

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related to solubility in hydrophobic solvents. Compressibility, in the context of pressure effects, becomes a measure of the ease with which the local hydrophobic region with the protein is compressed.

The converse of pressure reversal of anaesthesia is the amelioration of the high pressure neurological syndrome by anaesthetics. This process seems much more complicated and it is not clear that it can be interpreted in terms of simple models with the same degree of success as for pressure reversal. Nevertheless, calculations have shown the expansion model to be in keeping with the broad features of the amelioration of pressure affects by a variety of molecularly simple agents (Miller 1983).

The more general effects of pressure acting alone on biological systems will not be discussed here but will be the subject of a later paper in this symposium by McDonald.

Decompression sickness

Nowhere has the application of physical principles to a physiological process proved more inviting than in the study of decompression sickness. Paradoxically, such principles have done little to clarify the mechanisms that underly this disease. It is worthwhile to speculate why so little progress has been made in the last 75 years and what new approaches might remedy this situation.

Theories of decompression sickness address three problems. First, they seek to elucidate the mechanisms that control the uptake of gases by the body when exposed to high environmental pressures and the manner by which the rate of gas uptake can be modified. Second, they attempt to understand processes that lead to the elimination of excess gas by the body after a reduction of the ambient pressure. Third, it is necessary to identify the critical conditions that must be satisfied if symptoms are to be observed.

The earliest plausible theory of decompression was due to Boycott *et al.* (1908), who made a series of simple assumptions. First the body was regarded as comprised of a number of hypothetical tissues for each of which the uptake of gas was exponential and characterized by a single half-life. The release of gas by the body was considered to be accounted for by the same kinetic model. Haldane (Boycott *et al.* 1908) assumed that the critical condition was set by the total volume of excess gas that could be tolerated. The application of the Henry law and Boyle law suggested that this principle should set a maximum value of the ratio of final to initial pressure between which a saturated diver could rapidly be decompressed. Since men could return safely from long exposure to 30 ft of sea water the ratio was estimated to be approximately 2. This ratio is only approximately constant and in general, though the Haldane theory was an important advance, its practical success was not such as to provide justification for the principles by which it was constructed. Many new theories have been advanced since Haldane's day, but our understanding of decompression sickness has made little progress.

The mechanisms for gas uptake have been considered. For perfusion, which is the transport of gas by the blood, the saturation of tissues is determined in part by the relative solubility of the gas in the tissues and in water (these may be very different for fatty areas). Hempleman (1952) proposed an alternative model in which diffusion of gas from the capillaries was the rate limiting process. Both this and Haldane's model lead to an uptake function that can be represented by a series of exponential terms and it is still uncertain which mechanism of uptake is most relevant for sites important in decompression sickness. The diffusion limited model has

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been developed on the basis that only one critical tissue is involved. With the Haldane type theories it has become fashionable to consider large numbers of tissues. Both models have been used in the calculation of decompression tables, but with extensive empirical modifications. The American Naval Tables were obtained by using a multi-tissue model (most recently with 15 tissues). The British Navy Tables were generated from calculations based on Hempleman's single tissue, diffusion-limited model. Both sets of tables have been widely used and found to be reasonably satisfactory. Certainly the differences cannot be used to establish the superiority of either model.

It is now clear that Haldane's (Boycott *et al.* 1908) assumption that gas release follows the same kinetics as uptake is not justified, at least as far as that portion of the gas causing symptoms is concerned. Gas release appears to be much slower than uptake, as can be seen from the evidence from sequences of dives. It is usually assumed that 'silent bubbles' are present even in non-symptomatic dives and that these can modify the kinetics of gas exchange. Such bubbles can be detected by ultrasonic methods. Decompression tables are empirically modified to allow for this factor.

Perhaps the most crucial element in theories of decompression is the critical end point, which, if passed, will lead to symptoms. Most theories have followed Haldane in assuming that the threshold of safe decompression can be determined by assuming that there is a critical volume of excess gas that can be tolerated by the body. This leads to the familiar ratio rule. However, some models have assumed that the *quantity* of excess gas is crucial. This leads to a maximum value of pressure difference for saturation decompressions rather than a fixed ratio. The evidence from practical diving experience suggests that neither model is correct. Indeed it is probable that the critical condition is not simple. Certainly this is true in certain complex diving schedules. If during a long dive of fixed depth and exposure a brief excursion is made to the surface it might be expected to reduce liability to bends at the end of the dive (compared to similar dives in which the excursion did not take place). Some excess gas must be released during the time at the surface. However, in certain circumstances the incidence of decompression sickness may be enhanced by the inclusion of a return to the surface (Griffiths *et al.* 1971; Gait *et al.* 1975). This has been shown to be due to bubbles, formed on the exposure to low pressure while at the surface, being compressed and thus enabled to move centrally and become more dangerous during the second period at depth. Such complexities indicate the difficulty of producing a general theory of decompression sickness.

The uncertainty in the nature of the critical end points also reflects considerable ignorance of how excess gas may be expected to behave in the body. The view that no gas separates out in symptomless decompression is not now generally held. Even in safe decompression the presence of silent bubbles has been deduced from the enhanced liability to decompression sickness on subsequent dives, and more recently by direct ultrasonic detection. The rate of separation of excess gas is, however, uncertain. Some models assume that the separation of excess gas within the body is instantaneous and that thermodynamic equilibrium will occur at all times (Hills 1969). So called thermodynamic theories of decompression have been developed on this assumption. Others believe that the rate of nucleation is the critical factor. Classical nucleation theory is only approximate, but it indicates that the rate is extremely sensitive to the conditions, the degree of supersaturation in particular. For the homogeneous condensation of a pure gas (a model for which calculations are available) it is estimated that in water vapour at 0 °C at a supersaturation ratio of 3.75, one nucleation event would occur in each cubic centimetre every

hour. At a ratio of 4.25 an event would occur every 10 ms (Burton 1977). Such sensitivity could be thought to explain why the critical supersaturation ratios observed in decompression sickness should appear relatively constant. However, this extreme sensitivity to the supersaturation ratio is not in keeping with the fact that the severity of bubble formation in decompression sickness is only moderately sensitive to the pressure change, and even in severe decompressions bubble formation appears to reach a limiting value. Furthermore, homogeneous nucleation is also extremely sensitive to, for instance, surface free energy. In the example quoted, a 10% change in surface free energy was calculated to change the rate of nucleation by a factor of 10^{10}. Such extreme sensitivity is not a feature of decompression experiments. The apparent limit to the quantity of bubbles formed even in severe decompressions suggests that nucleation states generated by the shear forces arising from blood flow may not be a major source of bubbles. The fact that pre-treatment with hydrostatic pressure appears to remove nuclei (Evans & Walder 1969; Daniels, this symposium) indicates a heterogeneous process (perhaps involving 'micro-pockets' of separated gas).

The failure of decompression sickness to reflect the patterns suggested by simple theories suggests that the rate processes governing phase separation are of crucial importance. This requires an understanding of the role of micronuclei, their nature and distribution within the body. It is to this area that much effort must be applied in the near future.

One further observation on research into decompression sickness is appropriate. If inert gas narcosis had been studied with only nitrogen and helium it is not clear that much progress would have been made. Fortunately the traditions of research into general anaesthesia led to a wide range of substances being employed. It might help progress in our understanding of decompression sickness if, in the same way, a wide range of gases was investigated. As yet only preliminary studies in this area have been made (Smith 1967; Lever *et al.* 1971). The manner in which the disease presents itself with gases of substantially different physical properties could provide important clues to the mechanisms involved. For the limited number of gases studied the degree to which they caused decompression sickness appeared to be related to fat solubility rather than total body solubility or water solubility.

Sufficient research has been undertaken to suggest that if monotonic schedules are appropriate then no substantial improvements in decompression schedules can be expected. Investigators starting from widely different models, and others working entirely empirically, have all produced schedules in which the times of decompression are roughly comparable. It is interesting to note, however, that if gas separation occurs and if complex redistributional phenomena, as described above, are present then a monotonic return to the surface may not be the optimum procedure. Under these circumstances it might be an advantage to decompress with an oscillating schedule. This is an area yet to be explored. Though the probability of success may not be high it at least offers a chance of substantial savings in decompression time.

Conclusions

Despite the practical successes in developing deep diving, which have taken men to pressures equivalent to over 2000 ft of sea water, the physiological problems that are encountered are far from fully understood. The basic mechanisms by which pressure, inert gas narcosis and gas supersaturation can affect man's capacity to dive have yet to be elucidated. However, the application of general physical principles to human and animal data and the study of model

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systems *in vitro* have led to theories of considerable predictive power and have indicated the constraints that the fundamental molecular mechanisms must satisfy.

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Discussion

T. R. Hennessy (*A.M.T.E. Physiological Laboratory, Gosport, U.K.*). Dr Smith pleaded that more inert gases, other than nitrogen and helium, should be used in decompression research. For experimental decompression on humans, neon is undoubtedly the next most important gas to study. The lipid solubility coefficient is about one-third that of nitrogen, which decreases the risk of neurological decompression sickness. The lipid–water partition coefficient is close to that of helium, whereas its diffusion coefficient in liquid is close to that of nitrogen. These ideal properties permit the design of a decompression experiment capable of distinguishing between perfusion and diffusion mechanisms in tissue. Unfortunately, pure neon is prohibitively expensive. So, rather than trying more gases on human trials, perhaps more effort should be expended in doing definitive experiments with mixtures of nitrogen and helium.

K. W. Miller (*Department of Anaesthesia, Harvard Medical School, Boston, Massachusetts* 02114, *U.S.A.*). The binding site model of anaesthetic action seems to predict that the degree of pressure reversal of anaesthesia should be greater in the presence of a gas phase than in the absence of such a phase (Franks & Leib 1982). In experiments on tadpoles, described in our paper (this symposium), we show the difference between pressure reversal of octanol anaesthesia in the presence and absence of a helium gas phase. Although the latent anaesthetic effect of helium complicates this experiment, identical results are obtained with the non-volatile agent urethane. This is contrary to the model's predictions. Furthermore, we have examined the effect of barbiturates, under pressure, on (^{3}H)-acetylcholine binding to a nicotinic receptor from *Torpedo*. This effect is known to be mediated by the binding of barbiturates to an allosteric site and thus it provides an actual example of their model. For this example, we find that the action of barbiturates is independent of pressure (unpublished data).

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