REVIEW ARTICLE

PRIESTLEY LECTURE 1986

On the science of deep-sea diving—observations on the respiration of different kinds of air[1]

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In 1776, Joseph Priestley performed his critical experiments on the respiration of air and clearly identified it as a "phlogistic process"(1). These investigations were the first steps in understanding the oxidative processes involved in metabolism. Some 4 yr earlier, Priestley had read before the Royal Society his substantial and important paper "Observations on Different Kinds of Air"(2). In this he describes the identification of nitric oxide and hydrogen chloride as well as the preparation of "fixed air" (carbon dioxide). The investigation of "airs" was to be the main theme of Priestley's work in the years that followed and led to the discovery of "dephlogisticated air," oxygen, in 1774.

The discoveries that will be discussed in the following pages result, curiously, from a bringing together of these two themes, respiration and "different kinds of air." Indeed, a most appropriate title for this lecture would be: "Observations on the respiration of different kinds of air." The research concerns respiration not of normal atmospheres but rather of exotic gases. Long after the biological role of oxygen had

[1]The Priestley Lecture was established following the 200th Anniversary, which was celebrated in 1974, of the discovery of oxygen by Joseph Priestley. The first lecture was given at Leeds in 1977 by then Secretary of State for Education, Mrs. Shirley Williams, and subsequent Lectures have been devoted to areas of science which have developed from Priestley's work. *Undersea Biomedical Research* is proud and pleased to have gained permission to reprint Dr, Smith's 1986 Lecture, which was published in *Chemical Society Reviews* 1986; 15:503–522.

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been elucidated it was found that even the most chemically unreactive gases could have unusual and dramatic effects when respired. These discoveries and the manner in which they were made would have been readily appreciated by Joseph Priestley. First, they required an interdisciplinary approach that was more characteristic of the research of Priestley's time. Second, they were discovered in the absence of a framework of scientific ideas and indeed their discovery would have endorsed his "Baconian conviction" that only facts are important. It is a field where serendipity has played a more important role than theoretical understanding; it is a field that still awaits its Lavoisier. A third factor which would have pleased Priestley is that it is a subject of very direct practical importance. He took great pleasure from his discovery of a practical way to make "Pyrmont" water by artificially carbonating water. He wrote, "what cost you five shillings will not cost me a penny." The practical application of the "respiration of different kinds of air" includes general anesthesia and deep sea diving. Surprisingly these two subjects are not unrelated.

DIVING

Almost three quarters of the surface of the earth is covered by water and it is natural that from the earliest times man would wish to gain access to these regions. It is claimed, on the basis of archeological studies, that Neanderthal man dived for food. By 4500 B.C. diving was well established and from the times of the ancient Greeks diving for sponges has been a recognized profession. The Greeks are said to have established laws governing the rights of divers to salvaged goods; the share increased with the depths from which the goods were recovered. Even today, breath-hold diving plays an important role in Asian pearl diving communities. It is a highly developed skill with many records of divers remaining under water for over 4 min. However, without specialized equipment, man's capacity to explore beneath the ocean is severely limited.

The first use of diving equipment is usually attributed in legend to Alexander the Great who was said to have been lowered into the sea, at the Straits of the Bosphorus, in a glass barrel during the third century B.C. (Fig. 1). The medieval portrayal of his adventure shows the sealed glass barrel with lamps burning within, suggesting that Alexander was lucky to survive! More practical development of the diving bell occurred in the sixteenth century; in the mid-seventeenth century bells were used to recover the cannon (which each weighed 1 ton) from the Swedish Warship *Wasa* sunk in 30 m of water. In 1691 Edmund Halley improved bell technology by devising a method of replenishing the air supply using weighted barrels. In the nineteenth century the traditional diving suit was developed—a closed suit with air supplied under pressure by a pump on the surface. This was to provide the basic technology of diving until the development of scuba gear by Cousteau and Gagan in 1943. In recent years, a sophisticated technology for saturation diving, in which divers remain at pressure for periods of many days or even weeks, has been developed. The scientific principles underlying modern diving technology will be the subject of this lecture.

Before moving to a consideration of the more subtle factors that place restraint on human undersea activity, it might be helpful to identify two very basic constraints. First, for the free diver the pressure inside and outside the diver must be essentially

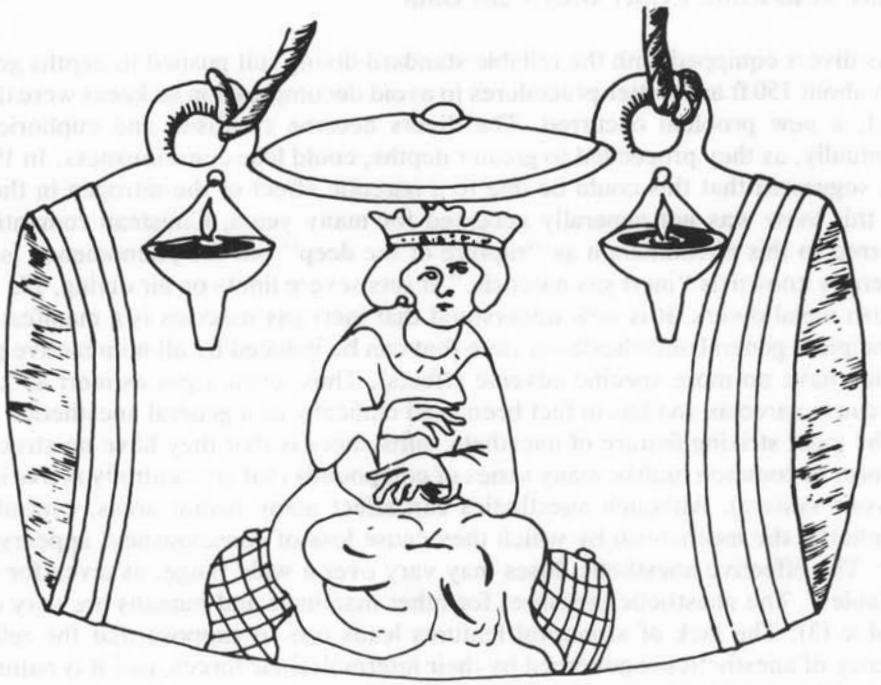


Fig. 1. A medieval view of Alexander the Great being lowered into the Straits of the Bosphorus in a glass barrel.

equal, otherwise his lungs would collapse. Divers must be supplied with air equal to their environmental pressure, which increases by 1 atm for every 10 m they descend below the surface. Second, the divers cannot breathe pure oxygen except in very shallow dives because oxygen has a number of adverse physiologic effects at pressures greater than say 2 atm and has the capacity to induce convulsions. These considerations mean that divers must be supplied with a gas mixture containing both oxygen and a dilutant gas or gases at pressure. This sets the context for deep diving research.

A further factor can limit deep diving—decompression sickness. As divers breathe gases under pressure, more gas dissolves in the body, according to Henry's Law. If a diver returns to the surface too quickly, excess gas can be released and can cause pain and even paralysis or death. The Hyperbaric Group at Oxford has been very active in investigating the problems of decompression sickness using ultrasonic detection equipment developed by my colleague, Dr. S. Daniels. However, in this lecture I will confine my attention to those problems that men and women face while at depth rather than those that restrict their return to the surface.

From early times attempts have been made to develop an alternative strategy for undersea exploration—pressure vessels and pressure suits. Recent advances have enabled divers in pressure suits to reach considerable depths, but it is generally accepted that the development of underwater resources requires the involvement of free divers, particularly in emergencies.

FIRST SURPRISE: INERT GAS NARCOSIS

As divers equipped with the reliable standard diving suit pushed to depths greater than about 150 ft and better procedures to avoid decompression sickness were developed, a new problem occurred. The divers became confused and euphoric and eventually, as they proceeded to greater depths, could lose consciousness. In 1935 it was suggested that this could be due to a narcotic effect of the nitrogen in the air, but this view was not generally accepted for many years. Cousteau romantically referred to this phenomenon as "rapture of the deep" but the phenomenon is now generally known as "inert gas narcosis." It sets severe limits on air diving, 185 ft for British naval divers. It is now understood that inert gas narcosis is a manifestation of incipient general anesthesia—a state that can be induced by all nonreactive gases (which have no more specific adverse effects). Thus, even a gas as inert as xenon can cause narcosis and has in fact been used clinically as a general anesthetic.

The most striking feature of anesthetic substances is that they have no structural features in common (unlike many series of compounds that are centrally active in the nervous system). Although anesthetics can affect many neural areas, central and peripheral, the mechanism by which they cause loss of consciousness appears specific. The effective anesthetic doses may vary over a wide range, as given for mice in Table 1. The anesthetic pressures for other mammals and humans are very comparable (3). The lack of structural features leads one to suppose that the relative potency of anesthetics is governed by their intermolecular forces, and it is natural to assume that anesthetic potency is controlled only by very general physicochemical principles. Underlying almost all current investigations is the so-called unitary hypothesis, which proposes that all general anesthetics act by the same mechanism.

TABLE 1
PARTIAL PRESSURES (IN ATM) REQUIRED TO PRODUCE GENERAL ANESTHESIA IN MICE

N_2	33	C_2H_2	0.85
CF_4	19	CF_2Cl_2	0.40
C_2F_6	18	CH_3CClF_2	0.25
Ar	15	CHClF	0.16
SF_6	6.1	cyclo-C_3H_6	0.16
Kr	4.5	$(C_2H_5)_2O$	0.030
N_2O	1.5	$CHCl_3$	0.0084
Xe	0.95	halothane	0.0077
C_2H_4	1.4	methoxyflurane	0.0022

FERGUSON'S PRINCIPLE

Not surprisingly, in view of the unitary hypothesis, much of the speculation about the mechanism of general anesthesia has come from physical chemists. The most general of these approaches is that of Ferguson (4). Ferguson's principle states that although the equilibrium concentrations of various anesthetics required to produce a

chosen level of anesthesia may vary widely, the thermodynamic activities corresponding to those concentrations lie in a relatively narrow range. This isonarcotic activity (defined relative to the pure liquid as standard state) for a number of common gaseous anesthetics is found to be approximately 2×10^{-2}. At equilibrium the thermodynamic activity of the anesthetic will be the same in all regions within the central nervous system, including the (as yet unidentified) site of action. Although the concentrations at different sites will differ due to different molecular characteristics, the activity at all sites would be the same. One serious implication of this argument is that if all anesthetics were equally potent under conditions of equal activity, it would be impossible to obtain any information about the nature of the site of action of anesthetics by studying the variation of potency with molecular properties. It is, therefore, important to examine carefully the validity of Ferguson's principle.

The general limitation of the principle becomes most apparent with gaseous anesthetics; it is equivalent to relating anesthetic potency to ideal solubility, which for any solute is independent of the nature of the solvent. It is therefore necessary to consider only the intermolecular forces between the anesthetic molecules, i.e., the solute molecules, and one can ignore those between the anesthetic and its site of action. Ferguson's principle may be written as:

$$a_{narc} = P_{narc}/P^{o}$$

where a_{narc} is the activity of an agent required to cause anesthesia, P_{narc} the equivalent partial pressure of the agent, and P^{o} its vapor pressure at the temperature of the experiment. Thus;

$$\log(P_{narc}) = -\log(1/P^{o}) + \log(a_{narc}).$$

A plot of log (P_{narc}) vs. log($1/P^{o}$) should yield a straight line of unit negative slope. $1/P^{o}$ is the ideal solubility of the anesthetic at 1 atmosphere partial pressure. If concentration at a particular site, rather than the activity, is the relevant variable, then we would expect Ferguson's principle to hold only for agents whose solutions at the site of action are ideal or deviate from ideality in a constant manner. To test Ferguson's hypothesis we must investigate the anesthetic potency of substances of unusual solubility properties.

FLUORINE COMPOUNDS

The unusual behavior of fluorine compounds, which has been the subject of considerable study by those interested in solubility, was suggested as a means of characterizing the site of action of general anesthetics. The phase in which anesthetics act can be identified only if special attention is directed, not to the general properties of anesthetics as in Ferguson's approach, but to situations in which the specific nature of the interaction between the molecules of the anesthetic and the molecules of the "solvent" area is manifest.

Such situations are provided by systems that deviate markedly from the ideal. Generally, fluorinated compounds have relatively weak intermolecular forces, and these lead to large positive deviations from ideality in mixtures with more typical nonpolar solvents. The largest deviations are observed when the solvent has strong intermolecular forces. Thus CF_4 and SF_6 have the lowest known solubility in water,

whereas their behavior in nonpolar solvents, though anomalous, is less exceptional. CF_4 and SF_6 are anesthetic at a thermodynamic activity that is greater than the activity of most common anesthetics by a factor of 10—a striking departure from Ferguson's rule. This departure from the Ferguson hypothesis suggested that fluorine compounds might provide a key to characterizing the site of action of general anesthetics.

HYDRATE THEORIES

In 1961, Pauling (5) and Miller (6) independently suggested that anesthetics act in the aqueous phases of the central nervous system. Although the two theories differ in detail, both seek to relate anesthetic potency to the stability of the gas hydrates that many anesthetics can form in aqueous solution. Because such hydrates are not stable under physiologic conditions, Pauling suggested that they might be stabilized by the charged side-chains of proteins in the encephalonic fluid. Hydrates, once formed, could increase the impedance of the neural network or occlude pores in membranes. They could also impair the reactivity of enzymes by a "cage" effect. Miller did not invoke the actual presence of hydrates within the body, but considered the effects of ordering which simple solutes are supposed to induce in neighboring water molecules (Henry Frank's "iceberg effect"). Miller suggested that these icebergs surrounding anesthetic molecules could impair neural function in much the same manner as the hydrates proposed by Pauling.

Both proponents suggested that a suitable test of their theories would be to determine the degree to which anesthetic potency of a substance is related to the stability of its hydrate as represented by the reciprocal of its dissociation pressure at 0°C. The examination of this relationship for a wider range of anesthetics shows that it is far from satisfactory (Fig. 2) (7). SF_6 would be 5 times more potent as an anesthetic if its hydrate stability were an accurate guide. The credibility of the theory is further reduced because it has proved impossible, to date, to make hydrates of a number of anesthetic substances, including both ether and halothane.

Advocates of the hydrate theory suggested a further test. If mixtures of gases which form the so-called class I hydrates and those which form class II hydrates were to be used to induce anesthesia, then a positive synergistic effect would be expected because a mixed hydrate could very readily be formed. This hypothesis was tested by Miller et al. (8) and Cullen et al. (9) in a study of the anesthetic potency of halothane-xenon and halothane-ethylene mixtures. The potency was found to be that expected if the effects of the two anesthetics were simply additive. These studies show that there is essentially no physicochemical evidence to suggest that the aqueous phases of the central nervous system are the site of anesthetic action.

LIPID SOLUBILITY THEORY

At the end of the nineteenth century, Meyer (10) and Overton (11) noted the striking correlation between anesthetic potency and fat solubility for a wide range of anesthetic substances. On the basis of this observation, they advanced the lipid solubility theory. The theory has been formulated in modern form by Meyer (12), "Narcosis commences when any chemically indifferent substance has attained a certain molar

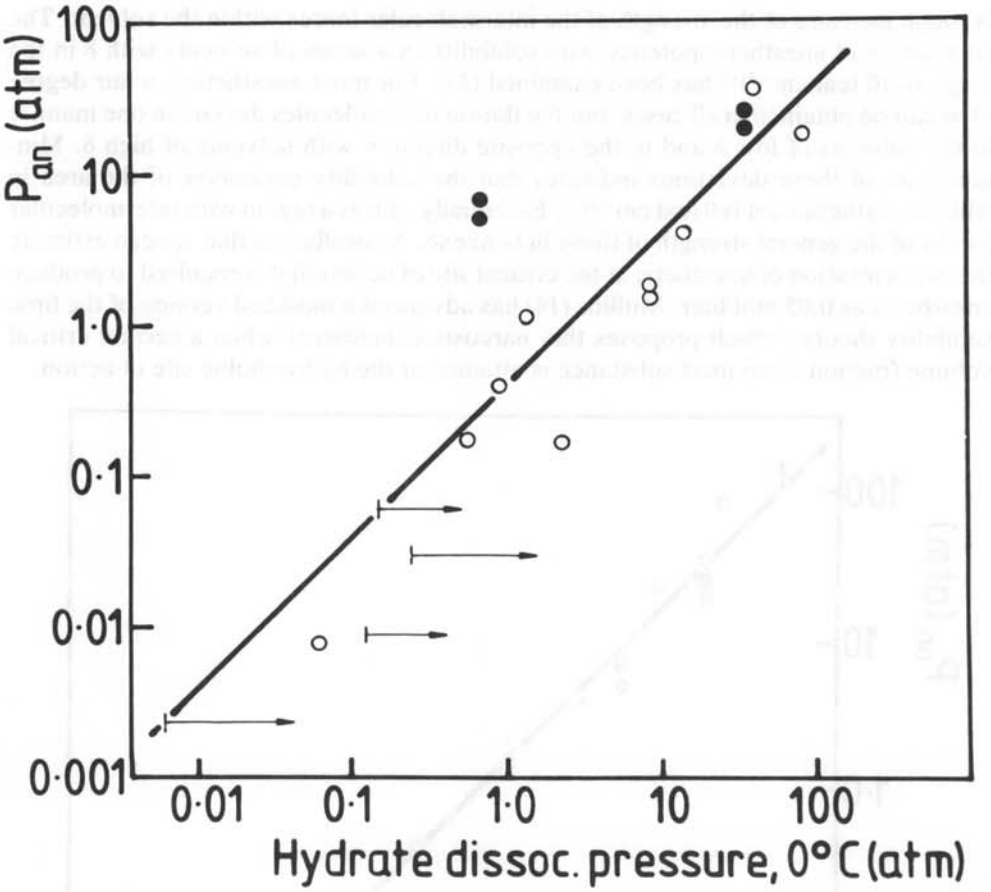


Fig. 2. Correlation of anesthetic pressures with hydrate dissociation pressures at 273 K. *Arrows* indicate anesthetic substances that do not form hydrates at their saturated vapor pressures. *Solid circles* represent fluorine compounds.

concentration in the lipid of the cell. This concentration depends on the nature of the animal or cell but is independent of the narcotic."

This model predicts that anesthetic potency should be directly proportional to solubility at the site of action. This relationship has been carefully tested using olive oil and other solvents selected to match the properties of the site of action. For olive oil the correlation between the solubility of anesthetic substances and their potency was shown to hold with remarkable accuracy and is predictive to ± 20% over a potency range of about a factor of 10^5—one of the more remarkable correlations in science (Fig. 3). Unfortunately, olive oil is not too well characterized from a physicochemical standpoint, and it is desirable to use simpler substances to establish the physical nature of the area in which anesthetics act. The most suitable property with which to characterize solvents is the solubility parameter, defined by:

$$\delta = \left[\frac{-E^{vap}}{V}\right]^{1/2}$$

where E^{vap} is the energy of vaporization of a substance, and V is molar volume, which

is rough measure of the strength of the intermolecular forces within the solvent. The correlation of anesthetic potency with solubility in a series of solvents with δ in the range 6–10 (cal $cm^{-3})^{1/2}$ has been examined (13). For most anesthetics, a fair degree of fit can be obtained in all cases, but the fluorinated molecules deviate in one manner in the solvents of low δ and in the opposite direction with solvents of high δ. Minimization of these deviations indicates that the solubility parameter of the area in which anesthetics act is 9 (cal $cm^{-3})^{1/2}$. Essentially, this is a region with intermolecular forces of the general strength of those in benzene. A corollary is that we can estimate the concentration of anesthetic at the critical site of action that is required to produce anesthesia as 0.05 mol/liter. Mullins (14) has advanced a modified version of the lipid solubility theory, which proposes that narcosis commences when a certain critical volume fraction of an inert substance is attained at the hydrophobic site of action.

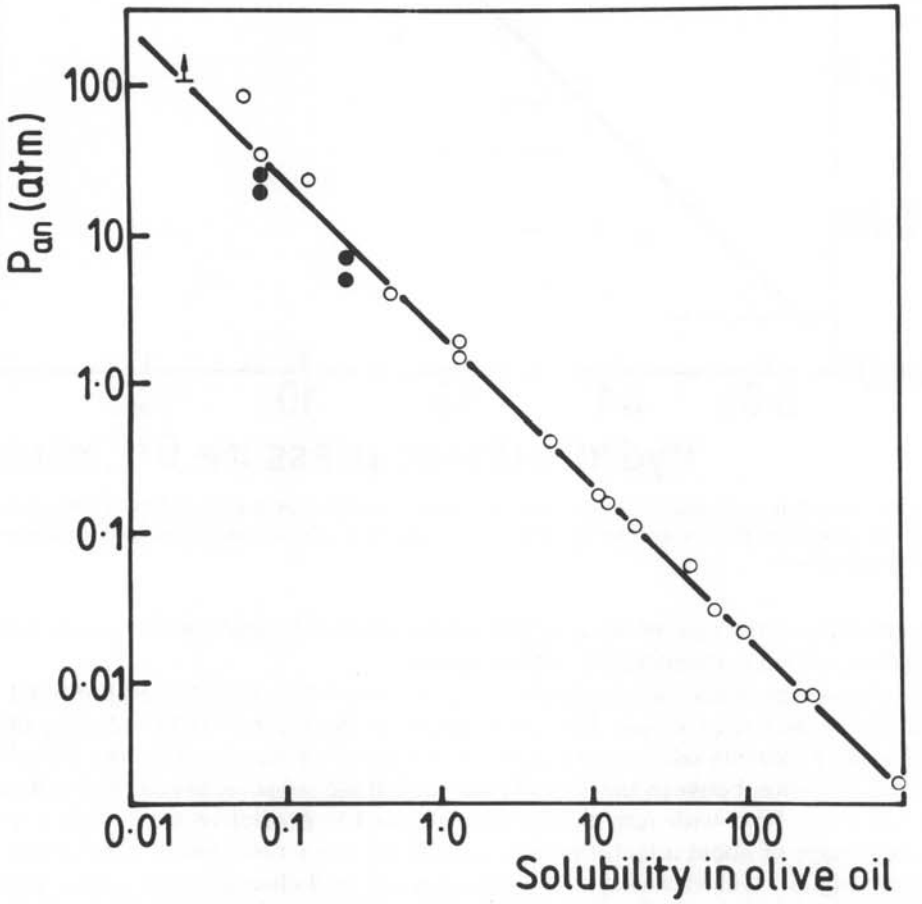


Fig. 3. Correlation of anesthetic pressures with solubility in olive oil. Anesthetic potency of a substance is seen to be directly proportional to its solubility in this solvent. *Solid circles* represent fluorine compounds.

THE MOLECULAR NATURE OF THE SITE OF ACTION OF ANESTHETICS

It is now generally accepted that the site of action of anesthetics is hydrophobic, but the molecular nature of the site is still a matter of debate. The traditional view is

that anesthetics act in the lipid region of the cellular membrane, to which is often added the belief that the induced perturbation interferes with ion transport through the membrane. However, studies of the effects of anesthetics on membrane processes lend little support to this view. Membranes are surprisingly resistant to the effects of anesthetics, and the changes that result from the application of anesthetics could easily be produced by variations of temperature within the physiologic range. In 1954, Johnson et al. (15) wrote, ". . . it is not necessary to assume that anesthetic action involved cell lipids." Indeed, on the basis of the results of experiments on the action of anesthetics on ion transport processes, we concluded some years ago that "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" (16). There seems no reason to modify this conclusion at the present time.

Possible alternative sites of action of anesthetics are hydrophobic regions of proteins. In recent years considerable efforts have been devoted to evaluating this possibility. For most of the proteins investigated no functional change was observed on the application of anesthetics. However, the light output of luminous bacteria is found to be inhibited by clinical concentrations of anesthetics and this inhibition appears to arise from a specific interaction of the anesthetics with luciferase, the enzyme responsible for light emission (17). This suggests that for this system a hydrophobic site within an enzyme may be the critical site of action of the anesthetic molecules. This does not conflict with the Meyer-Overton model because the interaction appears to be closely related to solubility in hydrophobic solvents. This has been confirmed by the recent experiments on firefly luciferase by Franks and Lieb (18). They showed that, over a 100,000-fold range in potency, the anesthetic concentrations required to reduce the activity of purified firefly luciferase by 50% were essentially identical to those that induce anesthesia in mammals. Anesthetics were shown to act competitively with the luciferase substrate. Franks and Lieb concluded that the volatile general anesthetics, despite their diversity, all acted by competing with the endogenous ligands by binding to specific receptors, and it was suggested that the anesthetic binding site can accommodate two small anesthetic molecules but only one molecule of anesthetics larger than hexanol.

Although the detailed mechanism of action of general anesthetics has yet to be established, the strong correlation of anesthetic potency with solubility in fatty solvents suggested that divers could avoid inert gas narcosis by breathing less soluble gases such as helium. The use of helium in diving was in fact suggested by Hilderbrand and others in the 1920s as a possible help in overcoming decompression sickness (inert gas narcosis was not identified at that time). Following many years of development, diving with helium was brought into service in spectacular circumstances in 1939. The U.S. submarine *Squalus* sank beyond the reach of air divers. The new experimental helium technique was brought into play and all the crew were saved and the vessel salvaged.

THE SECOND SURPRISE: THE HIGH PRESSURE NEUROLOGIC SYNDROME

As divers using helium went to even greater depths in the 1960s, new problems arose. At about 200 m divers were observed to experience new symptoms quite

different from those of inert gas narcosis: tremors, nausea, numbness, etc. (19). Similar effects were also observed in mice and other experimental animals exposed to high pressures of helium (20). Somewhat higher pressures than those to which divers have been exposed can induce convulsions in animals.

At first it was not clear if these effects were due to special properties of the helium used in the diving mixture or due to pressure per se. The classic work of Regnard (21) and others showed that with aquatic animals the first effect of pressure is a stimulation of the central nervous system at pressures above 50 atm. At higher pressures (200–300 atm), spontaneous muscle contraction occurs and the animals become paralyzed. Still higher pressures (400 atm) prove lethal. The interpretation of experiments performed on aquatic or amphibious animals is unequivocal since the hydrostatic pressures may be applied in the absence of potentially narcotic gases; however, in experiments with mammals it is not always easy to distinguish between the effects of pressure per se and the narcotic effects of the gases breathed. In experiments with the amphibious Italian great crested newt the same response was observed if pressure were applied using helium (or indeed neon) or hydrostatically. Thus it was concluded that helium, and probably neon, acted essentially as pressure-transmitting agents and that if these gases were anesthetic, their anesthetic pressures were greater than the tolerable mechanical pressure (22). This indicated that the symptoms observed in men and animals breathing helium-oxygen mixtures at high pressures were due directly to the effects of pressure; the effects are now known as the high pressure neurologic syndrome (HPNS) which constitutes a serious barrier to diving to great depths.

THE THIRD SURPRISE

It was found that the symptoms of HPNS were less severe if slow compressions were employed, and using this technique divers reached depths of well over 1000 ft. Nevertheless, it appeared that the effects of pressure per se would set the ultimate limit on the depths at which divers could work. However, there was a third surprise awaiting research workers in this field: pressure and anesthetics appear to be mutually antagonistic. The first observation of this remarkable effect was made by Johnson and Flagler in 1951 (23). They showed that pressure could restore the luminosity of luminous bacteria exposed to an anesthetic agent, and they conducted a similar experiment on tadpoles treated with ethanol. Tadpoles exposed to 2.5% ethanol ceased swimming due to narcosis. Swimming was also inhibited by pressures of 200–300 atm. However, if pressures of approximately 100 atm were applied to tadpoles treated with ethanol, their swimming activity was restored (Fig. 4). Pressure reverses the effects of ethanol which is acting as a general anesthetic.

This phenomenon, the pressure reversal of anesthesia, has since been observed with other animals using helium as the pressurizing agent (24, 25). For the Italian great crested newt, 34 atm of nitrogen acts as an anesthetic and reduces the ability of the animals to right themselves (Fig. 5). As additional pressure is applied, the performance of the animals is restored to normal levels. Similar observations have been made using nitrous oxide as the anesthetic. This antagonism has been observed in mammals, where mice have remained active at 280 atm at concentrations of anesthetic that would normally be lethal. Since 280 atm is about twice the normal

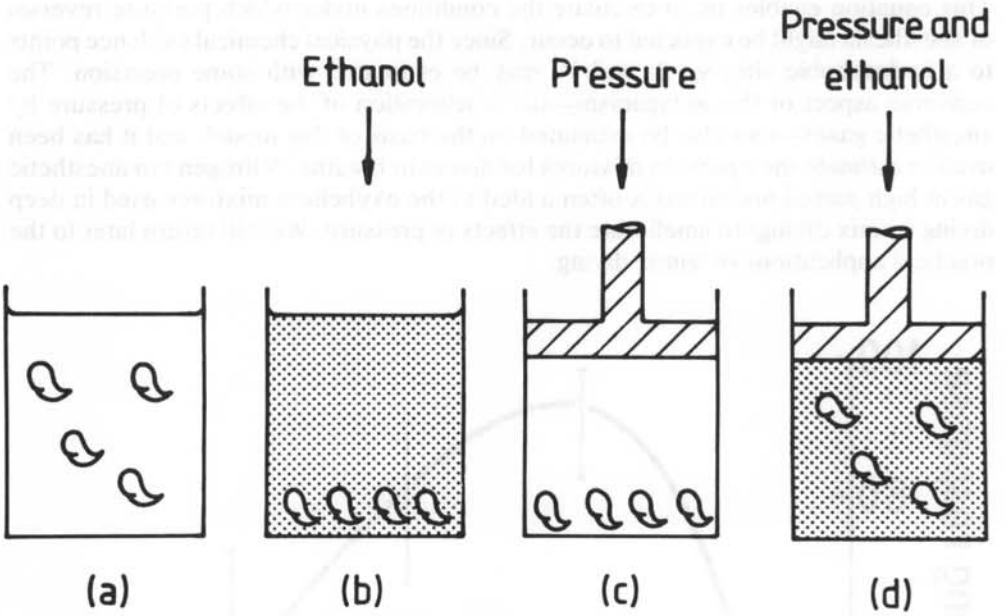


Fig. 4. Effects of pressure and ethanol on the swimming activity of tadpoles.

lethal pressure for mice, we see that not only does pressure reverse the effects of anesthetics but that anesthetics also ameliorate the effects of pressure.

One hypothesis that seeks to account for this antagonism assumes that the concentration of molecules at the active site is the critical factor determining anesthetic action. Thus it is natural to suppose that pressure reverses anesthesia by "squeezing out" the dissolved molecules, and calculations show that between 300 and 1000 atm would be required to reverse anesthesia. However, these values are too high when compared with the experimental value of approximately 100 atm, and the squeezing-out effect cannot be regarded as an adequate theory to account for the pressure reversal of anesthesia.

An alternative model that gives reasonably good prediction of the antagonism is the so-called critical volume hypothesis (25). This assumes that when the anesthetic gas dissolves at the (undefined) hydrophobic site of action of general anesthetics, it produces a fractional expansion given by:

$$\Delta V/V = \gamma_a P_a V_a$$

where γ_a, P_a, and V_a are the solubility coefficient, the partial pressure, and the partial molar volume of the anesthetic gas. The application of hydrostatic pressure will lead to a reduction in volume by:

$$\Delta V/V = \beta P_T$$

where β is the isothermal compressibility and P_T the total pressure. We suppose that the function of the site is unimpaired if the effects of the anesthetic and the pressure maintain the site at its original volume. Then:

$$\gamma_a P_a V_a = \beta P_T$$

This equation enables us to calculate the conditions under which pressure reversal of anesthesia might be expected to occur. Since the physical chemical evidence points to a hydrophobic site, γ_a, β, and V_a may be estimated with some precision. The converse aspect of this antagonism—the amelioration of the effects of pressure by anesthetic gases—can also be estimated on the basis of this model, and it has been used to estimate the optimum mixtures for divers to breathe. Nitrogen (an anesthetic gas at high partial pressures) is often added to the oxyhelium mixtures used in deep diving (trimix diving) to ameliorate the effects of pressure. We will return later to the practical applications of trimix diving.

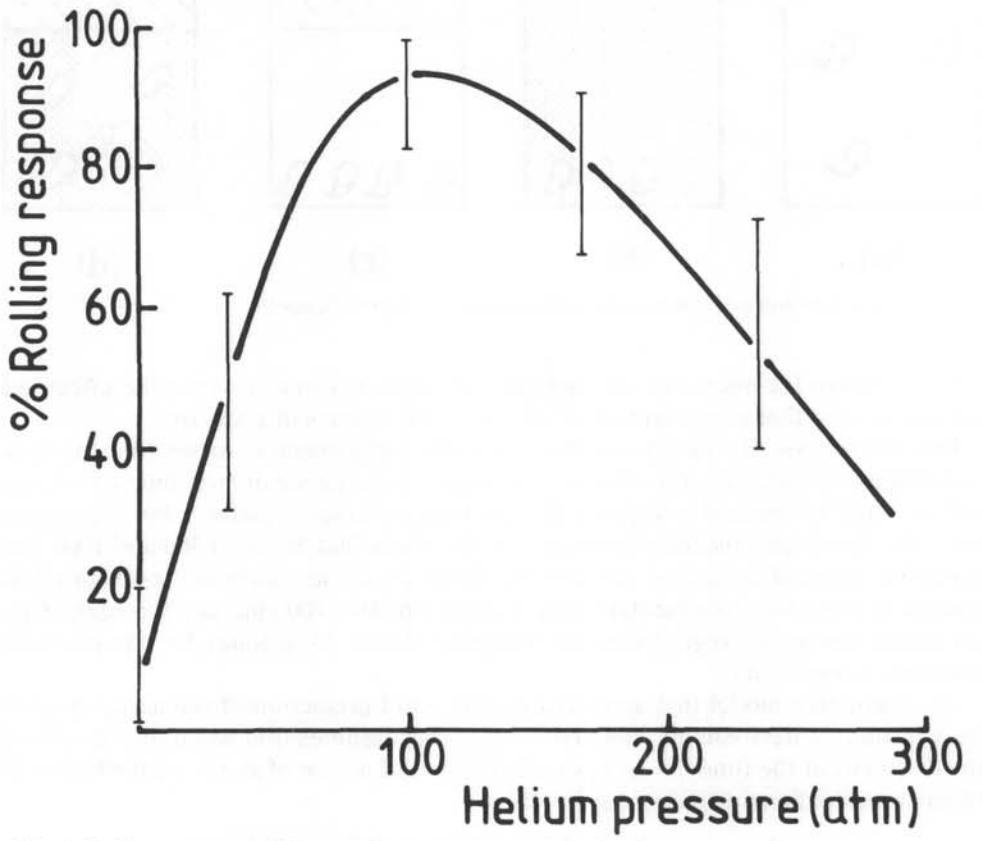


Fig. 5. Rolling response (ability to follow the slow rotation of their container) of newts exposed to 34 atm of nitrogen in the presence of additional pressures of helium. The nitrogen acts as an anesthetic and its effect is reversed by increasing pressure.

MECHANISM OF THE ACTION OF PRESSURE

In recent years much effort has been put into elucidating the neurophysiologic changes induced by high pressures. Much of this work has of necessity been indirect, with pharmacologic investigations playing the major part. The observation of HPNS in the lower vertebrate species and in immature mammals which have yet to develop

cortical activity suggested that its symptoms arise from effects on the subcortical regions of the central nervous system (26). Neuroanatomic studies have confirmed this view that pressure has a subcortical site of action (27, 28). Neuroanatomic studies have also indicated that pressure has no direct action on the cortex, but it is now recognized that the cortex provides a substantial measure of inhibition to counter the convulsive activity that arises from subcortical regions. The observation that reserpine, a monoamine-depleting alkaloid, abolishes the compression-rate dependence of HPNS (and is therefore most effective in lowering convulsion onset-pressures, during slow compressions) (27) suggests that the cortical control mechanism may account for the compression-rate dependence of HPNS and the remission of signs that frequently occur on prolonged exposure to pressure. The descending inhibition originating from the cortex appears to be primarily dependent on noradrenaline (29).

The subcortical action of pressure suggested an analogy with the convulsants picrotoxin and strychnine. Picrotoxin is thought to antagonize the actions of the inhibitory neurotransmitter GABA at subcortical sites. It was observed that some agents that potentiate the effects of GABA postpone the onset of high pressure seizures. However, the discovery that muscimol (29), a GABA agonist, and baclofen (28), a GABA analogue, do not confer protection against HPNS indicates that GABA may not play a direct role in determining HPNS. Recently it has been shown that a number of more specific GABA-enhancing agents do not prevent the behavioral changes associated with high pressure (30). Experiments with the rat cervical ganglion have shown that pressure does not affect the GABA-sensitive response of this preparation (31).

Although these results indicate that some drugs that influence the actions of GABA and other neurotransmitters can effect the onset of HPNS (32), there is at present no evidence that these transmitters mediate the primary effect of pressure. However, in the case of glycine there is more reason to suppose that it plays a major role in determining the effects of pressure on the central nervous system. In this context, the centrally acting muscle relaxants related to mephenesin have proved particularly interesting. We found that (27):

1. The aromatic propandiols mephenesin and methocarbamol give excellent protection against seizures caused by pressure. They are also effective against seizures induced by strychnine.

2. The aliphatic analogues meprobamate and carisoprodol fail to protect against the convulsions associated with HPNS or those due to strychnine.

3. The relative potencies of the ortho, meta, and para isomers of mephenesin are the same for protection against both pressure- and strychnine-induced convulsions.

4. This correlation of the effectiveness of drugs acting against pressure and strychnine is not matched with other convulsants such as Metrazol (Table 2). Strychnine is believed to act postsynaptically on glycinergic inhibitory neurons, and the pharmacologic evidence outlined above suggests that pressure acts in a similar manner.

Further evidence for this point of view can be obtained from the dose-response curves for strychnine measured at high pressures of helium (33). It was found that the effects of strychnine and pressure in inducing seizures were strictly additive, thus suggesting that both agents may share a common mechanism in the production of convulsions. In contrast, with picrotoxin which is thought to antagonize GABA, highly nonadditive behavior was observed (Fig. 6). This figure also illustrates the effects of simultaneous administration of strychnine and pressure in the presence of

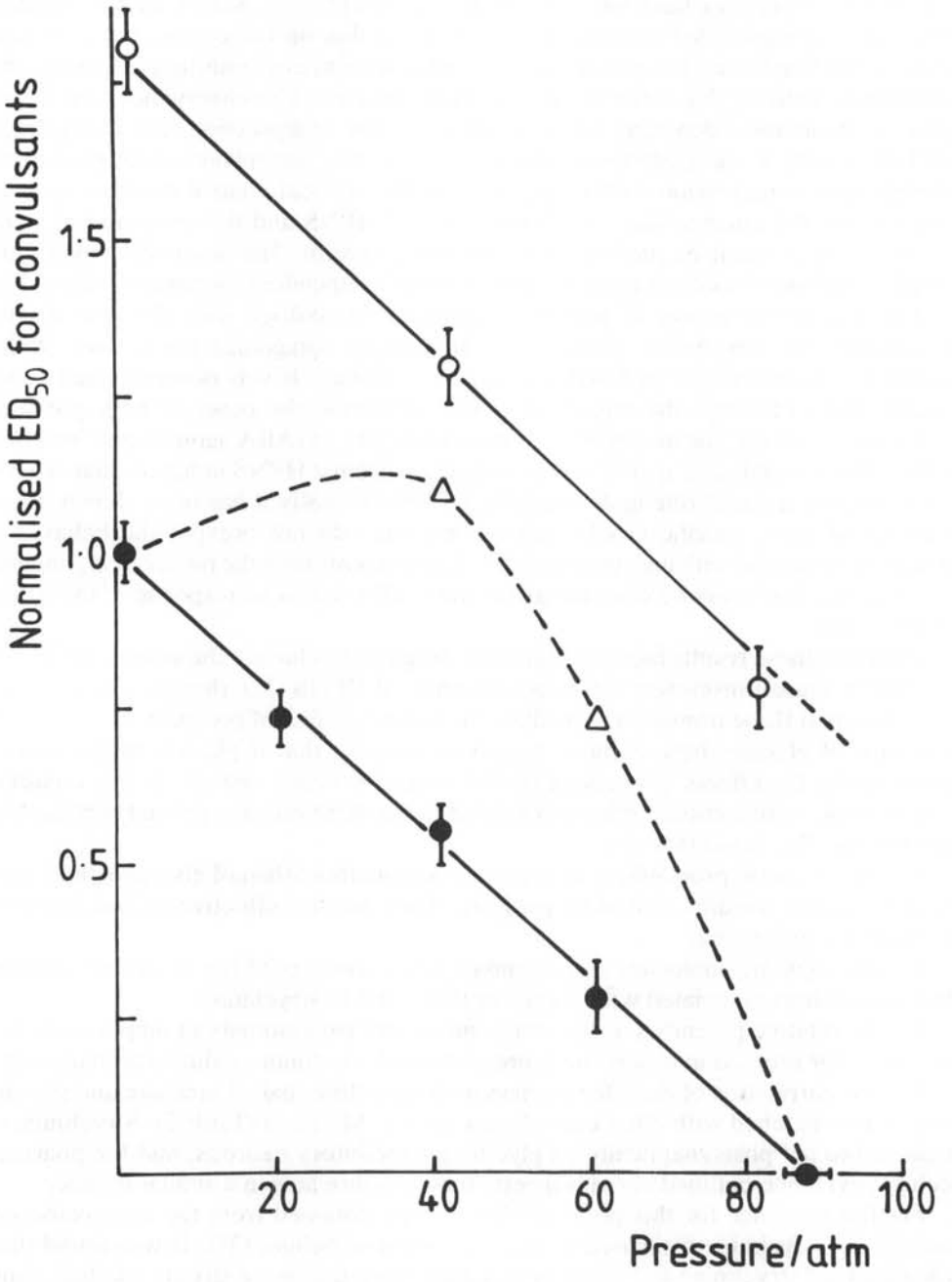


Fig. 6. Effect of pressure on the doses of strychnine (○) and picrotoxin (Δ) required to produce seizures in mice. *Upper line* (●) shows the results obtained for strychnine after the administration (130 mg · kg^{-1} s.c) of the anticonvulsant drug mephenesin.

mephenesin. The effects of both strychnine and pressure are ameliorated to the same degree by mephenesin, so that the effects of the two agents remain additive, although higher doses are required in the presence of mephenesin to obtain the original level of response. The actions of strychnine and pressure are both characterized by extremely steep dose-response curves for which $ED_{33}/ED_{67} = 1.10 \pm 0.05$. It is interesting to note that dose-response curves of this unusual degree of steepness are also observed with general anesthetics.

TABLE 2
ACTION OF ANTICONVULSANT DRUGS*

Drug	Protection Against Seizures Induced By:			
	Pressure	Strychnine	Picrotoxin	Metrazol
Phenobarbitone	↑ ↑	↑ ↑	↑ ↑	—
Valproate	↑ ↑	↑ ↑	↑ ↑	↑ ↑
Diphenylhydantoin	0	0	0	0
Ketamine	↑ ↑	↑ ↑	—	—
Althesin	↑ ↑	↑ ↑	—	—
Mephenesin	↑ ↑	↑ ↑	0	0
Methocarbomol	↑ ↑	↑ ↑	—	0
Meprobamate	0	0	—	↑ ↑
Carisoprodol	0	0	—	↑ ↑
Baclofen	0	0	—	↑ ↑
Diazepam	↑	↑	↑ ↑	↑ ↑
Flurazepam	↑	—	↑ ↑	—

* ↑ ↑ = Good protection; ↑ = moderate protection; 0 = little effect; — = no information available. The author is indebted to Dr. F. Bowser-Riley for this assessment based largely on published results.

The fact that glycine is not thought to act as a neurotransmitter in certain invertebrates (34) provides the possibility of investigating its involvement in the effects of pressure more directly. In particular, we have sought to elucidate its role in the pressure reversal of anesthesia. A comparative study was carried out on the effects of pressure and anesthetics on the electrically stimulated swimming activity of freshwater shrimps *(Gammarus pulex)* and tadpoles (larvae of *Rana temporaria*) under similar experimental conditions (35). The measure of swimming activity was the fraction of the total population not resting on the bottom when this region was exposed to an electrical stimulus. The anesthetics chloroform, diethyl ether, ethanol, halothane, and sodium pentobarbitone were employed and their potencies were found to be very similar for both species. However, the effect of pressure was strikingly different for shrimps and tadpoles. For the tadpoles, pressure clearly reversed the effects of the anesthetics and enhanced the swimming activity (Fig. 7). Conversely, in shrimps no pressure reversal was observed and pressure enhanced the inhibitory effects of the anesthetics (Fig. 8). In light of our previous investigations on the action of pressure, it is possible that the lack of pressure reversal may arise from the absence of glycine as a neurotransmitter in the crustacean CNS.

Whether any aspects of the action of anesthetics are also mediated by glycine so that the antagonism of anesthetics and pressure results from opposing perturbations of the same process or whether the antagonism is purely physiologic has yet to be established.

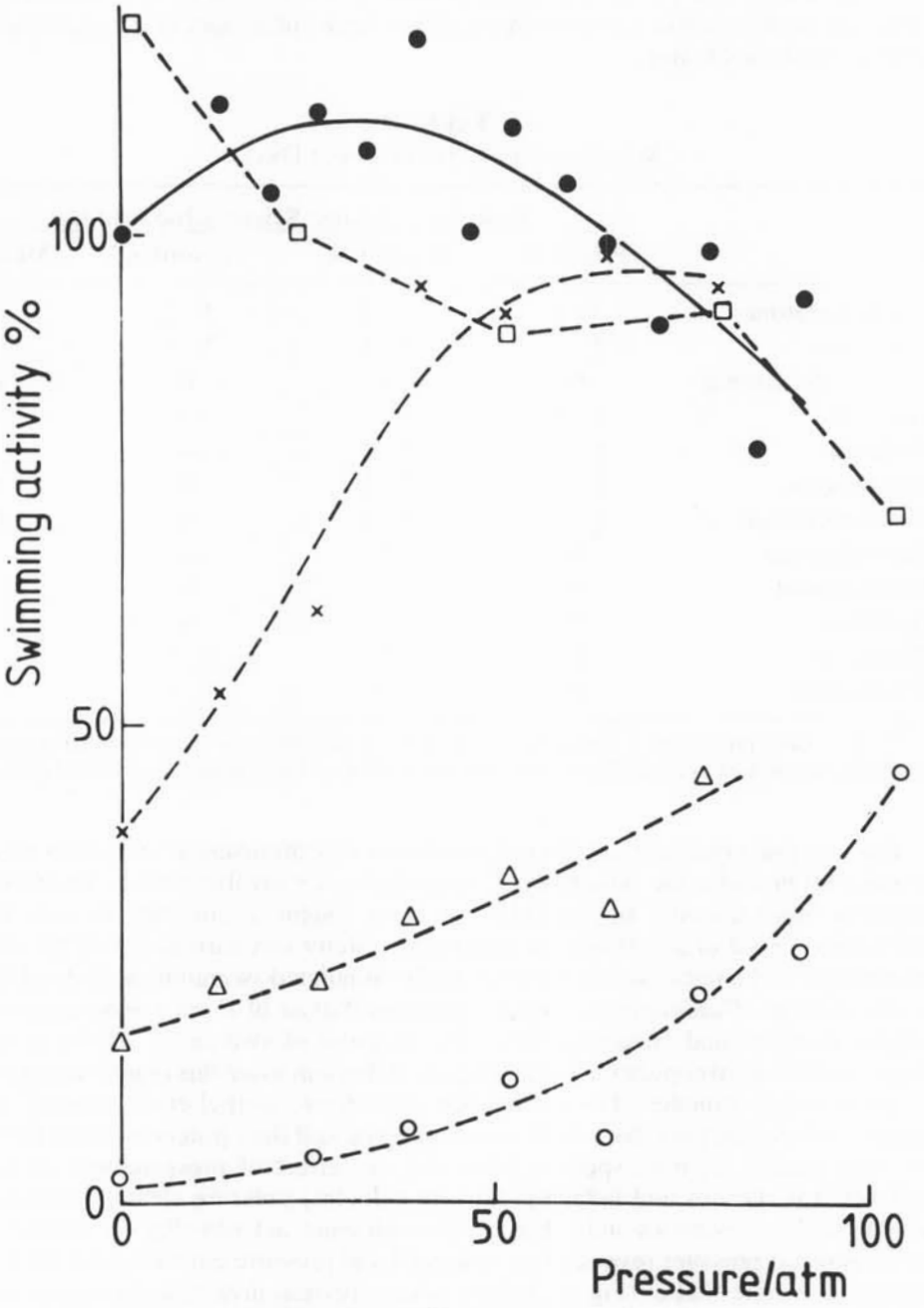


Fig. 7. Swimming activity of tadpoles (larvae of *Rama tempoia*) in the presence of ethanol: ● = no ethanol; ◘ = 0.01 *M* ethanol; x = 0.20 *M* ethanol; Δ = 0.265 *M* ethanol; ○ = 0.275 *M* ethanol.

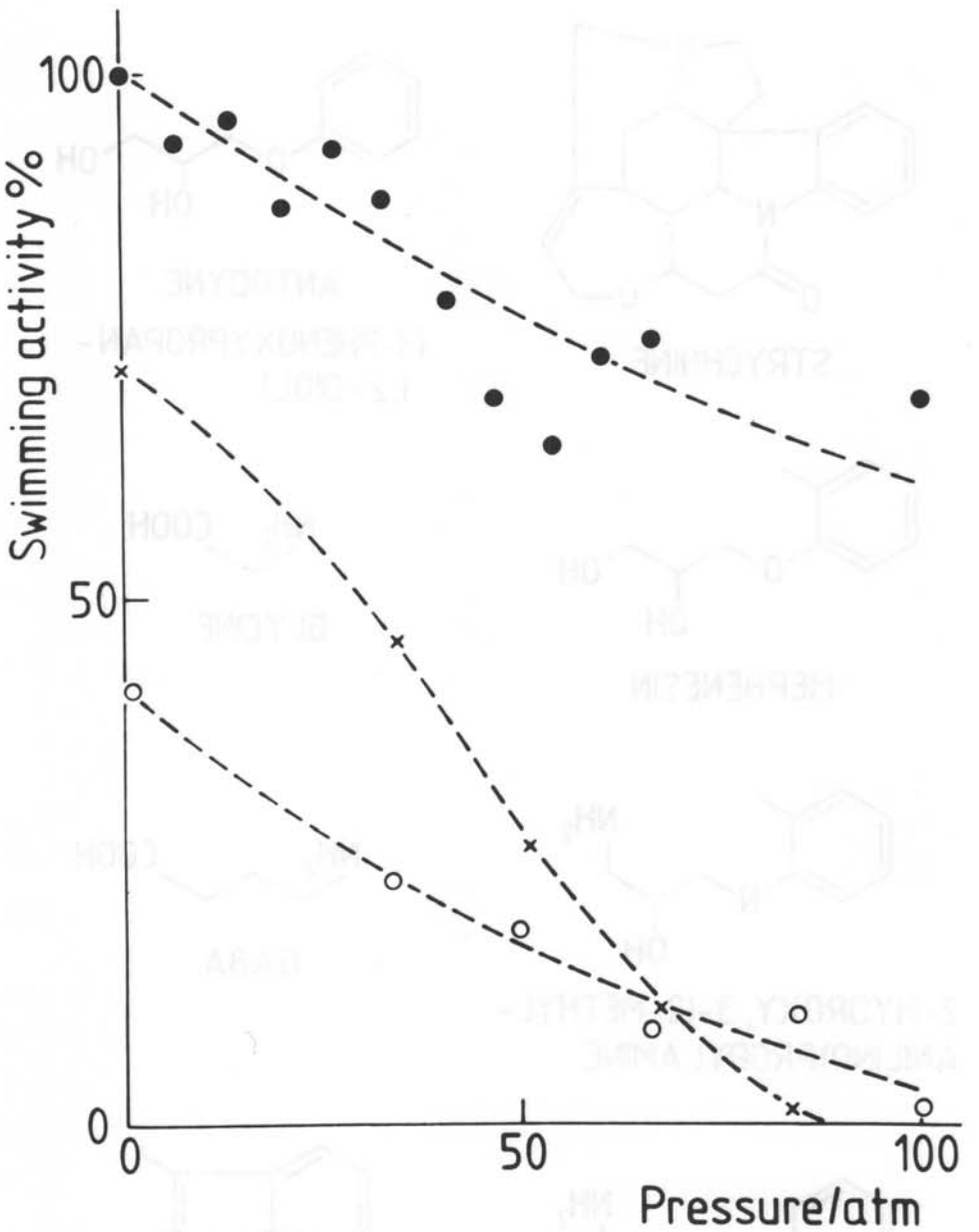
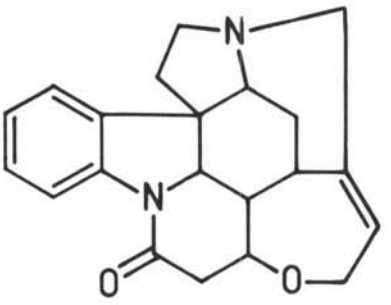


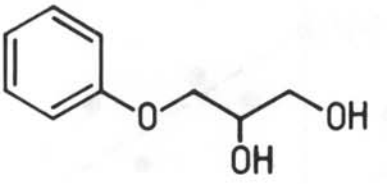
Fig. 8. Swimming activity of shrimps *(Gammarus pulex)* in the presence of ethanol; ● = no ethanol; x = 0.15 *M* ethanol; ○ = 0.25 *M* ethanol.

DESIGN OF DRUGS TO PROTECT AGAINST THE EFFECTS OF PRESSURE

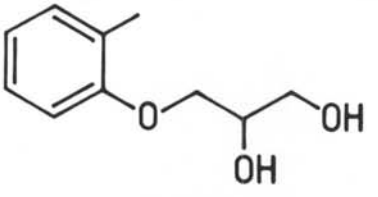
The observation that strychnine and pressure may share a common mechanism, involving the glycine receptor protein, by which they produce convulsions has been



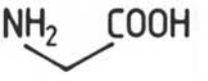
STRYCHNINE



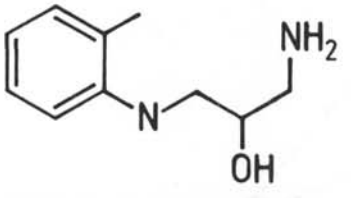
ANTODYNE
(3-PHENOXYPROPAN-
1,2-DIOL)



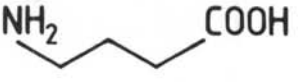
MEPHENESIN



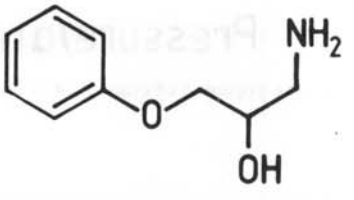
GLYCINE



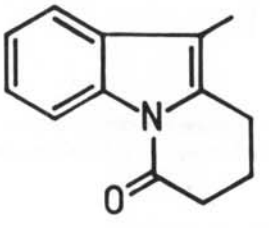
2-HYDROXY, 3-(2-METHYL-
ANILINO) PROPYL AMINE



GABA



2-HYDROXY, 3-PHENOXY-
PROPYL AMINE



INDOLE-2-BUTYROLACTAM

Fig. 9. Structures of substances referred to in text.

used in the design of drugs that might protect divers against the effects of high pressures. Inspection of the structures of strychnine and a number of other glycine antagonists (36) suggests that they share as a common structural feature a benzene ring linked to an oxygen or nitrogen atom with a positively charged nitrogen atom some 4.5 Å distant. For strychnine (Figs. 9 and 10) computer modeling indicates that the N-N distance is 4.65 Å and that the two nitrogen atoms and the benzene ring lie in the same plane. The simplest model would suggest that the nitrogen of the strychnine molecule, which is positively charged at body pH, binds to the site that would normally be occupied by the nitrogen of the glycine molecule (Fig. 11).

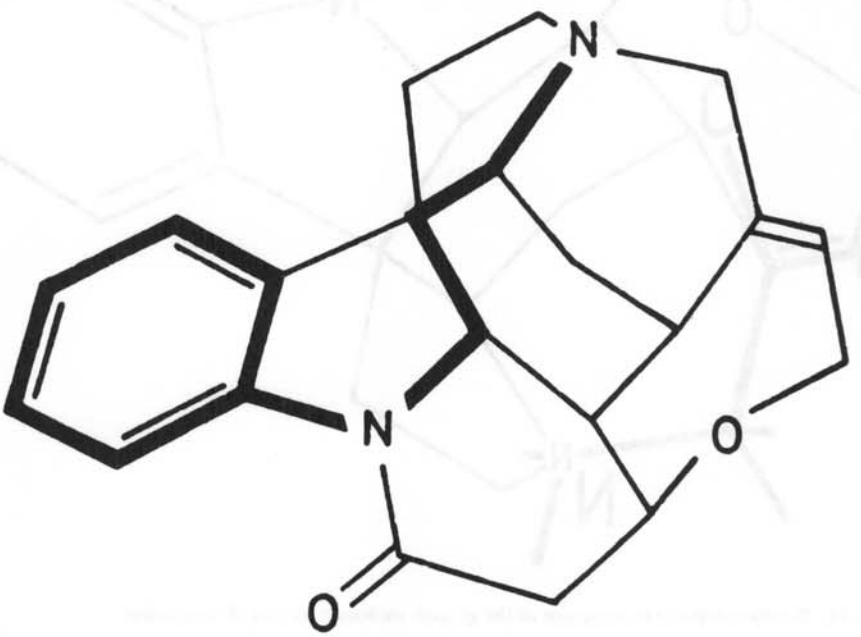


Fig. 10. Structure of strychnine, with that part of the molecule believed to confer convulsant activity emphasized.

Other simple compounds which incorporate these structural features have been shown to act as convulsants. Berger and Lynes (37) synthesized a number of "dinitrogen" convulsants such as 2-hydroxy-3-(2-methylanilino)propylamine (Fig. 9) which contained basic features common to many glycine antagonists. Substances that replace the anilino nitrogen with an oxygen atom such as 2-hydroxy-3-phenoxypropylamine (Fig. 9) also act as convulsants.

In contrast, compounds in which the positive nitrogen is replaced by an oxygen, such as antodyne or mephenesin (Fig. 9), prove effective antagonists of both strychnine and pressure. Computer modeling indicates that one of the most energetically stable configurations of mephenesin matches closely that of strychnine but leaves the region of the positive nitrogen clear, thus enabling glycine to bind (Fig. 12*a*). In this conformation, mephenesin has its secondary hydroxyl group near the probable binding site of the carboxylate moiety in glycine and may thus promote glycine binding. A slightly higher energy conformation of mephenesin would place an oxygen atom close to the site of the positively charged nitrogen, which could also enhance glycine binding (Fig. 12*b*). We have used these models of structure-activity relationships as

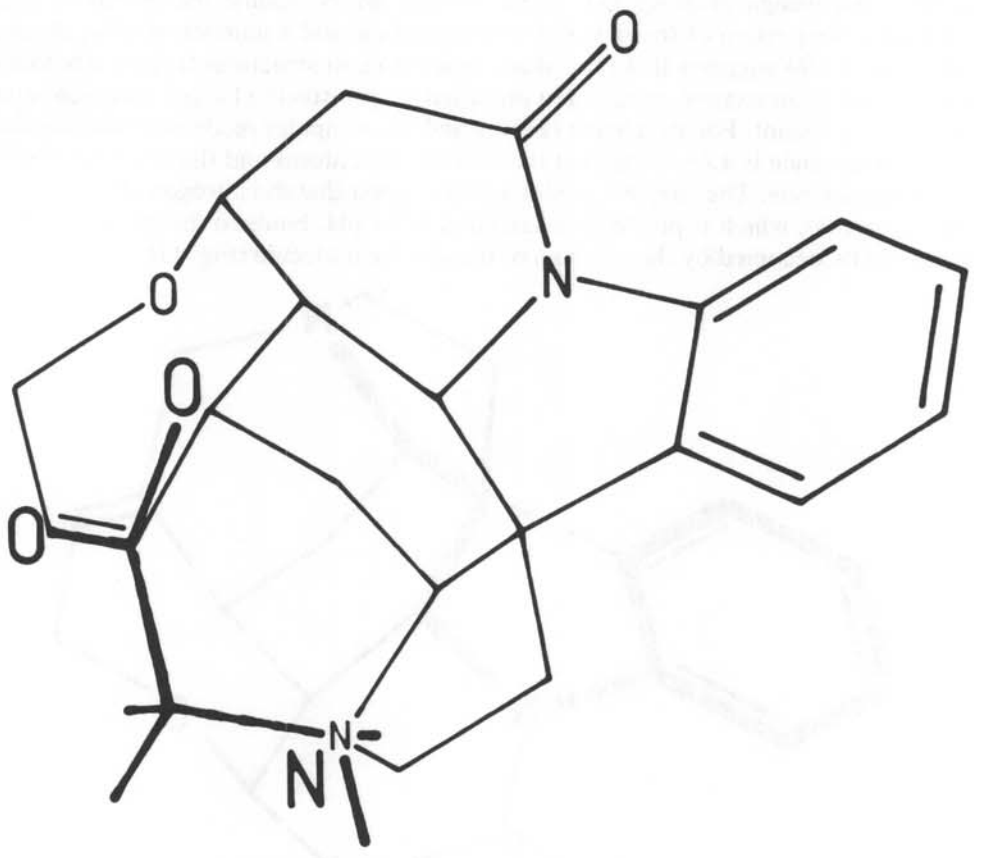


Fig. 11. Relationship of the structure of the glycine molecule to that of strychnine.

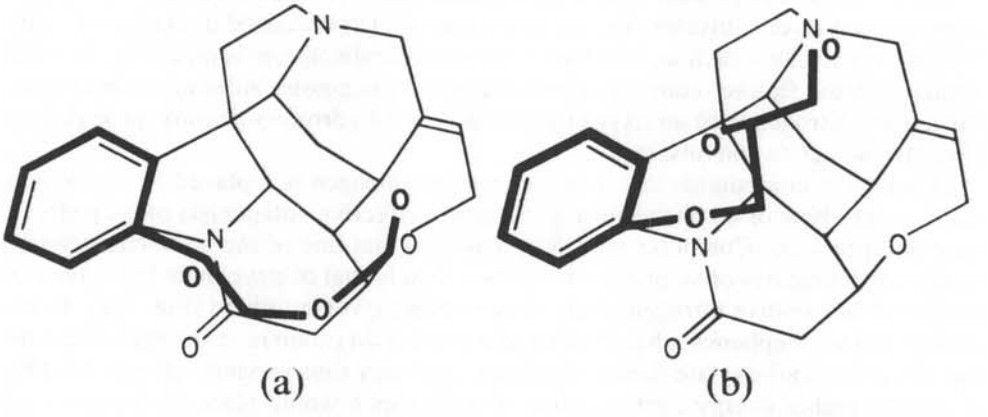


Fig. 12. Two possible fits of the mephenesin molecule to that of strychnine. Structure (*a*) represents the lowest energy conformation.

a guide when synthesizing a range of some 40 compounds capable of modifying the action of pressure on the CNS (38). The results of tests on mice confirmed that any compound that placed a positive nitrogen atom approximately the same distance and direction from a benzene ring as occurs in strychnine, potentiated the effects of pressure. Conversely, compounds that placed a negatively charged group in this location were found to protect against the effects of pressure, as did compounds that were capable of hydrogen bonding to the carboxylate group of glycine. Compounds that mimic much of the structure of strychnine but do not possess groups capable of interacting with the glycine binding site might be expected to antagonize the effects of strychnine without conferring protection against the effects of pressure. An example of this behavior was found with indole-2-butyrolactam (Fig. 9). This serves to confirm the view that pressure acts by causing a conformational change in the glycine receptor protein which inhibits glycine binding. In fact, binding studies suggest that glycine and strychnine bind at separate but allosterically linked sites. It would be consistent with this view to identify the pressure-sensitive site as the locus for allosteric coupling.

To develop new drugs to combat the effects of pressure we hope to combine the binding but nonconvulsant regions of the strychnine molecule with the structurally active features of the pressure antagonists related to antodyne. This approach will, we hope, produce powerful strychnine antagonists and drugs that will find practical application in protecting divers against the adverse effects of high pressure.

CONCLUSION

In recent years, divers in both the United States and Great Britain using trimix (nitrogen added to the helium-oxygen mixture to take advantage of the anesthetic-pressure antagonism) have reached depths of 2200 ft in simulated dives. Dives to such depths are difficult and expensive operations; it can take a week to reach depth and as much as 2 wk to decompress. Nevertheless, many divers have survived at depths over 2000 ft. The scale of this achievement can be put in context by remembering that in the relatively recent past the British naval diving limit was set at 185 ft. This great advance in diving capability is to a considerable extent due to the efforts of chemists. They have helped in the clarification of the mechanisms involved in narcosis and the effects of pressure. There is every reason to suppose that the application of physicochemical principles will lead to further advances and perhaps to the discovery of a fourth and even more unexpected surprise that will enable divers to proceed to even greater depths.

Much of the work discussed in this lecture has been carried out by past members and current members (Dr. S. Daniels, Dr. F. Bowser-Riley, Mr. W. A. G. Hill, and Mr. D. J. Price) of the Oxford Hyperbaric Group supported by grants from the Wellcome Trust, the Medical Research Council, and the U.S. Office of Naval Research. The syntheses described in the final section were carried out in collaboration with Dr. E. W. Gill, and assistance with the computer modeling was provided by Dr. W. G. Richards, Mr. D. Ricketts, and Mr. A. Novaks.

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