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Patterns of interaction of effects of light metabolically inert gases with those of hydrostatic pressure as such—a review

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Brauer RW, Hogan PM, Hugon M, Macdonald AG, Miller KW. Patterns of interaction of effects of light metabolically inert gases with those of hydrostatic pressure as such—a review. Undersea Biomed Res 1982; 9(4):353–396.—This review of available literature attempts to interpret net effects of metabolically inert light gases (He, H_2, and Ne) as the resultant of hydrostatic pressure and intrinsic pharmacological effects associated with exposure to these gases, and to assess the relative importance of each component with respect to a number of biological responses. A common pattern is recognizable for pressure reversal of anesthesia, high pressure convulsions, high pressure bradycardia, and certain characteristics of liposome model systems. Using the method of analysis proposed, these lightest gases can be shown to conform to the pattern of relation of potency to physical properties characteristic of more potent gaseous anesthetics, including N_2, N_2O, and Xe. The relations between effect produced and partial pressure of the acting gas are approximately linear to total pressures of 100 ATA for anesthesia or pressure reversal of anesthesia and (or to a much smaller extent) for the liposome model systems, but not for high pressure convulsions. As a result of these general factors no single gas can be expected to neutralize the effects of hydrostatic pressure with regard to all of the biological responses tested over any significant pressure range. A series of experiments with single cells and tissue cultures have revealed interactions between high pressure and inert gas that do not conform to the pattern set by the responses mentioned so far. These responses cannot yet be shown to constitute a homogeneous group and may represent at least two subgroups. Responses falling into this second heterogeneous category include cell motility, development of cell abnormalities and lysis, and cell and perhaps virus replication or multiplication. The implication of these results for the formulation of biophysical hypotheses to explain interactions between inert gas and high pressure, for considerations of high pressure effects as a safety hazard, and for the problem of experimental approaches to the study of pressure acclimation are discussed briefly.

anesthesia, pressure reversal of; high pressure neurological syndrome; HPNS; bradycardia, pressure; lipid bilayers, fluidity of; phospholipid bilayers; phase transition temperature; liposomes; cell multiplication, pressure effects on; sinus node, beat frequency of; liquid-breathing mice; *Symphurus plaguisa; Notophthalmus viredescens; Triturus cristatus carnifex; Tetrahymena pyriformis; Acholeplasma laidlawii;* protozoa; helium; hydrogen; nitrogen; argon; neon; nitrous oxide;

hydraulic pressure; echo II virus; herpes simplex virus; *Spirostomum ambiguum; Echinosphaerium nucleofilum;* bacteria—marine (EP-4); cell morphology, pressure altered; toxic effect, inert gases; convulsions, high pressure; trimix; mixtures of inert gases, neutral; survival times, high pressure; critical volume hypothesis

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A. INTRODUCTION

1. General

The last two decades have witnessed a striking resurgence of interest in the effects of high pressures on biological functions in intact animals, in isolated tissues, and in single-celled organisms. This interest has been manifested in large measure by two sets of investigators approaching the subject from two substantially different vantage points: on the one hand, investigators interested in underwater physiology have become sensitive to the fact that as

diving technology has developed, human subjects are increasingly being exposed to real or simulated depths at which hydrostatic pressure effects contribute to changes in their physiology in ways that may adversely affect safety or performance. In virtually every case, compressions of interest to these workers have involved the use of various gas mixtures as the primary pressure transducing fluid, so that to this community pressure effects are almost invariably manifested as the net effects elicited by high concentrations of one or several gaseous components of the compression atmosphere, modified in various ways by the simultaneously exerted hydrostatic pressures.

A second group of investigators took as its point of departure an interest in the adaptive (or acclimational) changes that make it possible for organisms to survive in the deep sea, where of necessity hydrostatic pressure is a major determinant of the characteristics of the environment. Observations and experimental work by investigators approaching the subject from this side have almost invariably involved application of hydrostatic pressure in aqueous systems without the intervention of any gas atmosphere, so that phenomena that have come to the attention of these workers tend to have been determined by hydrostatic pressure alone.

A gray zone between these two approaches, however, emerges when attention is focused on the lightest gases, helium, neon, and hydrogen. These display pharmacological effects that are of the same order of magnitude as those corresponding to the hydrostatic pressures required to attain effective concentrations of these gases, so that for a number of responses the net qualitative effects of compression in helium, neon, or hydrogen atmospheres are identical with those of hydraulic compression, and inert gas effects are manifest only as quantitative modifications of hydrostatic pressure effects.

This review is designed to deal primarily with gases in this gray zone and has two principal objectives: In the first place, it makes an attempt to assess the quantitative relations between the effects of these lighter gases and those of hydrostatic pressure without entering into theoretical discussions of the mechanisms underlying interactions between these two factors; at the present time, these are only rarely described with sufficient precision to determine confidently which of several hypotheses may represent the true mechanism underlying their interaction. In the second place, this review explores to what extent these interactions between hydrostatic pressures and inert gases follow a single pattern, or to what extent it may become necessary to recognize diverse patterns of interaction of inert gas and hydrostatic pressure to characterize various ones among the variety of responses that have been studied to date.

To attain either one of these objectives, the experimental material to be included must allow a meaningful comparison between the effects of the different modes of compression on the same test object. The number of responses it is appropriate to consider in this paper is therefore limited to approximately two dozen among the several hundred systems of biological interest known to be affected by high hydrostatic pressures.

2. Historical background

Changes in locomotor activity in animals exposed to high hydrostatic pressures were first observed and recognized as such around the turn of the century (1). These observations were made in aqueous systems, and in the sequel they spawned several spurts of investigations. The earliest among these were series of studies of the effects of high pressures on nervous and muscular activity, largely carried out during the 1920s and 1930s (e.g., refs. 2–4), as well as highly significant studies of pressure effects on locomotion and cell division in unicellular organisms (5, 6). These efforts in turn led to a number of biochemical studies concerning pressure effects on protein integrity and enzyme function (7, 8), which in their turn provided

the factual basis for theoretical studies of thermodynamic and kinetic effects of high pressures on living systems (9) that have remained the theoretical basis for much of the later thinking.

On the other side of the fence, workers interested in the effects of high pressure gases on air-breathing species during this same period gradually picked their way through the complications of the phenomena of inert gas narcosis and by the early 1940s had evolved a coherent set of observations and a number of largely equivalent descriptive hypotheses that provided a coherent and, on the whole, satisfying description of the relation of the narcotic potencies of these various gases to their molecular properties (10–12). In this effort, workers interested in underwater physiology were early joined by pharmacologists and anesthesiologists interested in the mechanism of general anesthesia (13, 14). Throughout the next decade it was this body of facts and speculation that informed the thinking of investigators in this general field and provided satisfactory answers so long as thinking was limited by effective diving depths of, at the most, 100 or 200 m (305 or 610 ft). When, as a result of efforts during the 1950s, diving technology progressed to the point where diving depths approached and exceeded 300 m (915 ft), new phenomena began to be encountered (15). In view of the success that had hitherto attained application of the pharmacological theories developed for dealing with inert gas effects, it is hardly surprising that such anomalies, when they began to appear, were at first interpreted as aberrant pharmacological responses. Thus the investigators who first described what we now recognize unmistakably as high pressure effects in man (16, 17) interpreted these (and, indeed, persisted in so interpreting them until at least 1970) as "helium tremors," presumably a manifestation of "helium toxicity." Recognition of these and related effects as high pressure effects was achieved presently on the basis of comparisons of the relative effectiveness of helium and hydrogen in producing these effects, and of comparison of those data with the results of experiments intended to measure the net anesthetic potencies of hydrogen and helium (18). At about the same time investigators at Oxford approached the subject from the opposite direction and, building upon earlier observations by Johnson and Flagler (19), demonstrated the reverse phenomenon—i.e., the reversal of inert gas narcosis by exposure of animals to high hydrostatic pressures (20). This phase of the discovery—or rediscovery—of the intrusion of hydrostatic pressure effects into diving physiology was completed by the demonstration that effects qualitatively indistinguishable from those of compression in helium-oxygen atmospheres could be elicited in mice that had been converted to liquid breathing by appropriate manipulation, and in which, therefore, it was possible to carry out the compression without the intervention of helium or any other gaseous component (21).

Following upon these initial observations, it could soon be demonstrated that the antagonism between hydrostatic pressure effects and the effects of inert gas anesthetics is not confined to the phenomenon of pressure reversal of anesthesia but extends to other manifestations of the hydrostatic-pressure–induced changes in central nervous system (CNS) activity (22, 23), as well as to at least certain pressure effects on contractile systems (24). Furthermore, it became clear that some of these effects are exerted not only by anesthetically powerful agents, like halothane or nitrous oxide, but also by nitrogen, as well as the even less potent hydrogen. On the other hand, similar effects attributable to helium are so feeble that it has only been in relatively recent times that conclusive evidence of their existence has been furnished. Many of the effects of compression of intact animals, as well as of isolated tissue systems under helium, are qualitatively indistinguishable from those of hydraulic compression, and quantitative differences can be detected only by special methods, involving in particular direct comparison in experimental designs such as the traditional pharmacologists' ABA design which allow one to compare in a single experiment the performance of the same system compressed in the presence, and in the absence, of helium (25).

While these investigations seem to have provided the main line of effort in these fields, isolated reports have continued to appear in the literature concerning systems that failed to conform to the predictions based on experience with the systems so far discussed (26, 27). As is shown presently, systems that have shown such seemingly aberrant responses constitute a heterogenous group, united largely by negative rather than positive criteria: they do *not* conform to the common pattern that unites behavior of intact animals, heart muscle preparation, certain lipid bilayer models, and others. In the discussion that follows, we initially review data bearing upon the first group of phenomena, which we designate as *Group A* phenomena, and seek to characterize them as nearly quantitatively as may be. The second part of this review then attempts to bring together reports on systems that do not conform; since at this point they seem to us to refuse to fall into a single category, we lump them together under the negative term *Group Non-A* responses. We believe that the distinction thus arrived at is a useful one, not only by calling attention to the complexity of interaction between inert gas and hydrostatic pressure effects, but also by defining what seems to us an area as yet only lightly explored. It seems to us that such information already on hand suggests that comparative studies involving the use of both hydrostatic pressures and inert gas agents may add an important tool to the exploration of this heterogeneous group of non-A responses. From a practical point of view, perusal of the data leads us to suspect that certain effects that fall into this category may presently emerge as significant contributors to the hazards of prolonged deep-diving operations. Finally, recognition of the differences between A responses and non-A responses is likely to help to clarify the complex phenomena that underlie the acclimation and adaptation processes permitting aquatic animals to exist successfully down to the greatest depths of the ocean.

3. General overview of sources of information available

Application of the criteria enunciated above limits the number of publications directly pertinent to the present discussion to no more than about 25 out of the hundreds of papers dealing with high pressure effects or HPNS-related phenomena. Table 1 provides a listing of the key papers used in preparing the present summary, together with a brief description of each. Responses have been grouped in two categories as discussed above. Detailed reasons for this categorization emerge in the course of the further discussion. It is probably worth noting at this point, however, that papers available to date dealing with the responses in Category A involve excitable tissues in vertebrates and one particular type of model system, whereas Category Non-A involves responses observed in single-celled organisms or in tissue culture.

Several responses to high pressure that are of potential interest have been excluded from the present considerations as a result of a lack of the necessary comparative data. These include the changes in metabolic rates associated with high pressure exposures in intact animals (28) and studies of pressure effects on enzymes or enzyme systems. Assessment of changes in metabolic rate associated with exposure of intact animals to high pressures is complicated by simultaneous changes in motor and in thermoregulatory activities that, to date, have not been disentangled from possible changes in metabolic rate directly attributable to the biophysical effects of compression. Studies in model systems such as tissue cultures that would allow such separation likewise have not, to the best of our knowledge, been reported. The available data with regard to enzyme systems seem to be limited to studies in which compression was carried out either only hydraulically (29) or only under helium (9), so that the kind of comparison pertinent to the present discussion is not yet feasible. (See Table 1.)

TABLE 1

PAPERS BEARING ON COMPARISON OF EFFECTS OF HYDRAULIC PRESSURE OF COMPRESSION ON HE, H_2, N_2 UPON VARIOUS BIOLOGICAL RESPONSES

Type of Response	Preparation Used	Pressure Range, ATA	Senior Author and Ref. No.	Data Bear on
A Group				
Pressure reversal of anesthesia	Rat; Isolated nerve	1–250	Carpenter (30)	Extrapolation to estimate ${}_a\pi_{He}$
	Various	1–250	Schreiner (11)	Extrapolation to estimate ${}_a\pi_{He}$
	Various	1–250	Featherstone (14)	Extrapolation to estimate ${}_a\pi_{He}$
	Mouse	40–135	Brauer (31)	Estimate of $({}_a\pi_{He} + {}_a\pi_p)$ from binary gas mixture effects
			Halsey (32)	Estimate of $({}_a\pi_{He} + {}_a\pi_p)$ He-H_2, He-N_2, He-N_2O
			Kent (33)	Estimate of $({}_a\pi_{He} + {}_a\pi_p)$ He-N_2O, Ne-N_2O, H_2 in newt
High pressure convulsions	Newt	180–210	Lever (20)	Loss of rolling response in hydraulic compression and in He
	Newt	180–210	Beaver (unpubl.)	Convulsion threshold pressure during hydraulic and He compression
	Flatfish	70–95	Beaver (34)	Convulsion threshold pressure during hydraulic and He compression
	Trout	101–151	Barthélemy (27)	Survival times under hydraulic or He pressure
	Crayfish	100–120	Roer (unpubl.)	Convulsion onset, compression, in presence or absence of He
	Mouse (liquid-breathing)	70–95	Lundgren (24)	Convulsion onset pressure in hypothermic mice on hydraulic compression
	Mouse	70–95	Brauer (35)	Convulsion threshold in He, hypothermic mice, as in preceding
	Mouse	70–130	Brauer (66)	Convulsion threshold pressure in He-H_2 and He-N_2 mixtures

TABLE 1—*Continued*

A Group				
High pressure bradycardia	Mouse	70–140	Örnhagen (25)	Degree of bradycardia on hydraulic or He compression
	Sinus Node	150	Örnhagen (36)	Degree of bradycardia on hydraulic compression or compression in He or N_2
Lipid bilayer (liposomes)	—	1–350	Johnson (61)	K^+ permeability as function of pressure hydraulic vs He compression
	—	1–350	Mastrangelo (37) Trudell (38)	Fluidity as function of pressure in presence or absence of Ne, H_2, or N_2.
	—	1–350	MacNaughtan (39)	Transition temperature as a function of pressure in presence or absence of He or N_2
Non-A Group				
Direct observation of behavior of protozoa	*Echinosphaerium nucleofilum*	30–120	Miller (40)	Comparison of effects of hydraulic or He, N_2, or Ar compression on axopod length and cell lysis
	Spirostomum ambiguum	30–120	Macdonald (41)	Comparison of effects of hydraulic or He, N_2, or Ar compression on swimming speed, reversal reaction cell lysis
(Cell) growth and replication	Echo II and herpes simplex	1–61	Chastel (42)	Virus replication and pathological effects under hydraulic, He, or N_2 compression
	EP-4 marine bacterium	1 and 500	Taylor (43)	Replication and metabolic rate under hydraulic or He compression to 500 ATA
	Acholeplasma laidlawii	200–300	MacNaughtan and Macdonald (44)	Growth rate under hydraulic or He compression
	Tetrahymena pyriformis	175–400	Macdonald (45)	Growth rate under He, H_2, or hydraulic compression
	Saccharomyces cerevisiae	1–150	Thom (46)	Growth rate under hydraulic or He compression

Data represent findings from Swedish, French, British, and American investigators. Our search has not revealed any papers dealing with the particular question formulation with which we are dealing here from other scientific communities which have had an active interest in high pressure phenomena, notably Japanese, USSR, German, and Belgian investigators.

B. GROUP A RESPONSES

1. Inert gas narcosis and pressure reversal of anesthesia

Perhaps the most widely known effect of exposure to inert gases at high pressures is the induction of narcosis (cf. Refs. 13, 14). Similarly, probably the earliest explicit analysis of the interaction of inert gas effects with those of high hydrostatic pressures involved the phenomenon of pressure reversal of anesthesia (cf. Ref. 20). It is true, nonetheless, that from the point of view of the present review these phenomena are the least satisfactory to deal with. The reason for this is that, while the qualitative phenomena are described well enough, to the best of our knowledge no experiments have been performed that would allow one to separate, on the basis of direct experimental observations, the contributions to the net effect—on the one hand by pharmacological effects of the inert gas, and on the other hand by effects of the hydrostatic pressure, inevitably associated with the use of the lightest (and least potent) inert gases. Nonetheless, because of the importance of these phenomena for the development of the whole field, we have felt it necessary to begin the present review with a discussion of this group of biological effects.

In the absence of direct comparisons of the effects of compression in helium with the effects of hydrostatic compression on anesthesia in vertebrate systems, recourse must be had to inferential methods utilizing a certain amount of hypothetical apparatus to derive comparative results from what data are available. Since these biological data consist largely of dose-response curves for gas mixtures of varying composition that produce anesthesia at some experimentally determined pressure, and since in gaseous systems the concentrations of the pharmacologically active gases are inescapably linked to the partial and total pressure applied, the principal hypothetical apparatus required will have to provide a description of the manner in which these factors interact to produce anesthesia.

a. Hypothesis of linear additivity of anesthetic effects of gaseous anesthetics and of pressure effects

The simplest form of such a hypothesis is to assume that these several factors are linearly additive. This hypothesis can be cast in the form of the following equation:

$$\sum_i {}_a\pi_i \cdot {}_aP_i + {}_a\pi_p \cdot \sum_i {}_aP_i = \epsilon \qquad (1)$$

where ${}_aP_i$ represents the partial pressures of the several gases present when the total pressure of mixture reaches a value producing anesthesia in 50% of the animals (i.e., ED_{50}), ${}_a\pi_i$ represents constants of proportionality that in effect are measures of the relative anesthetic potencies of each of the gases per se, and ${}_a\pi_p$ is the corresponding constant describing the manner in which hydrostatic pressures affect development of anesthesia. The hypothesis of linear additivity then implies that it is possible to define constants π such that whenever the summation on the left side of Eq. *1* attains or exceeds a common critical threshold value ϵ, anesthesia will ensue. Absolute numerical values for the several π thus are determined by the value of ϵ chosen. In most of the discussion to follow, it is convenient to set ϵ at such a level that ${}_a\pi_{N_2}$, the intrinsic anesthetic potency for nitrogen, is equal to 1.00, and to relate the intrinsic anesthetic potencies of other gases to this intrinsic anesthetic potency for nitrogen. The resulting relative intrinsic anesthetic potencies, ${}_a\pi_i/{}_a\pi_{N_2}$, is designated by the sumbol ${}^{N_2}_aR_i$. (See Appendix for listing of symbols used.)

In actual biological situations, experiments comparing anesthetic potencies of gases by the methods here under discussion of necessity contain at least three components, i.e., the two metabolically inert components to be compared, and oxygen. The agents of interest in the present discussion, however, require total pressures of 30 or more atmospheres to exert biologically measurable anesthetic effects, whereas oxygen partial pressures throughout are limited to 0.5 ATA or less by the appearance of manifestations of oxygen toxicity and, furthermore, are constant in any given series of experiments. Thus only a small and in practice negligible error is introduced here by ignoring the presence of oxygen and treating the data as though they were derived from the use of binary inert gas mixtures alone.

With this added assumption, the hypothesis of linear additivity of anesthetic effects takes the form:

$$\epsilon = {}_a\pi_A P_A + {}_a\pi_B P_B + {}_a\pi_p(P_A + P_B) \qquad (1a)$$

which can be rearranged to the important equation:

$$P_A = \epsilon \div ({}_a\pi_A + {}_a\pi_p) - P_B \cdot [({}_a\pi_B + {}_a\pi_p) \div ({}_a\pi_A + {}_a\pi_p)] \qquad (2)$$

The hypothesis of linear additivity, then, is seen to become equivalent to the experimentally testable statement that at ED_{50} the partial pressures of two gas components in a series of mixtures of these two gases will be linearly related to one another, the slope of the line being given by the ratio of $({}_a\pi_B + {}_a\pi_p)$ to $({}_a\pi_A + {}_a\pi_p)$. Equation *2*, furthermore, reveals that comparative studies limited to the use of gaseous compression environments do not yield information on the relative intrinsic anesthetic potencies ${}^{N_2}_{a}R_i = {}_a\pi_i/{}_a\pi_{N_2}$ but only concerning the relative net anesthetic potencies $({}_a\pi_A + {}_a\pi_p)$ and $({}_a\pi_{N_2} + {}_a\pi_p)$. Thus Eq. *2* provides both a test of the validity of the hypothesis of linear additivity of inert gas effects and a definition of the experimental problem, resolution of which must presently occupy us.

b. Limits to the validity of the hypothesis of linear additivity

Experiments designed to test the hypothesis of linear additivity, as well as to provide estimates of relative net anesthetic potency for various metabolically inert gases, have been conducted in several laboratories. Brauer and Way (31) showed that within the pressure range explored by them a relation analogous to Eq. *2* satisfactorily describes the data for the systems $He\text{-}N_2$, $H_2\text{-}N_2$, and $N_2O\text{-}N_2$, but they noted that at the lowest N_2 concentrations increasing proportions of their animals underwent convulsions, vitiating the tests at these lowest N_2 concentrations. Halsey and co-workers (32) and Kent and co-workers (33) showed that at moderate pressures similar linear forms are applicable to describe partial pressure relations at anesthesia for the systems $H_2\text{-}N_2O$, $Ne\text{-}N_2O$, and $He\text{-}N_2O$. At higher pressures, however, the experimental results of these workers became incompatible with the hypothesis of linear additivity. Equation *2* does not describe the behavior of the system $He\text{-}N_2O$ at pressures higher than about 100 ATA (32) and breaks down at even lower pressures for He-Ar and $He\text{-}N_2$ (47). Such results may reflect one of two alternatives: either they reveal that ϵ is not a constant, as hypothesized, and hence that the basic hypothesis is at the best only an approximation of limited applicability, or they indicate that at pressures of 100 or more atmospheres biological effects other than anesthesia come into play to complicate the measurements. It seems clear that the latter alternative may apply to at least some of the results, as inferred by Brauer and co-workers for the low N_2 segments of their $He\text{-}N_2$ and $H_2\text{-}N_2$ data where high pressure convulsions were observed. Lever and co-workers (20) clearly recognized the possibility of such interference of spastic phenomena with their experiments on newts that revealed a

maximum in the pressure tolerance of their animals at some intermediate N_2 concentration: the rolling response used by these workers was perceived to not discriminate loss of righting reflexes due to paralysis or spasms from a similar end result due to anesthesia. It seems doubtful that similar explanations can be applied to all of the deviations from linearity reported by the San Francisco investigators, and the possibility, therefore, must be recognized that the hypothesis of linear additivity proves at best an approximation, albeit a most useful one that seems to account acceptably for the phenomena of inert gas anesthesia at pressures up to 100 ATA or even 140 ATA.

c. The case of helium

The proper point of departure for this discussion is the discovery, some 10 years ago, that there exists a striking conflict between the predicted anesthetic potency of helium (and of neon) and the actually observed effects of this gas: while all predictions based on correlation of physical properties with anesthetic power pointed to a small but positive anesthetic effect (cf. Refs. 14 and 30), direct observation of the effects of this gas in binary inert gas mixtures showed it to exert a well-defined antagonistic effect against the anesthesia produced by other more potent inert gas narcotics (cf. Refs. 22, 32, 48). This effect was presently associated with the previously observed phenomenon of pressure reversal of anesthesia (20) and led investigators to suggest that the net effect of helium in intact vertebrates was the resultant of several separable elements: the effect of hydrostatic pressure, tending to reverse the anesthetic effects of agents like ethanol or nitrogen; a small narcotic effect of its own; and some as yet ill defined stimulatory effect either of hydrostatic pressure or helium, which produced convulsions in nonnarcotized animals.

Together with the demonstration that up to about 100 ATA the interaction between mixtures of inert gases can be described by the linear relations of Eqs. *1* and *2*, these observations provide a basis for a strategy to assess the relative contributions of hydrostatic pressure and of the anesthetic effect of these several light inert gases. The course of the argument is simple enough: if the net effect observed can be apportioned as in Eq. *1*, then the contribution of pressure per se can be estimated from the difference between actually observed and theoretically inferred or extrapolated anesthetic potencies. To do so, one may use a relation derived from Eq. *2*:

$$ {}^{N_2}_{a}R_p = {}_a\pi_p \div {}_a\pi_{N_2} = Q \div (1 - Q) \tag{3} $$

where Q is defined by the equation:

$$ Q = ({}_a\pi_{He} + {}_a\pi_p) \div ({}_a\pi_{N_2} + {}_a\pi_p) - ({}_a\pi_{He} \div ({}_a\pi_{N_2} + {}_a\pi_p)) \tag{3a} $$

Of the two terms making up Q, the expression $({}_a\pi_{He} + {}_a\pi_p) \div ({}_a\pi_{N_2} + {}_a\pi_p)$ is the experimentally determined net anesthetic potency of helium relative to the net anesthetic potency of nitrogen, while ${}_a\pi_{He} \div ({}_a\pi_{N_2} + {}_a\pi_p)$ is the inferred intrinsic narcotic potency of helium, relative to the net narcotic potency of nitrogen, as deduced from the molecular properties of helium by comparison with those of series of much more powerful anesthetics for which the contribution of ${}_a\pi_p$ is negligible.

Once ${}^{N_2}_{a}R_p$ has been determined, other values for ${}^{N_2}_{a}R_i$ can be derived from experimentally determined relative net anesthetic potencies [i.e., $({}_a\pi_i + {}_a\pi_p) \div ({}_a\pi_{N_2} + {}_a\pi_p)$] by application of Eq. *4* derived from Eqs. *3* and *3a*:

$$ {}^{N_2}_{a}R_i = \frac{{}_a\pi_i + {}_a\pi_p}{{}_a\pi_{N_2} + {}_a\pi_p} \cdot \frac{1}{1-Q} - {}^{N_2}_{a}R_p \tag{4} $$

It should be noted that these calculations are based solely on the experimentally verified hypothesis of linear additivity implicit in Eqs. *1* and *2*, and, by the same token, lose their validity at pressures where these relations no longer describe the experimental facts—i.e., at pressures > 100 ATA.

Application of these equations to the estimation of ${}^{N_2}_{a}R_{He}$ depends on a fact well recognized since the time of Meyer and Hopff (49), namely, that the potency of anesthetic agents varies in a predictable fashion with the specific physical properties of the various molecular species. From time to time various physical properties have been singled out for this purpose, including oil solubility (30), oil-water partition coefficients (50), the constant a in the van der Waals equation for nonideal gases (31), the ratio of molecular polarizability to molecular volume (14), and others.

While these several functional relations between molecular properties and anesthetic potency were primarily derived to describe relatively powerful anesthetic agents, they invited application to weaker anesthetics, including the gases here under consideration. Perhaps the earliest attempt at such extrapolation was that by Carpenter in 1954 (30). On the basis of oil solubility measurements, this investigator predicted a net anesthetic effect for He between 10% and 15% as strong as that for N_2. Subsequent extrapolations based on other molecular properties predicted values of 16% (partition coefficient), 20% (van der Waals a), and 8% (polarizability to molecular volume ratio). All of these predictions imply a small but positive anesthetic effect for He; by averaging the available data, the most likely predicted value for the anesthetic potency of He relative to N_2 is about 0.14 with a range from 0.08 to 0.20. This value, then, would appear to provide an acceptable estimate of ${}_a\pi_{He}/({}_a\pi_{N_2} + {}_a\pi_p)$ for use in Eq. *3*.

Attempts to determine directly the net effective narcotic potency of He by itself—i.e., $({}_a\pi_{He} + {}_a\pi_p)/({}_a\pi_{N_2} + {}_a\pi_p)$—have been uniformly unsuccessful. As shown by Zaltzman (17) and by Brauer and co-workers (18), instead of becoming anesthetized, mice and monkeys compressed under heliox atmospheres undergo tremors, convulsions, and, as shown subsequently, death (23, 51). Similar results were obtained in newts (20).

To obviate this difficulty, Brauer and Way (31) resorted to the use of series of binary gas mixtures containing He and N_2 in varying proportions and showed that addition of He increased the amount of N_2 required to induce anesthesia. The relations were found to obey the hypothesis of linearity over a substantial range of N_2 concentrations, and they led to an estimate for the negative effective narcotic potency for He relative to N_2 of between 0.05 and 0.08. Lever and associates (20), using a single partial pressure of N_2 in newts, found 100 ATA He to reverse the anesthetic effects of 34 ATA N_2. More recently, Halsey et al. (32), using the system He-N_2O, obtained data confirming Brauer and co-workers' observations regarding the applicability of linear relations and regarding the antagonistic effects of He and N_2O. Their data imply a negative relative anesthetic potency for He equivalent to 0.7% that of N_2O. Taking the mean of published values for the ratio of narcotic potencies of N_2O to N_2 as about 28 (cf. Ref. 31), the data of Halsey and associates imply a rather greater negative potency for He than those of Brauer and co-workers, the value of $({}_a\pi_{He} + {}_a\pi_p)/({}_a\pi_{N_2} + {}_a\pi_p)$ thus computed being about -0.16. Reasons for the difference in these results are not clear but may reside in differences in chamber temperature or in the method used to establish the endpoint. In any event, all three series of data imply a small negative net anesthetic potency, and for the time being the most probable numerical value for $({}_a\pi_{He} + {}_a\pi_p)/({}_a\pi_{N_2} + {}_a\pi_p)$ would appear to be the mean of the results of the two determinations, i.e., -0.11, with a range of values from -0.05 to -0.16.

Thus, for helium we have obtained ${}_a\pi_{He} \div ({}_a\pi_{N_2} + {}_a\pi_p) = 0.14$ and $({}_a\pi_{He} + {}_a\pi_p) \div ({}_a\pi_{N_2} + {}_a\pi_p) = -0.11$, and we can compute, from Eq. *3*: $Q = (-0.11) + (-0.14) = -0.25$ and $1 \div$

$(1 - Q) = 0.80$. Therefore, ${}^{N_2}_{a}R_p = -0.25 \div 1.25 = -0.20$ for the (negative) anesthetic effect of hydrostatic pressure, and ${}^{N_2}_{a}R_{He} = -0.16 \cdot 0.80 + 0.20 = 0.072$ for helium.[1]

Insertion of these results into Eq. *4* yields the relation

$$ {}^{N_2}_{a}R_i = 0.80 \cdot ({}_a\pi_i + {}_a\pi_p) \div ({}_a\pi_{N_2} + {}_a\pi_p) + 0.20 \qquad (4a) $$

as a basis for deriving relative intrinsic anesthetic potency values from experimental data for other gaseous anesthetics to which the hypothesis of linear additivity is applicable.

d. Neon

Miller and Miller (52), tabulating information available in 1974, concluded that neon must be a very feeble anesthetic, though probably more potent than helium. They estimated a neon partial pressure for anesthesia in excess of 140 ATA. Kent et al. (33) in 1976 performed direct tests using a series of Ne-N_2O mixtures and found that Ne, like He, not only does not decrease P_{N_2O} required to produce anesthesia, but rather increases it slightly—i.e., that it too exerts a negative net anesthetic effect. The effect is small, 100 ATA Ne corresponding to an increase in the required P_{N_2O} by only about 0.1 ATA. Using the factor 28 as above to translate this figure to a nitrogen-equivalent anesthetic potency, one arrives at $({}_a\pi_{Ne} + {}_a\pi_p)/{}_a\pi_{N_2} + {}_a\pi_p) = -0.03$. Inserting this value into Eq. *4a*, ${}^{N_2}_{a}R_{Ne} = 0.18$ for the intrinsic narcotic potency of neon relative to N_2.

e. Hydrogen

Lazarev in 1943 carried out a small number of experiments concerning the anesthetic potency of hydrogen (48). He was led to infer that this gas is a feeble anesthetic, less than half as potent in this respect as nitrogen. Brauer and co-workers (18, 31) in 1970 and 1971 reported that in monkeys and mice attempts to measure the anesthetic partial pressure of hydrogen directly, as in the case of helium, were frustrated by the appearance of high pressure convulsions. These, however, did not occur in 100% of the animals, and at an average pressure higher than in the case of helium, supporting the prediction, on the basis of physical properties, that H_2 would prove a substantially more potent anesthetic than He. As in the case of He, these workers therefore studied the effects on mice of compression in a series of H_2-N_2 mixtures of increasing N_2 content (31). Relations between P_{N_2} and P_{H_2} at anesthesia were found to be linear, and application of Eq. *2* resulted in an estimated value for ${}^{N_2}({}_a\pi_{H_2} + {}_a\pi_p)/({}_a\pi_{N_2} + {}_a\pi_p)$ of 0.26 for the mouse. Miller and co-workers (53) in 1973 studied the effect of compression of newts in hydrox and reported failure to obtain anesthesia at 200 ATA. Finally, Kent et al. (33) in 1976 performed a series of studies using H_2-N_2O mixtures in a manner analogous to that of Brauer et al. for H_2-N_2. Their data confirm the linearity of P_{H_2}-P_{N_2O} relations at anesthesia and permit the estimate that 100 ATA H_2 lessen the amount of N_2O required by 0.8 ATA. Recalculating to nitrogen equivalence as before, one obtains a value of 0.22 for the net narcotic potency of H_2 relative to N_2, in reasonable agreement with the value of 0.26 derived from the data of Ref. 31. Taking the mean of these two values, the best estimate for $({}_a\pi_{H_2} + {}_a\pi_p)/({}_a\pi_{N_2}$

[1] A recent paper [Fumariuk ZW Jr., Dodson BA, Miller KW. Is helium pressure equivalent to hydrostatic pressure: an in vivo test of the critical volume hypothesis. Fed Proc 1982;41:1620 (Abstr).] uses pressure reversal of urethane in tadpoles by hydraulic or helium compression to compare relative potencies of these agents. Helium was only 70% as effective as hydrostatic pressure in this respect, in very satisfactory accord with the values derived from mammalian data in the text and included in Table 2.

$+ {}_a\pi_p$) at present, therefore, is 0.24. Insertion of this value into Eq. *4a* then yields ${}^{N_2}_{a}R_{H_2} = 0.39$ for the relative intrinsic narcotic potency of H_2.

f. Summary of data relating to interaction of inert gas narcosis and hydrostatic pressure

The results of the analyses presented so far can be conveniently summarized in the form of Table 2, which compares the estimates of net anesthetic potency, derived experimentally for the five metabolically inert gases mentioned so far with their intrinsic anesthetic potencies as derived by the application of Eqs. *1–4* to these data. The net effect of these manipulations is perhaps best appreciated by plotting the logarithm of the estimated narcotic potency against the several physical properties in the manner shown by previous workers to correlate with corresponding functions for more potent anesthetics. Figure 1 illustrates these results for the case of the parameter preferred by Featherstone and Muehlbacher (14), i.e., polarizability ÷ molecular volume. Such a plot shows that experimentally determined net potencies for helium and neon cannot be fitted into such a plot at all (logarithms of negative numbers imply $\log 0 = -\infty$ for some intermediate value, and are themselves imaginary numbers), while that for hydrogen falls substantially below the extrapolated straight-line relation prevailing for the more potent anesthetics. By contrast, the intrinsic anesthetic potencies of the last column of Table 2 show a close fit to the straight-line relation, down to helium itself. Similar relations can be demonstrated for oil solubility, for constants *a* of the van der Waals equation, and for the oil-water partition coefficients. Relative intrinsic anesthetic potency for helium derived by extrapolation from these several curves yields values of 0.07, +0.14, +0.12, and +0.05, respectively, with a mean value of 0.10, close enough to the value of 0.07 deduced above from the application of Eq. *4* to the experimental data. In addition to thus admitting of a unified treatment for all metabolically inert gaseous anesthetics, the results of Table 2 have the advantage that for the first time they provide a measure of the comparative effect of hydrostatic pressure as such on a specific biological response.

The data of Table 2 thus define a clear-cut and distinctive pattern for the interaction of inert gas anesthetics with high hydrostatic pressures with respect to anesthesia: all inert gases are found to possess some narcotic effect; the magnitude of the effect increases regularly; the progression is helium, neon, hydrogen, nitrogen; and pressure was found to antagonize the effect of all of these agents with a potency intermediate between those required to neutralize the narcotic effects of neon and hydrogen.

TABLE 2

ESTIMATES OF NET AND INTRINSIC ANESTHETIC POTENCIES OF THE LIGHTER GASES, AND OF THE EFFECT OF HYDROSTATIC PRESSURE THEREON

Agent	Relative Net Anesthetic Potency*	Relative Intrinsic Anesthetic Potency**
Hydrostatic Pressure	—	−0.20
He	−0.11	0.07
Ne	−0.03	0.18
H_2	0.24	0.39
N_2	1.00	1.00
N_2O	28	22.6

Common units of pressure throughout. *$({}_a\pi_i + {}_a\pi_p)/({}_a\pi_{N_2} + {}_a\pi_p)$. **${}_a\pi_i/{}_a\pi_{N_2} = {}^{N_2}_{a}R_i$

In deriving these results, hypothetical apparatus has been kept to a minimum: the assumptions made are those underlying Eq. *1*. They include: 1) the assumption of linear additivity of anesthetic effects of each gas in a gas mixture; 2) the assumption that the antagonistic action of pressure is directly proportional to the total hydrostatic pressure applied and can be added to the inert gas effect to yield the net anesthetic effect of a given gas or gas mixture; 3) the assumption implicit in the constancy of ϵ, i.e., that when whatever changes induced in the animals by the superposition of inert gas and high pressure effects attain a critical value, anesthesia will supervene. It is implied that this critical value will be constant for all conditions to which Eq. *1* is applicable. The several experimental series testing Eq. *1* showed that for a variety of binary inert gas systems over a range of pressures from 1 ATA to about 100 ATA, assuming a common value of ϵ yields concordant results. Thus those data confirm the validity of all three of the above hypotheses within the pressure limits set and justify the formal manipulations that have led to the results incorporated in Table 2 and Fig. 1.

The conceptual apparatus thus developed provides a skeleton upon which any proposed mechanism to account for the interaction of hydrostatic pressure with inert gas anesthesia must be built. A number of such hypotheses have been proposed, the most successful among them to date probably being the "critical volume hypothesis," enunciated by Miller and coworkers (53) in the early 1970s. This hypothesis focuses its attention on some lipophilic cell constituent and assumes that the volume changes in this constituent are brought about by either compression under the action of hydrostatic pressure or expansion by the dissolution in its substance of inert gas. Anesthesia is presumed to supervene when the volume increase has

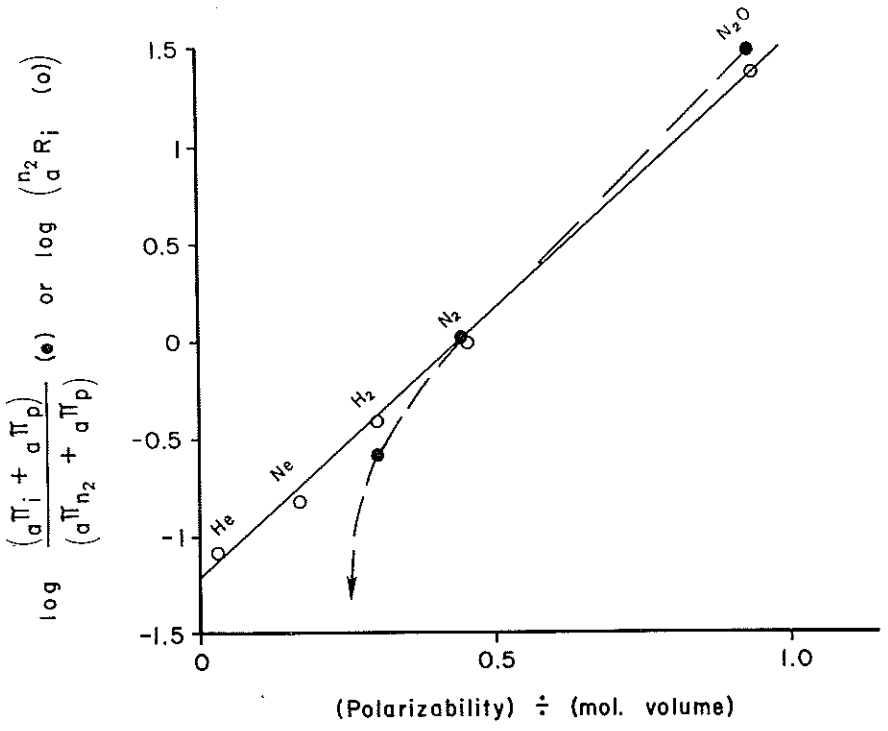


Fig. 1. Anesthetic potencies of He, H_2, N_2, and N_2O as a function of polarizability ÷ molecular volume. ●, Relative effective potencies $[({}_a\pi_i + {}_a\pi_p)/({}_a\pi_{N2} + {}_a\pi_p)]$. ○, Relative intrinsic potencies $({}_a\pi_i/{}_a\pi_{N2} = {}^{N2}_{a}R_i)$, ———, extension of regression line from data of Ref. 14.

attained a critical value. This initial expansion can be calculated from the partial molar volumes and solubilities of the several inert gases in any solvent for which data are available. Traditional solvent models such as olive oil work well. Alternatively, a solvent may be chosen by optimizing the fit of fully fluorinated gaseous anesthetics with the inert gases. Once the isonarcotic expansions for gas mixtures for all pressures have been calculated, values of compressibility can be attained, and the results provide an acceptable description of the experimental facts within the region over which Eq. *1* is applicable. The relative anesthetic potencies derived by this model are in the same ranking order as those derived herein for the linear model. Indeed, the two models are formally equivalent but differ in the explicit mechanism assumed by the critical volume hypothesis, which enables more detailed predictions to be made. It is possible to define conditions at the site of action that permit $({}_a\pi_{He} + {}_a\pi_p)$ to be positive (e.g., see Ref. 54).

The region of applicability of Eq. *1* terminates somewhere between 100 and 130 ATA. Since corrections for nonideality appear too small to account for this breakdown of Eq. *1* (cf. Ref. 53), one must assume either that the hypothesis of a constant ϵ in Eq. *1* (or of a constant value for compressibility of the critical phase in terms of the critical volume hypothesis) ceases to apply above the pressure range where Eq. *1* applies, or that other phenomena supervene to complicate the results. As has been pointed out in the past (55), anesthesia, the manifestation that is used as an endpoint in these experiments, is a complex event that to date has defied reduction to phenomena seen at the cellular level. It may well be that it is this complexity that underlies the observed breakdown. Even in the early development of data to support the critical volume hypothesis, it was realized that phenomena other than anesthesia might account for some of the quantitative relations observed (20), and it is these phenomena, then, to which it now seems appropriate to turn our attention.

2. High pressure convulsions

a. Historical background

Changes in locomotor behavior of animals exposed to high pressure were probably the earliest manifestations of high pressure effects to come to the attention of investigators (1). In vertebrates, USSR investigators in the early 1960s noted the occurrence of tremors and convulsions in animals exposed to helium at high pressures (17), but they misinterpreted these phenomena as manifestations of helium toxicity. In the U.S. in 1965, studies intended to elucidate narcotic relations between helium and hydrogen revealed convulsions—rather than anesthesia—and on the basis of differences in the effects of these two gases, these effects were interpreted as high pressure convulsions, development of which was assumed to be partly antagonized by narcotic effects of hydrogen (cf. Refs 15, 18). High pressure spasms or paralysis was also observed in newts in the course of studies of pressure reversal of anesthesia and, here again, evidence was obtained that inert gas narcosis may protect the animals against these effects of high pressure (20). This early stage of investigation was rounded out by observations in liquid-breathing mice showing that convulsions can be elicited by purely hydraulic compression at pressures that are roughly comparable to those required in heliox atmospheres to elicit similar convulsions (21). It appeared clear on the basis of these several investigations that high pressure, as such, is capable of producing convulsions in liquid-breathing animals, that compression in helium and hydrogen elicits convulsions in mice and monkeys, and that admixture of some anesthetically effective gas, such as nitrogen or nitrous oxide, to heliox

atmosphere can substantially increase the pressures at which high pressure convulsions occur. In the sequel it became clear that high pressure convulsions can be elicited in representatives of all vertebrate orders (56), and that, at least in some species of mammals, high pressure convulsions can be categorized into two different types that respond very differently to different drugs and, to some extent, to general anesthetic agents (57). Because the bulk of the data pertinent here are confined to one component of the convulsion phase of the high pressure neurological syndrome, in the discussion following below, the term *convulsions* throughout is intended to imply the first high pressure convulsive seizure event observable in a given animal during compression; thus in rodents attention is focused on what Brauer and associates term *Type I HPNS seizures* (57).

b. Liquid-breathing animals

As a first step in trying to define more sharply the dose-response relations governing the interaction of hydrostatic pressure with metabolically inert lighter gases in the production of high pressure convulsions, it is in order to consider relations between the effects of hydraulic compression and of compression in helium. Such experiments can be carried out either with aquatic animals or in mammals converted by proper manipulation to liquid breathing.

Lever and co-workers (20) found suppression of the rolling response in newts at pressures in the neighborhood of 200 ATA and recognized that this effect was associated not with anesthesia but with spasms or paralysis ("the animals all balled up"). Mean threshold pressures for this event at 25°C were 210 ATA under helium and 190 ATA in hydraulic compression. Beaver and co-workers (34), using the newt *Notophthalmus viredescens,* in the same family but of a different species from that used by Lever and co-workers, compared convulsion threshold pressures in animals compressed hydraulically with those seen in animals compressed in water saturated with helium at the test pressure. Mean convulsion thresholds for the two types of compression at 21°C were indistinguishable from each other, amounting to 195 ± 12 ATA at a compression rate of 40 atm/h, and 197 ± 8 ATA at a compression rate of 1000 atm/h. Both studies imply that anticonvulsant effects of helium under these conditions are small or nonexistent. For *Symphurus plaguisa,* a flatfish lacking a swim bladder, convulsion threshold pressures at 30°C for this species under conditions of hydraulic compression at 40 atm/h were 77 ± 9 ATA (cf. Ref. 34); upon compression in helium-saturated water, the animals convulsed at 86 ± 5 ATA (R.W. Beaver and R.W. Brauer—unpublished observation). The difference between the two threshold pressures lacks statistical significance.

Barthèlemy and collaborators (27) developed an ingenious arrangement that allowed them to vary total pressure and inert gas pressure (up to a maximum of the total pressure used) independently of one another. Although their work was carried out using trout, a fish that has a swim bladder, the authors contend that their data were not affected by the presence of such a gas-filled structure, inasmuch as results obtained in cystectomized trout, or in trout with catheterized swim bladders, could not be distinguished from those obtained in intact animals. They obtained observational, electrophysiological, and survival data on animals compressed at various partial pressures of helium and nitrogen.

In these trout, survival times were greatly reduced by exposure of the animals to pressures between 100 atm and 150 atm. Animals exposed in water saturated with helium to various partial pressures survived significantly longer at all pressures tested, and survival times increased progressively as helium partial pressures were increased. At nitrogen partial pressures up to between 40 and 60 atm, a qualitatively similar situation appears to obtain, animals in nitrogen-saturated water surviving longer at any given pressure than animals in water containing less

than 1 atm of nitrogen; here again there is a suggestion of increased survival times with increasing partial pressures of nitrogen at a hydrostatic pressure of 101 atm; at higher hydrostatic pressures these effects become blurred. Whatever the nature of the effect responsible for the short survival times at pressure, there appears to be little or no difference between the effectiveness of helium and of nitrogen, respectively, in blocking or reversing it. In the case of nitrogen, at partial pressures beyond 40 atm, survival times at all pressures decreased again, presumably as a result of excessively deep anesthesia. In the absence of more detailed quantitative and physiological data to clarify what phsiological events were being observed, these results, while of interest, do not allow inferences pertinent to the present discussion.

An alternative to working with naturally aquatic or amphibious animals is to use mammals that normally breathe air converted by appropriate manipulation to liquid breathing along the lines of the title of one of Kylstra's earliest papers, "Of mice as fish" (58). This has the advantage of making use of an animal with substantially higher brain development than is available among naturally water-breathing species. One does so, however, at the expense of utilizing an animal whose physiological state differs very substantially from that to which the regulatory mechanisms inherent in the animal are geared: in particular, there is a severely limited rate of carbon dioxide elimination, resulting in CO_2 accumulation; markedly hypothermic body temperatures occur that are unavoidable if the animals are to survive under the conditions of limited respiratory exchange; and there is an extremely limited respiratory reserve to cope with any increase in oxygen consumption secondary to changes in activity. The idea of utilizing such a preparation for comparison of the effects of helium and hydraulic compression suggests itself readily and was incorporated into an early report by Kylstra and co-workers (21) concerning high pressure convulsions in mice breathing fluorocarbon. The authors confirmed the conclusions of Ref. 18 based on studies in mice subjected to high heliox pressures—namely, that these motor disturbances are indeed high pressure convulsions; they established that such seizures can be demonstrated in liquid-breathing animals in the absence, or near-absence, of helium. As reported, animals in hydraulic compression convulsed "between 50 and 100 ATA," while animals compressed on heliox convulsed "between 69 and 86 ATA." Beyond their qualitative import these data, unfortunately, do not contribute to our present discussion.

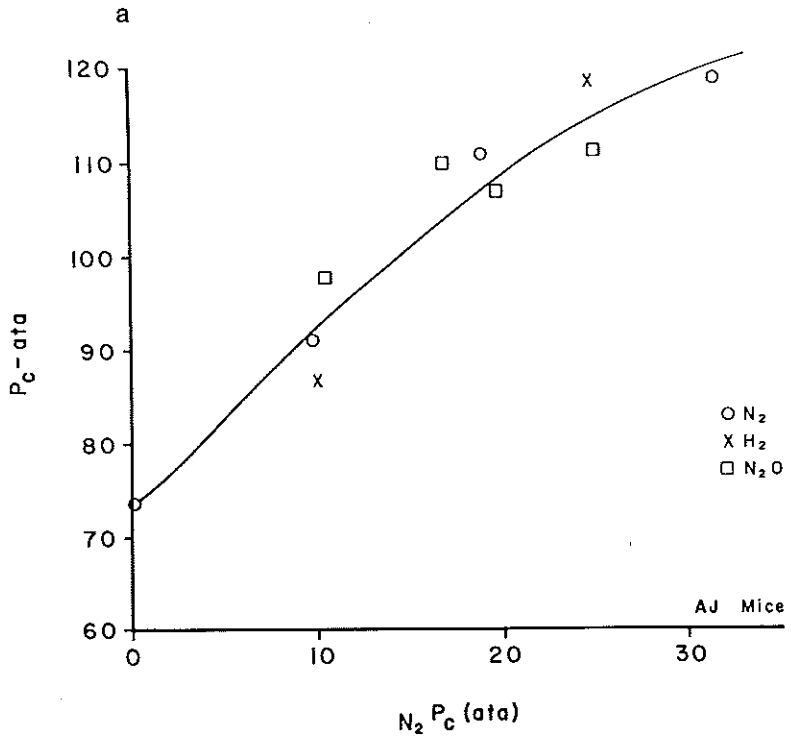
A much more careful study was undertaken by Lundgren and Örnhagen (24), who carried out their studies over a range of rectal temperatures from 17°C to 31°C and reported convulsion onset pressures and maximum tolerated pressures. Changes in compression rates over the range from 60 to 260 atm/h produced no recognizable changes in convulsion onset pressures in their mice at 21°C. At a compression rate of 120 atm/h, and at rectal temperatures anywhere between 21°C to 27°C, mean convulsion onset pressure was 85 ± 15 ATA, the threshold being determined visually and confirmed electromyographically. These data can be compared with results obtained in another laboratory: helium-breathing hypothermic mice of the same strain (35) compressed at 80 atm/h and at ambient temperatures of 25°C to 27°C and, consequently, with rectal temperatures between 26°C and 29°C, had threshold pressures for the first HPNS convulsion of 88 ± 7 ATA. Comparison of these data with those of Lundgren and Örnhagen (24) fails to reveal any significant difference between the effects of helium and of hydraulic compressions. A direct comparison of both heliox-breathing and of liquid-breathing mice in the same laboratory clearly would be desirable to test the validity of this conclusion.

In reference to the several studies reported in which a direct comparison between compression under helium and hydraulic compression on the same type of test object has been feasible, it would appear that the burden of the studies must be that significant differences between the two modes of compression were not observed. The conclusions to be drawn at the present

time, therefore, would appear to be that, in the terminology used in the preceding section, the intrinsic anticonvulsant potency of helium with respect to hydrostatic pressure convulsions is indistinguishable from zero.

c. Estimation of relative intrinsic anticonvulsant potencies

Turning now to the question of intercomparison of anti–high-pressure convulsant effects of other metabolically inert gases of interest here, it is first necessary to consider the basis for such comparison. As pointed out in the discussion of the antagonism between high pressure and anesthesia, direct experimental determination of physiologically meaningful relative intrinsic potency values for any factors directly affecting a given biological response presumes that the experimental data can be described in terms of the hypothesis of linear additivity of all these effects. If this hypothesis has to be rejected, the set of potency values will cease to be constant, and comparison of different agents with each other must follow a different course. As in the case of inert gas narcosis, the question whether the hypothesis of linearity can be applied to the interaction of inert gases with hydrostatic pressure in eliciting high pressure convulsions can be answered by reference to appropriate comparisons of convulsion threshold pressures of a given species in series of binary gas mixtures. Such data are available for the system He-N_2 for three well-defined mouse strains (cf. Refs. 22, 51). Plots of convulsion threshold pressures as a function of N_2 partial pressure at the onset of convulsions are shown in Fig. 2*(a–c)* and at once reveal marked strain differences in the shape of the dose-response curve. Figure 2*d* shows plots of helium partial pressure vs. nitrogen partial pressure, both at the times of onset of a convulsion. As shown previously (cf. Eq. *2*) such plots should be



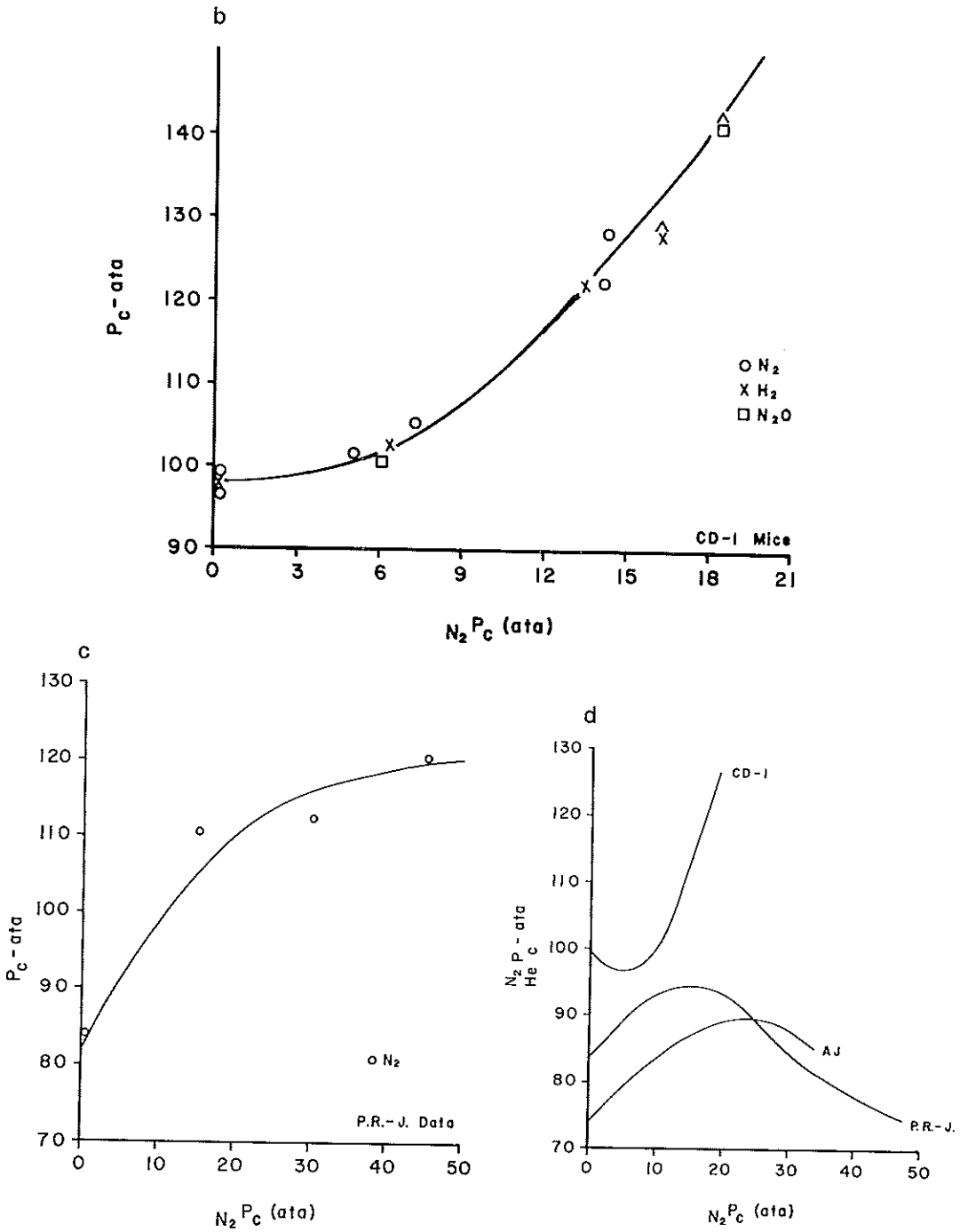


Fig. 2. Dose-response curves for anti-HPNS convulsant effect of metabolically inert gases (Type I HPNS seizures in mice). *a*. Convulsion threshold pressures as a function of partial pressure of H_2, N_2, or N_2O in CD-1 mice. Scales of abscissas adjusted to superpose the families of data for the several gases, the scaling factors being those shown in Table 3 (data from Ref. 22). *b*. Same, but for AJ mice (data from Ref. 22). *c*. Same, but for N_2 only (data from Ref. 51). *d*. Test for adherence to linearity; helium partial pressure at convulsion onset plotted against nitrogen partial pressure at the same point for the sets of data shown in *a*, *b*, and *c*.

straight lines if the hypothesis of linearity applied, and their slopes would provide measures of the ratio of the relative net potencies of the two components of the gas mixture. Clearly, the three strains illustrated in Fig. 2 do not satisfy the requirements of the linearity hypothesis; moreover, they differ profoundly among themselves in the dose-response patterns revealed.

While the data clearly preclude using the linearity hypothesis for the analysis of high pressure convulsion data, an alternative mode of analysis is suggested by the data of Ref. 22 included in Fig. 2, *a* and *b:* the experiments in question had been designed to compare three gases besides He—i.e., H_2, N_2, and N_2O—with a more than 100-fold span of anesthetic potencies (cf. Ref. 31). On analysis it became plain that although the two mouse strains yielded rather different dose-response curves, the data for the effects of the several gases on each strain could be superposed by adjusting the scale of the abscissa, leaving the ordinate unaltered. Figure 2, *a* and *b,* shows the results of such superposition. The excellent fit of the several dose-response curves thus obtained within a given strain has important implications: when one compares pairs of binary gas mixtures containing helium and a second and pharmacologically active gas, i.e., He-A and He-B, and of such composition that both mixtures will produce seizures at the same total pressure, the scaling property of the data implies that the ratio of the partial pressures of A to B will be substantially constant over the entire range of convulsion threshold pressures for which the similarity in dose-response curves applies. Without making any further assumptions than that the net effect observed in each case under these conditions is the sum of a pressure-determined effect and an effect determined by the inert gas, and retaining the definition that the effective potency of the pharmacologically active gas component in any such binary mixture is the ratio of the effect actually produced (for instance, ΔP_c) to the partial pressure of gas producing the effect, we can cast the conclusion just arrived at in the following form:

$$\begin{aligned}(\Delta P_c) &= ({}_c\pi_A \cdot [A] + {}_c\pi_{He} \cdot [He]_A) \cdot P_c + {}_c\pi_p \cdot P_c \\ &= ({}_c\pi_B \cdot [B] + {}_c\pi_{He} \cdot [He]_B) \cdot P_c + {}_c\pi_p \cdot P_c.\end{aligned} \tag{5}$$

In these relations the terms ${}_c\pi_p \cdot P_c$ are the same in both equations under the conditions of comparison, and the terms ${}_c\pi_{He} \cdot [He]$ can be ignored, since we have already shown that ${}_c\pi_{He}$ is indistinguishable from zero. Eliminating ΔP_c, therefore, we obtain:

$$\frac{{}_c\pi_A}{{}_c\pi_B} = \frac{[B]}{[A]} \tag{6}$$

for the convulsion threshold pressure P_c. The fact that simple adjustment of the abscissa of Fig. 2, *a* and *b,* allows superposition of the data for the several gas mixtures implies that this ratio will be constant for a given pair of nonhelium gases over the entire range of convulsion threshold pressures to which the statement applies. Thus, ${}_c\pi_A \div {}_c\pi_B$ turns out to be uniquely defined for this set of data for each of the two strains of mice. Specifically, these relations imply that the relative anticonvulsant potency of a gas referenced to N_2 (i.e., ${}^{N_2}_{c}R_{H_2}$ or ${}^{N_2}_{c}R_{N_2O}$) can be uniquely defined and measured for either strain of mice, regardless of what changes the absolute anticonvulsant potencies ${}_c\pi_i$ may undergo as gas composition is varied. The required mean values for the scaling factors are most convincingly derived from a simple transform of the raw–dose-response data, replotting these to show P_c as a function of $\log {}_iP_c$ and determining mean displacment relative to the N_2 dose-response curve of the H_2 and N_2O dose-response curves on the $\log {}_iP_c$ axis. Finally, it should perhaps be noted that the procedure used here is strictly analogous to that used in comparing drugs characterized by nonlinear dose-response curves that satisfy the proportionality requirement (59).

The results of the application of these procedures to the data for high pressure convulsions in two mouse strains (AJ and CD-1) illustrated in Fig. 2*a* and *b* are presented in Table 3. Despite the marked differences in the shapes of the dose-response curves for the two strains, the patterns of variation of intrinsic anticonvulsant potency with the chemical nature of the gas are very similar in the two models and, it may be noted, also closely resemble the patterns derived in the preceding section for reversal of anesthesia.

To complete the description of the dose-response pattern for high pressure convulsions in the present context, information is needed concerning relative potency of hydrostatic pressure as such with respect to elicitation of high pressure convulsions. It is obvious that in view of the nonlinear character of the dose-response curves, it may be impossible to establish any single value for the anticonvulsant potency of hydrostatic pressure that could apply to the response curves for the whole dose. In two strains that have been described [AJ and the strain used by Rowland-James et al. (51)] it would appear, however, that dose-response relations at low partial pressures of the pharmacologically active gas component approximate linearity. In these cases, a meaningful though restricted estimate of ${}_c\pi_p$ can be derived by applying to the data a variant of Eq. *3* (see footnote to Table 3). The data of Rowland-James et al. (51) and, for the AJ strain the data from Brauer et al. (22), thus yield relative intrinsic anticonvulsant potencies of -0.56 and -0.63 in very satisfactory agreement. In the case of CD-1 mice, the dose-response curve at low partial pressures of the pharmacologically active gases is anomalous and nearly flat (Fig. 2*a*). Application of the relations derived from the hypothesis of linearity here provide the biologically meaningless value of -1.19 for the potency of hydrostatic pressure relative to nitrogen (cf. negative slope of the corresponding curve in Fig. 2*d* near ${}_{N_2}P_c = 0$), implying that compression in nitrogen alone could produce high pressure convulsions. In actual fact, at nitrogen partial pressures greater than 10 ATA, admixture of nitrogen to helium atmospheres results in substantial increases in high pressure convulsion thresholds (also in CD-1 mice). A more meaningful estimate of relative anticonvulsant potency of hydrostatic pressure in this strain, therefore, might be derived by computing a mean slope $\Delta P_c/{}_iP_c$ for the data for nitrogen partial pressures greater than 10 atm. The results of such calculation yield a value for ${}_c\pi_p/{}_c\pi_{N_2}$ of about 0.6 (included in Table 3 in parentheses), closely in accord with the values for the two mouse strains with less-complex dose-response curves.

TABLE 3

ESTIMATES OF RELATIVE CONVULSANT AND ANTICONVULSANT POTENCIES OF HYDROSTATIC PRESSURE AND OF METABOLICALLY INERT GASES

	AJ Mice ${}_c^{N_2}R_i$	CD-1 Mice ${}_c^{N_2}R_i$	Rowland-James et al. (51) ${}_c^{N_2}R_i$
Hydrostatic Pressure	-0.56*	-1.19* (-0.63)**	-0.63*
He	—	0	—
H_2	0.45†	0.23†	—
N_2	1.00†	1.00†	1.00
N_2O	53†	30†	—

*From slope of curves ${}_{He}^{N_2}P_c$ vs ${}_{N_2}P_c$ near ${}_{N_2}P_c = 0$, and the relation ${}_c^{N_2}R_p = {}_c\pi_p \div {}_c\pi_{N_2} = {}_{N_2}P_c \div ({}_{He}P_c - {}_{He}^{N_2}P_c - {}_{N_2}P_c)$ derived from the basic equation $({}_c\pi_{He} + {}_c\pi_p) \cdot {}_{He}{}^iP_c + ({}_c\pi_i + {}_c\pi_p) \cdot {}_iP_c = \epsilon_c$, and the assumption ${}_c\pi_{He} = 0$, justified in text. Since ${}_c\pi$ are conceived as *anti*convulsant potencies, ${}_c\pi_p$ (and consequently ${}_c^{N_2}R_p$, will be negative. **Same calculation but for mean slope $\Delta{}_{He}^{N_2}P_c \div \Delta^{N_2}P_c$ for $P_{N_2} >$ 10 ATA and $P_{N_2} = 0$. †From mean displacement along log ${}_iP_c$ axis of P_c vs log ${}_iP_c$ curves (see text).

d. Summary of results regarding high pressure convulsions

The preceding review of the limited amount of data available concerning interaction of lighter metabolically inert gases with high pressure convulsions permits a number of conclusions:

1. High pressure convulsions can be elicited in representatives of all vertebrate orders tested.
2. With the exception of helium, all other metabolically inert gases tested antagonize the convulsant effects of high pressures.
3. The anticonvulsant effect of helium is not distinguishable from zero and on the basis of available data is unlikely to exceed a potency 5% as great as that of nitrogen.
4. Dose-response curves relating convulsion threshold pressures in mice with partial pressures of nonhelium atmosphere components do not satisfy the requirements of the linear additivity hypothesis. The curves for different gases in two mouse strains can be superposed by adjusting the linear concentration scales of the abscissa for the several inert gases, and this permits unambiguous estimation of relative anticonvulsant potency values for the several gases.
5. Because of the nonlinear character of the dose-response curves relative convulsant potency of hydrostatic pressure may vary with varying composition of the chamber atmosphere; estimates for relative potency of hydrostatic pressure can be derived for appropriately selected segments of the dose-response curves, to yield values relative to N_2 around 0.6.
6. The data reveal a potency pattern similar to that derived for pressure reversal of anesthesia except for 1) somewhat higher relative potency of hydrostatic pressure, and 2) the more marked departure of the dose-response relations from linearity.

3. High pressure bradycardia

a. Historical background

In the course of their experiments on liquid-breathing mice, Lundgren and Örnhagen noted pronounced bradycardia that appeared to be correlated with the pressure to which their animals were subjected (24). Quantitative interpretation of these results was complicated by the relatively unstable hypothermic conditions of the animals, unavoidable in liquid-breathing mice, and by the great sensitivity of heart rate to tissue temperature. Similar bradycardia has also been observed in helium-breathing mice (60). While the general form of the relation between heart rate and pressure was similar in the two series of experiments, the liquid-breathing animals were tested at much lower body temperatures, had correspondingly low heart rates at 1 ATA, and at any given pressure showed proportionally much more pronounced bradycardia than the helium-breathing animals. In the absence of data for overlapping body temperatures, it is not possible to evaluate the role of helium in modifying the magnitude of pressure bradycardia in these intact animals.

Following up on the observations by Lundgren and Örnhagen (24) concerning heart rate changes, Örnhagen and Hogan (36) turned to an investigation of pressure effects on the isolated mouse sinus node preparation where problems of temperature effects on beat frequency could be avoided by careful technique. The preparation used was the superfused sinus node, and experimental arrangements were devised with the intent of excluding helium from the perfusion medium. The experimental design utilized was presently found to contain a source of helium leakage, and the investigators concluded that the original experiments conducted with this arrangement would have involved at least 50% saturation of their perfusion medium with

helium. The leak was corrected and a second set of data was obtained in which, presumably, the original intent was realized—i.e., exposing the preparation to hydraulic pressure in the absence of helium (36). The results of the two series of experiments showed that at 150 ATA, cardiac frequency was decreased by 43% of the 1-ATA rate under hydrostatic pressure alone and decreased by 36% in the presence of helium, with standard errors of +4.5 and +2.6, respectively. The difference in results between the two series failed to be of statistical significance ($P<0.1$). Örnhagen carried these experiments further under more rigorous conditions that permitted comparing preparations exposed to purely hydraulic compression with preparations exposed to compression under conditions assuring saturation of the medium with helium or other inert gases (25). Under these conditions, the addition of 140 atm of helium to a perfusion solution maintained in bradycardia at 150 atm hydrostatic pressure always resulted in a slight (though statistically not secured) increase in heart rate. Significant additional slowing of beat frequency was, however, observed regularly upon removal of helium—i.e., upon return to purely hydrostatic compression. Alternation of helium-free and helium-saturated perfusates produced unmistakable increases in beat frequency during periods of helium exposure. Thus, in our opinion, as in Örnhagen's, the data leave little doubt that a real protective effect of helium against high pressure sinus bradycardia was being observed. Similar results were obtained for nitrogen and nitrous oxide, at lower partial pressures.

Quantification of the results of these experiments must be approached cautiously, since the experimental results do not contain serial data to permit comparison with each other of different levels of more than one potentially active gas in any given experiment. The data suggest that the beat frequency during compression fell at a rate approximately proportional to the increase in pressure, and (at least in the case of nitrogen) data for partial pressures from 35 to 140 ATA do not contradict the hypothesis that the net increase in beat frequency attributable to the inert gas at a constant total pressure of 150 atm is linearly related to nitrogen partial pressure. Taken together, these characteristics have suggested that high pressure bradycardia data can profitably be analyzed on the basis of the hypothesis of linearity as applied to a quantifiable response, in this case beat frequency.

On this hypothesis, then, estimates of the relative potencies for the several gases can therefore be derived by setting up the appropriate proportionalities between the intensity of observed effect and changes in partial pressure, or hydraulic pressure, corresponding to a particular level of displacement or beat frequency:

$$ {}^{N_2}_{b}R = \frac{{}_b\pi_i}{{}_b\pi_{N_2}} = \frac{P_{N_2}}{P_i} \cdot \frac{\Delta^i b}{\Delta^{N_2} b} \quad \text{and} \quad \frac{{}_b\pi_p}{{}_b\pi_{N_2}} = \left(\frac{\Delta^p b}{\Delta^{N_2} b - \Delta^p b}\right) \cdot \frac{P_{N_2}}{P} \qquad (7) $$

where $\Delta^i b$ is the change in beat frequency attributable to gas i at a given total pressure P.

b. Analysis of the data

This interpretation of the data presumes, as we do, that inherent changes in beat frequency of the preparation occur with time at a steady rate throughout the period during which the preparation is exposed to any given pressure, and data are, therefore, computed in each case by noting displacements against this basal frequency line as drawn. The quotient ${}_b\pi_i/{}_b\pi_p$ has thus been calculated for helium, neon, nitrogen, (3 values), and nitrous oxide (3 values, but complicated by overshoot phenomena in two of these). Agreement among the values for nitrogen is quite satisfactory: the computed values for ${}_b\pi_{N_2}/{}_b\pi_p$ are 1.43, 1.43, and 1.56 with a mean value of 1.48. For nitrous oxide agreement is less satisfactory but still good enough to get an approximate estimate of potency; the values obtained are 36.3, 36.4, and 25.9 with a mean value of 32.6. Recalculating all of these values so as to reference them to the narcotic

potency for nitrogen is effected by simple division and yields the series of values shown in Table 4.

c. Summary of results on high pressure bradycardia

The quantitative analysis attempted above and summarized in Table 4 confirms the qualitative conclusion by Örnhagen that the data indicate measurable antagonism by all inert gases to the effect of hydrostatic pressure on sinus beat frequency, and a progression in intrinsic potency in ascending order from helium to neon to nitrogen to nitrous oxide. The numerical values of ${}^{N_2}_{b}R_i$ are not significantly different from the corresponding values for ${}^{N_2}_{a}R_i$ and probably not from those for ${}^{N_2}_{c}R_i$. To these conclusions the quantitative analysis adds the information that the relative intrinsic potency of hydrostatic pressure in eliciting bradycardia is comparable to that for high pressure convulsions but substantially larger than that for pressure reversal of anesthesia.

4. Lipid bilayers

a. Effects of hydrostatic pressure

The types of pressure effects considered so far all relate to changes in the properties of excitable tissues, and it seems a plausible—though not necessarily inescapable—hypothesis that they have in common pressure-induced changes in the membranes that lend special qualities to such tissues. Gradations in inert gas effects have long been associated with the gradation of lipophilic qualities of the gas molecules, and hence it is not surprising that a number of studies have dealt with pressure effects on lipid bilayers as a model of those regions of cell membranes considered as probable targets of interaction of pressure and anesthesia.

(1). On K^+ permeability. Among studies on permeability of K^+ only a few deal with the light gases in a manner pertinent to the present discussion. Johnson et al. (61) prepared liposomes containing radioactive potassium ion in the enclosed aqueous phase and studied the rate of valinomycin-activated leakage of this ion into the surrounding aqueous phase under various compression conditions. The properties of such preparations vary somewhat from one time to the next and, consequently, the effects of any experimental procedure could be studied effectively only by comparing leakage rates in any particular preparation in successive steps of the same experiment. Potassium leakage rates decreased with increasing pressure in an approximately linear fashion, provided pressure was applied hydraulically. Compression under

TABLE 4
ANTAGONISM OF HIGH PRESSURE BRADYCARDIA IN SINUS NODE PREPARATION

	${}^{N_2}_{b}R_i$
Hydrostatic Pressure	−0.68
He	0.17
Ne	0.33
N_2	1.00
N_2O	22.7

*Relative potency of metabolically inert gas "i" with respect to bradycardia.

helium resulted in what appeared to be a nonlinear decrease of permeability with pressure: little significant change of permeability was seen from 1 atm to about 120 atm, while at pressures beyond 150 ATA permeability decreased at about the same absolute rate as in preparations subjected to hydraulic compression. Thus, on the average, permeability decreased under helium at a rate approximately 20% less than under hydraulic compression.

(2). On membrane fluidity. Trudell et al. (38) studied fluidity changes in compressed lipid bilayer preparations by electron spin resonance spectroscopy in the presence or absence of helium, hydrogen, or nitrogen. Hydraulic compression resulted in substantial, and presumably stable, decrease in membrane fluidity. When various inert gases at the pressures prevailing in the system were introduced following compression, there resulted gradual and partial reversal of this pressure effect; fluidity increased until a new equilibrium was reached. Results are conveniently expressed in terms of ΔS, i.e., the change in degree of orderliness of the membrane resulting from each step in the procedure. At a total pressure of 102 ATA and a temperature of 20°C, equilibrium values of ΔS were $+0.018$ for hydraulic compression from 1 ATA to 101 ATA; $+0.015$ for similar compression under helium; $+0.005$ under hydrogen; -0.02 under nitrogen, and ~ -0.32 under xenon. In a subsequent publication Mastrangelo et al. (37) provided data that when replotted to show ΔS as a function of partial pressure of xenon, showed significant deviation from linearity (Fig. 3). Estimates of relative potencies from the spin resonance data thus cannot be derived, without risk of error, from the values for ΔS corresponding to compression with the pure gases to a single fixed pressure. Mastrangelo and co-workers chose to substitute determination of the partial pressures of the gases required to neutralize the hydrostatic pressure effect in a series of binary mixtures with helium, all used at the same total pressure, a procedure analogous to the scaling applied to the high pressure convulsion data above. Alternative estimates can be derived from the slopes of the curves, at constant total pressure, of ΔS vs. P_i for small concentrations of the gas under test. The two approaches yield series of potency values that do not differ widely from each other: setting ${}_F\pi_{N_2} = 1.0$, the computed potencies are ${}^{N_2}_F R_p = -0.76$ and -0.63; ${}^{N_2}_F R_{N_2O} = 31$ and 21, respectively. The differences show that the quantitative effect of deviations from linearity in this system at 100 ATA are not too serious, and, at present, mean values derived from the two modes of calculation appear to be the most reliable approximation to significant ${}^{N_2}_F R_i$ values for N_2, N_2O, and Xe. For estimation of ${}^{N_2}_F R_{He}$, and ${}^{N_2}_F R_{H_2}$, one has to rely on single values for ΔS resulting from compression in undiluted gases to total (and partial) pressures of 102 ATA, since these are the only published data. The actual ${}^{N_2}_F R$ values probably can be computed most reliably from those data by comparison with P_{N_2} values yielding the same ΔS at what was essentially the same total pressure in the serial determinations of Mastrangelo et al. (37). Values for ${}^{N_2}_F R_i = {}_F\pi_i/{}_F\pi_{N_2}$ estimated in this fashion become for He, 0.12, and for H_2, 0.25 (Table 5, Row 2).

TABLE 5

INERT GAS-HYDROSTATIC PRESSURE INTERACTION IN LIPOSOME PREPARATIONS

Property Explored	Relative Potency				
	HP*	He	H_2	N_2	N_2O
K^+ permeability	1.0**	−0.2**	—	—	—
Membrane fluidity	−0.70	0.12	0.25	1.0	26
Phase transition temperature	1.6	0.2	—	1.0	38

*Hydrostatic pressures. **$\pi_i/\pi_p = {}^{P}R_i$ (all other values are ${}^{N_2}R_i = \pi_i/\pi_{N_2}$).

An important point was added to these considerations by the work of Finch and Kiesow (62). These workers used erythrocytes as the source of the membranes to be studied and compared the behavior of three different spin labels in which the active component of the labeled molecule is buried to different depths within the cell membrane. Using this technique, they compared the effects of hydrostatic pressure per se, helium, and nitrogen, as well as of a number of organic anesthetics. It was found that the results obtained depended critically on the depth to which the nitroxide moiety of the spin label was buried in the membrane. Thus hydrostatic pressure per se substantially increased the order parameter ΔS for the shallowest and for the intermediate burial site but produced no significant effect when the most deeply buried probe was used. The effects of helium were not significantly different from those of pressure alone on any of the three probes used; indeed, for the shallowest probe helium gave a slightly larger increase in ΔS than pressure alone, while for the intermediate one the mean value was slightly lower, the difference being void of statistical significance. In the case of nitrogen, a qualitative difference was observed in that for the shallowest probe nitrogen at 100 ATA pressure elicited a net increase in ΔS, while for the intermediate probe it produced a net decrease. No change was observed for the most deeply buried probe with nitrogen, nor indeed with any of the other anesthetics that were tested. The data probably reflect an especially high degree of anisotropy in the red cell membrane and suggest that the concept that fluidity changes under the action of pressure or pressure antagonists can be directly related to observed biological effects is likely to prove a serious oversimplification. Further tests of this point in actual biological systems would appear to be highly desirable.

(3). On phase transition temperature. MacNaughtan and Macdonald (39), using liposome preparations similar to those employed by Johnson et al. (61), and by Mastrangelo and Trudell and their co-workers (37, 38), examined changes in the temperature threshold for the phase transition in their system, using changes in the optical properties of their suspensions to define the transition point in each case. Increasing hydraulic pressure elicited increases in transition temperature, the shift being linearly related to the pressure applied throughout the entire range tested up to 350 ATA—and shown by others to be so up to 500 ATA (63). A temperature increase of 1°C was found to correspond to a pressure increase of just under 44 atm. These data were then compared to the effects of compression under helium, nitrogen, and nitrous oxide. Helium and also nitrogen compressions, like hydraulic compression, increased the transition temperature, though less than hydraulic compression, while nitrous oxide and more potent anesthetics reduced transition temperatures, again in direct proportion to their partial (and total) pressures applied. Potency estimates can be derived from the resulting data by setting

$$({}_T\pi_i + {}_T\pi_p) \cdot P_i = -\Delta T_m \qquad (8)$$

which is compatible with the numerical results presented, though a critical test of linear additivity would require serial gas mixture data that are not available. As in the other liposome studies, ${}_T\pi_p$ can be deduced directly from the results of hydraulic compression experiments, allowing computation of the remaining ${}_T\pi_i$ values. Normalization by dividing through by ${}_T\pi_{N_2}$ then yields the corresponding ${}^{N_2}_{T}R_i$ values. The results of such analysis are: ${}^{N_2}_{T}R_p = -1.6$; ${}^{N_2}_{T}R_{He} = 0.20$; ${}^{N_2}_{T}R_{N_2} = 1.00$; and ${}^{N_2}_{T}R_{N_2O} = 37.6$, (Table 5, Column 3). The numerical results for relative intrinsic potencies of He, N_2, and N_2O derived from these results are in excellent agreement with those derived from anesthesia, bradycardia, and high pressure convulsion data (Tables 2 and 3). The intrinsic potency of pressure, however, is substantially greater in this series than the opposing effect of nitrogen on the transition temperature. The authors surmise that the high value of ${}_T\pi_p/{}_T\pi_{N_2}$ may reflect the fact that solubility of inert gases in the lipid

phase of the bilayer may be substantially lower than in a bulk solvent phase of similar composition. The data of Smith and co-workers (64) tend to lend some support to this view. It is not clear, however, why a similar explanation should not apply to the other endpoints reviewed in the present section, nor why it should fail to apply to in vivo systems.

b. Summary of lipid bilayer data

The lipid bilayer data can now be assembled (Table 5) to show that the numerical results for the relative potencies of the several gases on the whole present a remarkably consistent picture resembling closely those derived for the several biological test systems discussed in previous sections. Estimated potencies for hydrostatic pressure as such are relatively high, ranging from 0.7 to 1.6, for reasons not clear at this time. On the whole it would appear that calculations based on the assumption of linear additivity of inert gas and hydrostatic effects are applicable to the various sets of artificial biomembrane data, at least in the lower pressure range. Two caveats, however, are in order in viewing these results: In the first place, the data of Mastrangelo and co-workers (37) concerning fluidity changes in the presence of xenon do not appear to support the assumption of linearity over any substantial segment of the concentration curves for xenon. In the second place, the data of Finch and Kiesow (62) point to highly significant qualitative differences in the behavior of different layers of erythrocyte membranes, calling attention to the possible complexity of the makeup of real biomembranes and the consequent difficulty of applying in a meaningful manner the concepts derived from the properties of bulk phases to the interpretation of the functionl behavior of such membranes.

5. Overall characterization of Group A responses and implications

It is now in order to take a comprehensive look at the interaction phenomena so far described. Table 6 summarizes the results for each of the sections presented in terms of relative potencies, all values being referred to the effects of nitrogen. Perusal of that table reveals that, insofar as relations between the several gases compared are concerned, the several sets of data show striking homogeneity: in all cases, the progression of potencies ascends from helium through neon, hydrogen, nitrogen, to nitrous oxide. In all cases, too, the numerical values of the relative potencies are similar, hydrogen being between ⅓ and ¼ as potent as nitrogen, and nitrous oxide between 20 and 40 times as potent as nitrogen. Potency values for helium are always low but show a somewhat greater spread from effectively 0 to ⅕ of the potency of nitrogen. In all series so far discussed the effects of hydrostatic pressure as such were found to be opposite in direction to those of the several gases. The numerical values of relative potency of hydrostatic pressure are more variable than those of the relative potencies for these gases, ranging from -20% of nitrogen for anesthesia reversal to -160% of nitrogen for the phase transition changes. The anesthesia reversal data stand out in this respect, pressure being substantially less potent relative to the gases than for any of the remaining responses.

A comment seems in order with regard to the definition of potency as here used. To the extent that this involves merely a comparison between the several gases, the concept, as has been shown in connection with the discussion of the high pressure convulsion data, can be remarkably free from arbitrary assumptions, and the comparisons between the several gases incorporated in Table 6 seem to us to be valid with a high degree of probability. When an attempt is made to assign a quantitative value to a potency of hydrostatic pressure as such, and to relate this to the relative potencies of the gases in antagonizing its effects, the relations become more complicated. As shown in connection with the discussion of linear additivity theory, unambiguous potency values for hydrostatic pressure can be defined only when linear

TABLE 6

SUMMARY OF RELATIVE INTRINSIC POTENCIES FOR INERT GAS-HIGH PRESSURE INTERACTIONS CHARACTERIZING VARIOUS GROUP A RESPONSES

	${}^{N_2}_{a}R_i$ Anesthesia and High Pressure Reversal	${}^{N_2}_{c}R_i$ High Pressure Convulsions	${}^{N_2}_{b}R_i$ High Pressure Bradycardia	${}^{N_2}_{F}R_i$ Fluidity of Liposomes	${}^{N_2}_{T}R_i$ Phase transition of Liposomes
High Pressure	−0.2	−0.6	−0.7	−0.70	−1.6
He	0.07	0.00	0.17	0.12	0.2
Ne	0.18	—	0.33	—	—
	>——<				
H_2	0.39	0.34	—	0.25	—
		>——<	>——<	>——<	
N_2	1.00	1.00	1.00	1.00	1.0
					>——<
N_2O	22.6	42	22	26	38

>——<, "Neutral" gas for each response.

additivity of effects prevails. This appears to apply to the phenomenon of pressure reversal of anesthesia up to pressures somewhere between 100 atm and 139 atm. Indeed, as shown in Fig. 1, in this case separation of the effects of pressure as such from those of the gases as pharmacological agents provides remarkably satisfying unification of a whole field.

In the case of high pressure convulsions the concept of linearity was found not applicable: in the one model for which detailed information is available three different strains of mice all show highly significant deviations from linearity and, furthermore, show patterns of deviations that appear to be strain specific. In this case, a coherent picture could still be formulated by focusing on the concept of relative potency of the gases, which, as has been shown, can still be defined unambiguously. To estimate the relative potency of pressure in this case requires additional assumptions: for instance, that pharmacological effectiveness of the gases is constant over the range of pressures tested, and by implication that it is the effective potency of pressure which is variable. This hypothesis is testable, but to date no direct test of any such formulation has been undertaken.

In the case of bradycardia, the available data are insufficient to test the hypothetical apparatus, although the data (such as they are) are compatible with the assumption of linear additivity. The behavior of liposomes has revealed complexities unexpected in view of the low level of organization of these preparations. As has been shown by Mastrangelo and co-workers (37), quantitative relations with regard to membrane fluidity changes deviate substantially from simple linearity even at quite moderate pressures (Fig. 3). Again, the phase transition data show an unexpectedly large apparent potency for hydrostatic pressure, and the authors have called attention to the possibility that different layers of the lipoprotein membranes may be affected in different ways by either, or both, hydrostatic pressure and the inert gas (39). The data of Finch and Kiesow (62) lend additional credence to this caveat.

The two biological responses for which the most complete data are available—anesthesia reversal and high pressure convulsion—are both highly complex responses, the apparent holistic character of the biological endpoint of which in each case conceals an extraordinarily complex array of anatomically, pharmacologically, and biochemically structured events (cf.

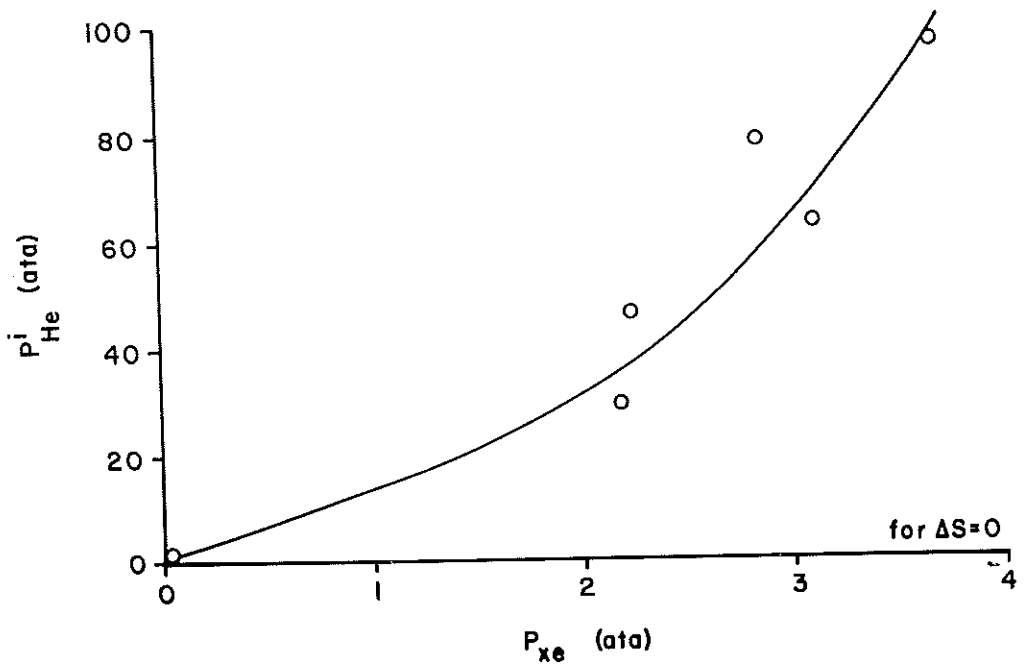


Fig. 3. Partial pressure of helium as a function of partial pressure of xenon for a series of binary Xe-He mixtures at total pressures where pressure-induced membrane fluidity changes are just neutralized (i.e., $\Delta S = 0$). [Data from Mastrangelo et al. (37).]

Ref. 55). Regarding anesthesia, this has been pointed out in the past to the point where the very nature of anesthesia in vertebrates even today remains very much in dispute. With regard to high pressure convulsions, clinical and radioautographic data certainly underscore the complexity of the convulsion phase of the high pressure neurological syndrome (65). Some of this complexity has been removed in the present discussion by focusing attention on a particular component of the convulsion phase. No data are available at the present time, however, to indicate whether the anatomical distribution of the CNS components involved in producing HPNS Type I convulsions remains the same when convulsion threshold pressures are modified by adding anesthetically effective agents to the compression conditions. Working with bolus injections of barbiturates, Brauer and co-workers (66) showed substantial differences in the sites of attack of these agents as manifested by anesthesia and the high pressure convulsions. Rowland-James et al. (51) and Miller et al. (67) have called attention to the several sites involved in producing successive stages of the high pressure neurological syndrome that show substantially different combinations of solubility and compressibility characteristics as derived on the critical volume hypothesis. One might be tempted to hypothesize similar anatomical or biochemical complexities to account for different slopes of successive portions of the nonlinear dose-response curves shown in Fig. 2. Direct test of these relations would seem to require experiments in which hydrostatic pressure and partial pressure of effective gas could be varied independently, in which the key parameters were varied over a sufficient range of values, and in which physiologically stable liquid-breathing preparations were utilized. Such studies have not been reported so far, to the best of our knowledge.

Despite all these complications, we judge that the attempt to separate numerically the effects of pressure as such from the pharmacological effects of the various gases has shown itself to

be worthwhile. Partly, it has been successful in helping to bring order and coherence to a large amount of data; and partly it has pointed up problems with simplistic solutions and helped to formulate testable alternative hypotheses without requiring premature enunciation of detailed biophysical mechanisms in a field that is still largely in a descriptive stage.

In particular, Table 6 provides strong support for the view that the several responses explored have some underlying characteristics in common that justify talking about a Group A of responses. All biologic effects that clearly fall into Group A relate to excitable tissues. It seems plausible to infer that the effects observed reflect pressure-induced changes in the properties of the excitable membranes characteristic of such tissues. The general conformance of the pattern of results for biological systems with those for liposomes would tend to lend additional credence to this inference. Most investigators have tended to take the general pattern shown by the inert gas potencies of Table 6 as indicative of a lipid phase, or at least of markedly lipophilic sites, as the target of interaction of hydrostatic pressure and inert gas. As pointed out by Featherstone and Muehlbacher many years ago (14), such a conclusion does not necessarily follow, since other types of intermolecular interactions (e.g., with polypeptide configurations) could give rise to a similar pattern and might underlie some of the differences in ${}^{N_2}_{P}R$ values shown in Table 6 to exist between the several responses. In addition, MacNaughtan and Macdonald (39) have reminded their readers that solubility and other properties of even the lipid phase in biomembranes or membrane models are likely to differ substantially from those of the corresponding lipid in bulk phase, a point dramatically confirmed by Finch and Kiesow (62). Thus, it would seem to remain true in 1982 as in 1962 that inferences from correlation of potency data like those of Table 6 with presumed physical properties of metabolically inert gases must be accepted very cautiously.

From a practical point of view, the observations reported above have a bearing on the protection of divers against the effects of high hydrostatic pressures by modification of the composition of the atmospheres they breathe. In Table 6, markers have been placed in each column to indicate at which point, on the basis of the mean values there reported, a particular effect of hydrostatic pressure would just be neutralized by one of the series of gases tested. It is evident that this point of neutrality varies, falling close to neon for anesthesia reversal; between hydrogen and nitrogen for convulsions, bradycardia, and liposome fluidity changes; and between nitrogen and nitrous oxide for phase transition changes. Thus, no single gas or gas mixture can be expected to be "neutral" with respect to all of the high pressure effects tested. Furthermore, in cases such as those illustrated in Figs. 2 and 3, where the dose-response relations are nonlinear, the apparent ratio of potencies between hydrostatic pressure and pharmacologically active gas varies continuously over the course of a single compression, so that maintenance of a neutral mixture even for a single endpoint would require continuous adjustment of the composition of the compression atmosphere.

In addition to these quantitative complications, there remains a more fundamental one: it is wholly uncertain at this time whether the observed antagonism of effects of highly inert gases is exerted at a molecular level or at an organismic level. Only in the former case can one speak of a true protective effect in which the net effects of the two agents do indeed cancel each other out. In the latter case actions at very different and possibly even anatomically remote sites might abrogate each others' visible manifestations without annulling the underlying changes. Observations such as those on liposomes very likely exemplify true molecular antagonism—although even here structural complications are beginning to be noticed. At the level of holistic responses in intact animals, either type of antagonism seems equally conceivable, and none of the available data help one to make a confident choice.

Yet the question is not void of practical significance: antagonism at the effects level only might well imply that divers using He-N_2 mixtures to minimize performance decrement due to

HPNS on deep dives might suffer substantial pressure-induced changes, the effects of which are merely masked by the N_2 effects. In that case, instead of providing protection, the trimix would merely encourage more severe exposure by masking manifestations of symptoms and thus increase the risk of acute or delayed pathological consequences of deep dives. Experiments to test this possibility would appear urgently needed. Comparison of residual effects following, for example, heliox (as against He-N_2-O_2) exposures to similar levels of symptomatic HPNS would not only provide guidance for further development of deep diving technology but also might provide what may be the first definitive indications of the real nature of the antagonism between high pressure effects and inert gases.

C. GROUP NON-A RESPONSES

The biological responses that have been considered so far have been the acute responses of organized multicellular systems, all of which in one way or another are related to responses peculiar to excitable tissues. A second group of responses to compression and their interaction with metabolically inert gases consists of manifestations observed in single-celled organisms or in tissue culture. These include changes in locomotion, changes in growth, cell division or replication, and gross morphological changes indicative of some type of cellular structural changes with deleterious implications. This is a heterogeneous group of responses; it is held together in part because all the responses are observed at the cellular level, and negatively it coheres because these responses deviate in one or several ways from the common pattern we have found characteristic of the Group A responses studied so far. If for the moment one might grant that events like anesthesia or changes in beat frequency of a regularly contracting muscle may well be traced to changes in the excitable membranes characteristic of these tissues—and perhaps secondarily to changes in transport or distribution of monovalent cations like sodium and potassium—then the series of events that form the subject of this section may well prove to involve changes in such other subcellular structures as microtubules, or in such subcellular organelles as those responsible for protein synthesis. Compression may affect these either directly or perhaps as a result of changes in the properties of composition of the intracellular fluid environment in which they must function. If emphasis in the previous series was at least tentatively directed toward hydrophobic tissue elements, then in this series one may find oneself in the presence of the effects of pressure on hydrophilic elements like proteins or on the state or arrangement of small molecules as they exist in solution. It is probably fair to state that at this time, despite a multiplicity of possible pressure effects that might explain the biological changes observed, the significance and the precise mechanisms of pressure effects on such systems—and implicitly the information that such effects may yield with regard to the fundamental properties of such systems—is extremely small. It may well be that the study of interactions of pressure and inert gas that are concerned with the lightest gases may add an important dissecting tool to the scientific armamentarium currently in use to help to develop these concepts.

The great majority of the responses discussed in the following pages occur at pressures that are rather higher than those which have occupied us so far, becoming noticeable only at pressures between 80 and 200 ATA and with data stretching on up to as much as 500 ATA. In addition, it is probably true that with one or two exceptions the phenomena discussed in the following pages will not even approximately yield to the model of linear additivity that has proved useful, at least as a yardstick, in analyzing the data reviewed so far. It is unfortunate that in none of the series presented below has it proved possible to obtain series of experimental data fully satisfactory for the purpose of determining what might be termed *dose-response*

relations. In drawing conclusions from this material, therefore, one is reduced to a series of qualitative statements about the presence or absence of pressure effects; about the presence or absence, and the sign, of inert gas effects; and about Boolean deductions to the effect that one gas may be more potent than another. Yet, as is seen presently, this kind of information is sufficient to allow a rough presorting of responses. In a number of cases it suffices to separate the response patterns conclusively from those characteristics of Group A responses; in the case of the other responses this type of analysis serves at least to formulate questions that invite further experimental work.

1. Protozoa at high pressures: motility and pathology

It seems appropriate to begin the review of this material by considering two papers that compare direct visual observations of two protozoa under hydraulic and under helium compression.

Echinosphaerium nucleofilum (40) is a heliozoan protozoan characterized by a series of elongate cytoplasmic protuberances, so-called axopods, stabilized by an embedded microtubular framework. Under hydraulic compression the mean length of these structures decreased during the first 10 to 20 min at pressure and thereafter returned to precompression dimensions. The minimum hydraulic pressure required to produce axopod shortening in *Echinosphaerium* was about 70 ATA; maximum response occurred at 130 ATA. Similar transient axopod shortening was observed under helium compression, but the threshold pressure for the effect was reduced to only a few atmospheres, and peak shortening was elicited at about 80 ATA; the maximum intensity of the effect was indistinguishable from that attained under hydraulic compression at 130 ATA. With nitrogen compression, the effects at low pressure were indistinguishable from those produced by helium; at about 40 ATA further axopod shortening was suspended, to be resumed only when pressure exceeded 80 ATA. As pointed out by the authors, axopod shortening in *Echinosphaerium* presumes partial disassembly of axopodial microtubular systems (cf. Ref. 68). In this situation then, helium not only does not antagonize the action of pressure but markedly potentiates it. At least at lower pressures, N_2 is no more effective than He in this respect; the biphasic response to N_2 may bespeak more specific stabilizing effects of N_2 at pressures greater than 40 ATA on the microtubular structures.

A second phenomenon observed in the course of these studies was the development of "leaky" cells, and of complete lysis and death in *Echinosphaerium* subjected to compression under inert gas. Such toxic reactions were absent in cells subjected to purely hydraulic compression up to 150 ATA. Nitrogen was substantially more toxic than helium (70% injured at peak, vs. 10% with He), and argon exceeded both to such a degree that observations on axopod shortening could not be completed. Leaky cells became detectable at 40 ATA for He, 30 ATA for N_2, and 10 ATA for Ar. Compression in He-Ar mixtures was far less toxic than in Ar alone. In the absence of control experiments using hydraulic compression in a medium containing known partial pressures of Ar, it is not possible to decide whether this effect represented pressure antagonism of Ar toxicity or a specific He-Ar antagonism.

Thus, the toxic effects of compression in *Echinosphaerium* present a pattern of dependence on inert gases that differs from both the pattern for the several excitable tissues of Group A responses as well as the pattern for axopod shortening in the same species (cf. Table 7, items 4 and 5). The appearance of leakiness followed by cell lysis suggests changes in permeability properties of the cell envelope. A possible role of autolytic changes preceding these events (and consequent alteration of the osmolarity of the cell contents) also cannot be excluded. The time course of these changes makes it improbable (but does not wholly rule out) transient

TABLE 7

QUALITATIVE AND SEMIQUANTITATIVE CHARACTERISTICS OF THE EFFECT OF HELIUM, HYDROGEN, NITROGEN AND HYDROSTATIC PRESSURE ON GROUP NON-A BIOLOGICAL RESPONSES, AND COMPARISON WITH GROUP A

Responding Organism, Response, and No. for Response	Relative to Hydrostatic Pressure							
	Pressure		Helium			Nitrogen		
	No Effect	Effect	No Effect	Antagonize	Potentiate	<He	=He	>He
Spirostomum ambiguum								
1. Swimming speed		X		X		X		
2. Reversal duration	X				X		X	
3. Toxicity	X				X			X
Echinosphaerium nucleofilium								
4. Axopod length		X			X	X (2nd phase)		
5. Toxicity	X				X			X
Echo virus								
6. Growth		(±)	X				X	
Herpes simplex virus								
7. Replication		(±)	X				X	
8. Pathological effects	X				X			X
Marine bacterium EP-4								
9. Growth		X		X		—	—	—

TABLE 7—*Continued*

Responding Organism, Response, and No. for Response	Pressure		Relative to Hydrostatic Pressure					
			Helium			Nitrogen		
	No Effect	Effect	No Effect	Antagonize	Potentiate	<He	=He	>He
Acholeplasma laidlawii 10. Growth		X		X		—	—	—
Tetrahymena pyriformis 11. Multiplication		X		X				X (but<H_2)
Saccharomyces cerevisiae 12. Growth		X		Over-compensation		—	—	—
All Group A Responses		X		X				X

imbalance in inert gas distribution that might elicit significant osmotic stresses and thus contribute to the overall effect (cf. Ref. 69).

A second paper dealing with visual observations of protozoa under hydraulic or inert gas compression used the actively swimming heterotrich ciliate *Spirostomum ambiguum* (41). Normally, forward swimming in this species is interrupted at intervals by reversal of ciliary beat and of swimming direction, so that its locomotion can be characterized by the speed of forward swimming and the frequency and duration of reversals. Swimming speed of *Spirostomum* was unaffected by pressures up to 80 ATA, regardless of whether these were applied hydraulically or under He or N_2. At higher pressures swimming speed decreased progressively, and again there were no discernible differences between hydraulic compression and compression under He or N_2. The reversal phenomenon, on the other hand, showed a different pattern: 120 ATA of hydraulic pressure failed to affect reversal duration; both He and N_2 substantially prolonged duration of reversal periods and did so to about the same extent. Toxicity and survival data for *Spirostomum* are virtually the same as for *Echinosphaerium:* changes induced by hydrostatic pressure were reversible, and no cell death was recorded. Exposures to 60–120 ATA of He resulted in persistent changes in the appearance and locomotor activities of the animals. Animals compressed under N_2 readily survived 60 ATA, but 85% failed to do so at 80 ATA. Under Ar, 50 ATA resulted in "bursting" of the cells within 2 min of exposure; cells exposed to 10 to 40 ATA survived. As in the case of *Echinosphaerium*, He-Ar mixtures at comparable Ar partial pressures were survived readily.

The characteristic effects of pressure and inert gas pressure on the several reactions of *Spirostomum* are summarized in items 1–3 of Table 7. Comparison with the unnumbered bottom line of that table shows that all three responses observed in *Spirostomum* varied with pressure in patterns that differ qualitatively from those for Group A responses. In addition the data bespeak significant differences even among the three response patterns within a single species. Toxic effects showed the same pattern in both *Echinosphaerium* and *Spirostomum;* axopod shortening in *Echinosphaerium* likewise responded to the several types of compression in a manner similar to the reversal reaction in *Spirostomum*. This may be significant in view of the recent association of pressure effects on the reversal reaction in another ciliate, and the effects of pressure thereon, with electrosensitive Ca^{2+} channels in the ciliary membrane (70).

2. Growth, replication, and multiplication

The available data concerning pressure effects on growth and replication are summarized in the order of complexity of the organisms studied.

The only paper pertinent to the present discussion that deals with virus multiplication reports on two viruses, the echo II virus, a simple RNA virus, and the more complex DNA virus of herpes simplex, both grown in tissue culture on vervet monkey kidney cells (42). Cultures were exposed for 24 h to pressures ranging from 11 to 61 atm in the presence or absence of helium or nitrogen, then decompressed and subcultured to obtain an estimate of virus titer in the pressure-exposed samples. In the case of echo virus, replication rates appeared to be slightly smaller in all preparations exposed to hyperbaric conditions than in the controls; there was no evidence of differences attributable to the presence of either helium or nitrogen. In the case of herpes virus, hydraulic pressure effects were insignificant; helium compression effects did not differ from hydraulic compression effects, but nitrogen at all partial pressures tested caused a substantial depression in replication rate. Electron-microscopic inspection of the tissue infected with herpes virus I showed substantially normal virus morphology in the hydraulically compressed, as well as in the nitrogen-compressed, samples but substantial (and

significant, in the opinion of the authors) abnormalities in the case of the helium-compressed tissue.

Taken together, the data seem to us to fail to demonstrate, up to a total pressure of 61 ATA, any significant pressure effects on virus multiplication, and to suggest that nitrogen might have a growth-inhibiting effect. Because of lack of correlation of the magnitude of this effect with nitrogen pressure, even this appears difficult to interpret. The interesting morphological data clearly invite further experimental study but at the present time do not, in our opinion, admit of any specific interpretation in terms of inert gas effects.

The only bacteriological study we have encountered comparing hydrostatic and helium-imposed pressure is an outcome of efforts to utilize the advantages of a gaseous environment for experimental manipulation of bacterial cultures in the study of properties and behavior of deep-sea bacteria (43). This series dealt with marine bacterium EP4, the growth and metabolic rate of which are severely inhibited (about 80% for replication rate and 50%–60% for both metabolic rate and incorporation of [^{14}C]glutamate) at 500 atm hydraulic pressure. When the cultures were grown under 500 ATA helium, growth and metabolic inhibition were far less marked than under hydraulic compression (65% of control for growth, and 60%–70% of control for [^{14}C]glutamate-incorporation rate). The data furnished, unfortunately, do not permit deducing dose-response relations, since only one level of pressure and of helium partial pressure was tested.

Very tentatively, then, one may conclude that in this case helium antagonizes the effect of hydrostatic pressure with a potency equivalent to at least one-half the numerical value of pressure as such.

Macdonald, working with the procariote *Acholeplasma laidlawii* (44), found that here, too, hydrostatic pressure inhibited normal increase in total cell mass as well as in membrane protein, resulting in complete suppression of growth at 300 ATA. Compression to 300 ATA under helium resulted in a transient period of growth inhibition followed by resumption of growth at a rate that was substantially equivalent to that before compression. In this preparation, therefore, helium, after an initial lag period, quite possibly due to delayed diffusion of helium throughout the medium, seemed to completely reverse the hydrostatic pressure effects on growth. Special interest may attach to this organism because of the demonstration that transition of its cell membrane to the gel (ordered) configuration inhibits cell division (44) and that the critical temperature for this transition is shifted upward by exposure to hydrostatic pressure in much the same way as in the liposome model discussed above (39,71).

A careful study has been reported regarding the rate of cell division in the ciliated eukariote *Tetrahymena pyriformis* (45). Multiplication rate in this organism appeared to be unaffected by pressures up to at least 100 ATA, but it was reduced progressively as pressures were increased about 175 ATA to the point where, at 250 ATA, cell replication was totally suppressed. Compression to 250 ATA under helium resulted in a transient period of reduced replication rate followed presently be resumption of regular division at approximately ⅔ the rate observed at 1 ATA.

Compression under hydrogen to 250 ATA and even 325 ATA elicited a brief lag period followed by resumption of multiplication at a rate indistinguishable from that observed under 1 ATA. At 400 ATA under H_2 cell division had come to a stop, while at 400 ATA hydraulic pressure cell numbers actually decreased. Indeed, cell lysis in this species appears to occur only at pressures greater than 350 atm and this effect, too, appears to be largely prevented by H_2. Nitrogen at 175 ATA counteracted pressure to an extent similar to helium at 250 ATA. This gas caused transient inhibition of cell division followed by marked recovery. All told, these data suggest that in regard to *Tetrahymena* multiplication the potencies of He and H_2 as

antagonists against the effects of pressure are within limits quite compatible with those of responses of Group A systems: ${}^{P}R_{He} = 0.4$, and ${}^{P}R_{H_2} \simeq 0.7$. On the other hand, the effect of N_2 seems less powerful than that of H_2 and thus deviates markedly from the systems analyzed in Table 6. The authors comment on the fact that in addition to these effects on cell division, the rate of ciliary swimming was reduced by hydrostatic pressure but appeared to be far less affected when compression was carried out under helium or hydrogen. Cells exposed to high pressures of hydrogen showed structural anomalies.

Relations are even more complex for *Saccharomyces cerevisiae,* a yeast with well-developed cell wall (46). In this species hydraulic compression exerted a remarkably strong inhibitory effect beginning apparently at pressures as low as 20 ATA and increasing linearly with the pressure. Helium at pressures up to 50 ATA more than reversed this effect so that such cultures grew more rapidly in high pressure helium than at 1 ATA. Beyond 50 ATA further growth was inhibited, the effect increasing with further increases of pressure at the same rate under helium as under hydraulic compression. Thus the curves describing growth rate as a function of pressure under the two types of compression parallel each other, the helium curve being displaced relative to the hydraulic one by about -75 ATA. The authors conclude that helium must specifically affect "some reactions in the growth" of these yeast cells.

3. Summary and characterization of Group Non-A responses

Having now reviewed the available information bearing on the comparison of the effects of hydraulic compression with compression under helium, hydrogen, and nitrogen on growth, cell division, motility, and preservation of cell integrity, it is in order to return to a comparison of these results with those previously summarized (Table 6) for excitable tissues. In making such comparison it must be admitted that the experimental material for changes at a cellular level, and upon which such comparison must be based, only rarely was gathered in a fashion that would permit deducing the type of dose-response relations essential for quantitative assessment of relative potencies. This is only in part attributable to the preferences of the investigators; in large measure it reflects the nonlinear character and the numerous apparently idiosyncratic reactions that time and again forced the investigators to develop enough descriptive and qualitative information to map the complex response patterns with which they had to deal. Instead of preparing a table of relative potencies analogous to Table 6, therefore, we propose to approach the analysis of this second group of responses by tabulating presence or absence of pressure effects, and semiquantitative evaluations of inert gas effects as *potentiating, antagonizing,* or *equal to* each other. In this way, Table 7 summarizes results for twelve response types observed variously for six organisms; for comparison Table 7 also includes the results for the three biological responses from Table 6, which, when subjected to the criteria of Table 7, reveal a single common pattern shown in the last line.

Of the responses listed in Table 7, only items 9, 10, and 11 are qualitatively compatible with the pattern shown by Group A responses. Among these, in turn, the marine bacterium is set apart from Group A reactions by the very high pressures required to inhibit growth (500 ATA!). The multiplication of *Tetrahymena,* on the basis of the data available, would seem to show a disparately small protective effect for nitrogen, as well as some curious acclimation effects. The data for *Acholeplasma,* finally, are as yet too incomplete to evaluate the extent to which its multiplication conforms to the pattern of Group A responses.

All other cellular responses shown in Table 7 differ in essential ways from the responses of excitable tissues to pressure or inert gas effects: thus responses 2–5, and 8 (i.e., the toxic effects observed in various organisms and the reversal reaction of *Spirostomum*) show a lack

of significant effects of hydraulic pressure as such. All of these responses, and in addition the shortening of *Echinosphaerium* axopods, were only observed under high pressures of inert gases, with nitrogen being no more potent (or, in the case of herpes simplex virus pathology, less potent) than helium. Such an inverted potency relation between helium and nitrogen was also observed in the case of the swimming speed of *Spirostomum*. In the case of *Saccharomyces* growth, there is the striking observation that helium markedly potentiated growth up to about 50 ATA, while elevated hydrostatic pressures invariably slowed the growth of this organism.

It may be in order at this place to recall the data of Barthèlemy and collaborators on survival times in fish (27), which appeared to consort poorly with the reactions shown in Table 6. While, on the basis of the qualitative criteria used in preparing Table 7, Barthèlemy's results, as far as they go, follow the pattern of the excitable tissue responses, yet the observed exaggerated antipressure potency of helium relative to nitrogen ($^{N_2}R_{He} = 0.6$) tends to set them apart from the remainder of the biological changes listed in Table 6.

In view of the results summarized in Table 7 it is hard to escape the conclusion that the effects of hydrostatic pressure and of high partial pressures of inert gases on cell motility, cell integrity, and probably on cell replication must involve reactions not primarily controlled by hydrophobic membrane constituents or by the ordering effect of pressure on membrane components, as shown by fluidity or transition temperature changes, setting this group of responses apart from the responses to the same stresses of the excitable tissues constituting our Group A.

In the case of the axopod shortening, such a result is hardly surprising: the effect depends on disassembling of microtubules (68), an effect known to be produced by hydrostatic pressures in the range of those here applied (72). It should be interesting to replicate experiments on pressure effects upon isolated microtubular elements in which compression under helium is substituted for hydraulic compression, to determine whether the resulting effects conform to the pattern characteristic of axopod shortening in *Echinosphaerium*. In connection with the reversal reaction of *Spirostomum* it is tempting to invoke the analogy with another ciliate—*Paramecium caudatum*. In this preparation, pressures higher than 68 ATA inhibit the reversal reaction (70); a reversal reaction could, however, be elicited in all animals by abrupt decompression from 170 ATA. Experiments with lysed and reconstituted paramecia and with a mutant strain led these investigators to infer that the pressure effects in this case depend on electroactive Ca^{2+} channels in the ciliary plasma membrane; they were not able to identify what molecular properties of these channels might account for the sensitivity of *Paramecium* to such comparatively low pressures. Here again, parallel experiments with helium compression should provide additional insight.

The several sets of data concerning the toxic effects of high pressure helium, nitrogen, or argon are interesting, but the information available does not suffice to formulate any testable hypothesis regarding possible mechanisms. In all of these cases it would appear that pressure as such lacked toxic effects. The time relations involved in the development of the cytological lesions appear too short to attribute these effects to disruption of biosynthetic mechanisms or stages in cell replication, yet they are rather too long to be compatible with osmotic effects attributable to possible transient maldistribution of the dissolved gases (69). By default one is driven to look to changes in permeability properties of the cell membranes, or to changes in the osmolarity of cell contents that are perhaps secondary to autolytic activity, or to alterations in transport functions not dependent on lipid membrane constituents.

The data on growth and cell replication present the most complex picture: in the case of the two protozoa, the possibility cannot be ruled out that membrane properties analogous to those that determine excitability in nerves of multicelled animals with differentiated tissues may exert a controlling influence on growth and replication rate. Unfortunately, the data available

to date do not permit associating the changes in growth and replication rates that were observed with any particular portion of the cell replication cycle. Thus any hypothesizing concerning these events seems premature. The pressures at which bacterial growth is inhibited are quite high enough to interfere with known processes of protein synthesis and replication (7). Finally, the aberrant behavior of *Saccharomyces* is of special interest because this species alone among those examined possesses a well-developed cell wall and can be expected to show the far-reaching changes in the transport properties of the cell membrane that differentiate such organisms (73) from typical cells bounded effectively only by their plasma membrane.

D. GENERAL CONCLUSIONS

Recapitulation of what has been learned regarding the interaction of hydrostatic pressure and of the lighter members of the chemically inert gases with various biological preparations leads us to the conclusion that the patterns of responses allow a distinction between a relatively homogeneous group (termed *Group A*) and a patently heterogeneous group, united only by their *not* conforming to the pattern shown by Group A responses. Group A is linked to changes in the functioning of excitable tissues and on the whole is understandable, if not predictable, in terms of the physicochemical properties of both, hydrophobic membrane elements and the several inert gases. The second group of responses are apparently determined by the properties of other cell constituents and involve changes such as differences in polymerization status of structural, contractile, or enzyme proteins (e.g., Refs. 7, 74), or alterations in interactions between proteins and smaller molecules (e.g., enzyme-substrate interaction, Ref. 29) that are attributable to pressure-induced alterations in protein configuration or in the solvent properties of the fluid medium in the cells.

The special characteristics of each of these groups and peculiar problems, as well as needed experiments suggested by the data, have been summarized for each of the two sections of this review. Here it is appropriate to consider briefly certain broader aspects of the subject. In the first place, this analysis has made use of interaction of the effects of hydrostatic pressure and inert gases to make a distinction between groups of biological responses that had not been made before and that surely could not have been made on an a priori basis. It seems to us that this provides a good example of the use of manipulations of hydrostatic pressure as a tool for recognizing and perhaps resolving biological problems. Review of the literature as a whole indicates that the usefulness of hydrostatic pressure as a tool to aid biological investigations is not recognized nearly as widely as it deserves, and it would please us if this review could contribute to a broader realization of the opportunities posed.

In the second place, one might perceive in the background of the outcome of this inquiry a distinction that has tended to be slighted in barobiology in the past, namely, the distinction between acute and chronic effects of high pressure exposures. It is implicit in the very existence of a large and diversified deep-sea fauna that prolonged exposure of living systems to high pressures both presumes and must lead to some degree of high pressure acclimation or high pressure adaptation. Physiological study of such phenomena has long been held back by inability to retrieve alive and to study in the laboratory deep-water animals acclimated to high pressure. In recent years, however, several series of experiments have addressed themselves to this question, and the inherent engineering problems are now coming near the point of being resolved. The resulting biological studies leave no doubt about the fact that animals that live at depths greater than about 500 m (164 ft), i.e., under pressures of 50 ATA or more, do indeed show evidence of pressure acclimation (or of pressure adaptation) both in terms of pressure tolerance and in terms of pressure dependence of specific physiological functions. There is a

suggestion in the data of a significant difference between the types of pressure acclimation that characterize animals living between depths of 500 and 2000 m (1641–6552 ft) and animals living at depths greater than 4000 m (13,124 ft), with some indications that the differences there entailed may be not unlike the differences between Group A responses—acclimation to which is characteristic of the fauna of relatively shallow water—and Group Non-A responses, which may come to dominate high pressure adaptation in the more truly deep-sea fauna. Laboratory experiments furthermore are suggesting that acclimation in the true sense of the word is possible—i.e., that shallow-water forms exposed for periods of days or weeks to high pressure regimes show persistent alterations in pressure tolerance and in pressure dependence of such functions as ion transport systems. While it is far too early to indicate to what extent this distinction will eventually prove valid, at any rate it would appear that the application of a methodology combining high hydrostatic pressure with high pressures of suitable metabolically inert gas components may have a significant contribution to make to the clarification of these problems.

From the point of view of their bearing upon the health and well-being of divers, these considerations raise some interesting points. It seems plausible to expect that exposure to conditions that cause significant changes in the function of excitable tissue would entail compensatory functional changes analogous to those encountered under a wide range of other experimental conditions that cause changes in central nervous system function, for instance. As a general matter these functional accommodations can be expected to be transient and relatively rapidly reversible. Other changes, however, that might be expected to occur under these conditions might well prove to be changes in multicellular systems analogous to the pathological and cell growth changes seen in some of the examples of Group Non-A responses discussed above. Such changes might be expected to be less transient and hence potentially of greater medical importance. This distinction becomes even more pertinent if one recalls that Group Non-A changes may not be antagonized or may even be potentiated by changes in the makeup of compression atmospheres intended to mitigate Group-A–type symptoms. These considerations gain further weight in view of current uncertainty regarding the basic nature of the antagonism between inert gas and hydrostatic pressure effects (cf. discussion under **Overall characterization of Group A responses**). One is thus led to surmise that one key area to which the considerations and the methodology of the present review need to be applied in the near future may well be the area of residual and late effects of high pressure exposures, and to phenomena associated with long-term exposures to elevated hydrostatic pressures.

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Brauer RW, Hogan PM, Hugon M, Macdonald AG, Miller KW. Modes d'interaction des effets des gaz métaboliquement inertes légers avec ceux de la pression hydrostatique en soi—une revue. Undersea Biomed Res 1982; 9(4):353–396.—Cette revue de la littérature existante s'efforce d'interpréter dans leur ensemble les effets des gaz légers métaboliquement inertes (He, H_2 et Ne) comme la resultante à la fois de la pression hydrostatique et d'effets pharmacologiques intrinsèques associés à l'exposition à ces gaz, et d'évaluer l'importance relative de chaque composante en tenant compte d'un grand nombre de réponses biologiques. Un schèma commun est reconnaissable pour la suppression de l'anesthésie par la pression, la bradycardie des hautes pressions, les convulsions des hautes pressions, et certaines caractéristiques de systèmes utilisant des modèles liposomiques. En utilisant la méthode d'analyse qui est proposée, on peut montrer que ces gaz les plus légers se conforment au genre de relation entre pouvoir et propriétés physiques caracteristique des anesthésiques gazeux plus puissants, y compris N_2, N_2O et Xe. Les relations entre l'effet produit et la

pression partielle du gaz qui âgit sont à peu près linéaires jusqu'à des pressions totales de 100 ATA pour l'anesthésie ou pour la suppression de l'anesthésie et (ou dans une mesure beaucoup plus modeste) pour les systèmes utilisant des modèles liposomiques, mais pas pour les convulsions des hautes pressions. Comme résultat de ces facteurs généraux, on ne peut attendre d'aucun gaz seul qu'il neutralise les effets de la pression hydrostatique, si l'on prend en compte toutes les réponses biologiques testées dans tout domaine de pressions d'importance. Une série d'expériences avec des cellules isolées et des cultures de tissus ont révélé l'existence d'interactions entre haute pression et gaz inerte qui ne sont pas conformes au type établi par les réponses mentionnées jusqu'ici. À ce moment on ne peut pas montrer qu'elles constituent un groupe homogène, et elles pourraient representer au moins deux sous-groupes. Les réponses tombant dans cette seconde catégorie hétérogène comprennent la mobilité cellulaire, le développement d'une pathologie de la cellule et sa lyse, et la replication et la multiplication des cellules et peut-être des virus. Les implications de ces résultats pour la formulation d'hypothèses biophysiques destinées à expliquer les interactions entre gaz inertes et hautes pressions, pour la prise en considération des effets des hautes pressions comme menace pour la sécurité, et pour le problème des approches expérimentales dans l'étude de l'acclimatation à la pression sont discutés brièvement.

anesthésie, suppression par la pression de; syndrome nerveux des hautes pressions; SNHP; bradycardie, pression; couches lipidiques bimoléculaires; couches phospholipidiques bimoléculaires, fluidité des; température de transition de phase; liposomes; multiplication cellulaire, effets de la pression sur; noeud sinusal, fréquence de battement du; souris en respiration liquide; *Symphurus plaguisa; Notophthalmus viredescens; Triturus cristatus carnifex; Tetrahymena pyriformis; Acholeplasma laidlawii;* protozoaires; hélium; hydrogène; azote; argon; neon; proboxyde d'azote; pression hydrostatique; virus echo II; virus herpes simplex; *Spirostomum ambiguum; Echinosphaerium nucleofilum;* bactérie—marine (EP 4); morphologie cellulaire, altérée par la pression; effet toxique, gaz inertes; convulsions, haute pression; trimix; mélanges de gaz inertes, neutres; temps de survie, haute pression; hypothèse du volume critique

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APPENDIX
SYMBOLS USED

Symbol	Meaning
${}_u\pi_p$	intrinsic potency of hydrostatic pressure in eliciting response u
${}_u\pi_i$	intrinsic potency of metabolically inert gas i with respect to response u
u	any of the several responses tested; identified by letter a, anesthesia b, bradycardia c, convulsions F, membrane fluidity change T, change in transition temperature
P_i	partial pressure of metabolically inert gas i
P_c	convulsion threshold pressure
${}^i_{He}P_u$	partial pressure of helium in binary gas mixture with inert gas i at response u
${}_iP_u$	partial pressure of gas i at response u
${}^{N_2}_uR_i$	relative potency of metabolically inert gas i with respect to response u, and referred to ${}_u\pi_{N_2} = 1.00$
[I]	concentration of I in a gas mixture, measured as mol fraction