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Inert Gas Narcosis, the High Pressure Neurological Syndrome, and the Critical Volume Hypothesis

Abstract. *The hypothesis that general anesthesia or pressure-induced convulsions occur when a hydrophobic region is expanded or compressed, respectively, by critical amounts is consistent with recent data obtained with mice. Calculations show that anesthesia occurs at an expansion of 1.1 percent and convulsions at a compression of 0.85 percent, the latter site of action being more compressible.*

The replacement of nitrogen by helium as the inert gas diluent in deep-diving breathing mixtures has removed the constraint of nitrogen narcosis (*1*), and simulated depths of 600 m (2000 feet, 61 atm) have been reached recently in France. However, a new barrier to deeper diving is the high pressure neurological syndrome, a hyperexcitability which first manifests itself in man at about 20 atm as a coarse tremor of the limbs (*2*). At higher pressures (60 to 100 atm) convulsions occur in experimental animals, including primates, and manned diving programs have consequently adopted cautious compression schedules (for example, 7½ days in the 600-m dive). Addition of narcotic gases to the breathing mixture has an ameliorating effect in animals (*3*).

Pressure reversal of anesthesia is another example of an effect of pressure on the central nervous system, and has led to the formulation of the critical volume hypothesis (*4*). This states that anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical volume by the absorption of an inert substance. An applied pressure opposes this expansion and reverses anesthesia. In this report, it is proposed that the hypothesis may be extended to include the high pressure neurological syndrome by assuming that convulsions occur when some hydrophobic region has been compressed beyond a certain critical amount by the application of pressure. Absorption of an inert gas will compensate for such compression and raise the convulsion threshold pressure. This extension of the critical volume hypothesis has the attraction of offering a unified description for the interaction between pressure and narcotic gases in the central nervous system with respect to hyperexcitability and anesthesia. It could also provide a theoretical foundation for the use of inert gas mixtures in deep diving. The few studies that have been made of the effect of anesthetics and pressure on simple membranes suggest that the hydrophobic region is membranous in nature (*5*, *6*).

The fractional expansion, E, that occurs when a gas at a partial pressure, P_a, dissolves in a bulk solvent is given by

$$E = V_2 x_2 P_a / \overline{V}_m \qquad (1)$$

where $\overline{V}_2$ is the partial molar volume of the gas in the solvent of molar volume V_m, and x_2 is the mole fraction solubility of the gas in that solvent when its partial pressure is 1 atm. In addition, physical compression of the liquid occurs according to its compressibility, β, and the total pressure, P_T (fractional compression $= \beta P_T$). In fact, for the less soluble gases, helium and neon, the compression term is larger than the expansion term and net compression results; hence they are not anesthetics. For the more soluble anesthetic or narcotic gases, such as N_2, Ar, and N_2O, net expansion occurs (*4*). Equation 1 must be corrected for gas imperfections and for the slight dependence of solubility on total pressure. The nature of these corrections has been given in a previous paper (*4*), in which the critical volume hypothesis accounted for pressure reversal of anesthesia data for newts. Here, the treatment will be applied to mammals.

Quantitative data for the pressure reversal of anesthesia in mice are available for three gas mixtures—He : N_2O, Ne : N_2O, and H_2 : N_2O (*7*)—while comparable data for the elevation of convulsion threshold are available for He : N_2, He : N_2O, and He : H_2 (*3*). The study of the high pressure neurological syndrome is complicated by the apparent dependence of the convulsion threshold on the strain of mice used and, to some extent, on the compression rate employed. While these variations deserve more detailed investigation, they are not large, and the data used in this study are internally consistent, having been obtained in one laboratory by a standardized procedure.

The expansion caused by dissolution of the inert gases (Eq. 1) was calculated for the experimental isonarcotic and isoconvulsion end points. This is shown in Fig. 1 as a function of pressure for the model solvent benzene. Such a plot should yield a linear relation where the slope gives the compressibility of the site of action and the intercept gives the critical volume

Table 1. Results of calculations according to the critical volume hypothesis for three solvent models for the pressure reversal of anesthesia (*8*) and the high pressure neurological syndrome (convulsions) (*3*) in mice. Physical parameters for these calculations have been given previously (*4*); in addition, the solubility of neon in carbon disulfide is 4.8×10^{-5} mole fraction (*17*). For hydrogen $\bar{V}_2$ was taken as 35 ml/mole for all solvents (*18*). The Bunsen partition coefficient of hydrogen in olive oil was 0.04 (*9*). The compressibilities of olive oil, benzene, and carbon disulfide are 6, 9, and 7×10^{-5} atm^{-1}, respectively (*4*). For the critical volume change and compressibility, values are means ± standard deviations.

Solvent	Effect	Critical volume change (%)	Compressibility ($\times 10^5$ atm^{-1})	Correlation coefficient
Olive oil	Anesthesia	+0.35 ± 0.03	3.2 ± 0.56	.85
	Convulsions	−0.39 ± 0.12	7.1 ± 1.20	.91
Benzene	Anesthesia	+1.1 ± 0.02	3.0 ± 0.39	.91
	Convulsions	−0.85 ± 0.14	13.9 ± 1.37	.96
Carbon disulfide	Anesthesia	+0.60 ± 0.04	3.8 ± 0.68	.84
	Convulsions	−0.60 ± 0.16	10.0 ± 1.59	.92

change required for anesthesia or convulsions at 1 atm absolute. Results of the calculations for three solvents, which experience shows are good analogs of the anesthetic site (*8*), are summarized in Table 1. All three model solvents produce a good fit of the data for both anesthesia and convulsions.

The most striking conclusion from Table 1 is that a particular model solvent gives a self-consistent description in terms of the critical volume hypothesis for the volume changes associated with anesthesia and convulsions; that is, a particular positive or negative change in volume at the sites of action is critical and results in profound effects in the central nervous system. The predicted percentage expansions vary somewhat depending on the solubility and molar volumes of the solvents. The compressibilities are close to those observed experimentally in each case, although the site mediating convulsions is two to five times the more compressible of the two, indicating that two separate sites of action exist for anesthesia and convulsions. Further discussion of the differences between the solvents seems unlikely to be profitable, and it would be more interesting to know the results of such calculations for real membranes, but the few physical data available (*9*) only allow one to conclude that the volume changes given by these solvents are of the order of magnitude to be expected in real membrane systems.

A further evaluation of the physical parameters of the site of action, which is independent of gas solubility data, may be made by using results of recent experiments (*10*) in which mice breathing an oxygenated fluorocarbon fluid were compressed hydraulically to produce convulsions. These experiments were conducted at a number of reduced rectal temperatures, allowing a short extrapolation to 37°C, which gives a convulsion threshold of 62 atm at compression rates comparable to those in Table 1. If we assume that the compressibility of the convulsive site is that given by each of the model solvents (Table 1), then these data yield critical volume changes of −0.44, −0.86, and −0.62 percent for olive oil, benzene, and carbon disulfide, respectively—values in good agreement with those in Table 1. These liquid breathing experiments raise a further intriguing possibility. Liquids that have been compressed to remove all gas nuclei may be subjected to negative pressures of several hundred atmospheres without cavitation (*11*). Would a similarly treated mouse thus be anesthetized by negative pressures of the order of 50 to 100 atm?

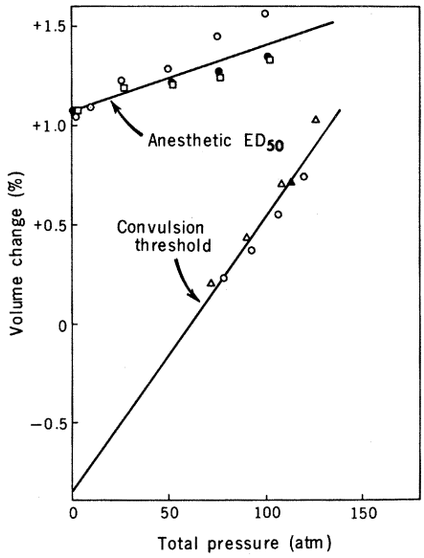

Fig. 1. Calculated expansion of benzene (Eq. 1) (*4*) caused by mixtures under isoanesthetic (*8*) and isoconvulsive (*3*) conditions at various pressures. The intercepts yield the critical volume changes, and the slopes, the compressibilities. The anesthetic ED_{50} is the dose effective in anesthetizing 50 percent of a group of animals.

For diving practice, the unified critical volume hypothesis suggests that the composition of the breathing mixture should be adjusted so as to produce no volume change at the site of action. Reversal of nitrogen narcosis by helium pressure has been observed in man (*12*), while the amelioration of the high pressure neurological syndrome in divers by adding anesthetic gas to their breathing mixtures is the object of active investigation (*3*). However, since the site for convulsions appears to be two to five times more compressible, it is clearly not possible to completely prevent volume changes at both sites by titrating the inert gas against the absolute pressure. Nonetheless, minimization of the changes should enable divers to maintain performance levels at considerably greater depths than those they currently achieve breathing helium-oxygen. Ultimately, it should be considered that the sites of action referred to here may only be the most sensitive of a spectrum of sites, as is suggested by the respiratory and cardiac problems encountered in mammals above 100 atm (*13*). This possibility, together with the different compressibilities in Table 1, suggests that more specific pharmacological intervention will eventually be required if man is to achieve depths of ever greater magnitude.

The success of the critical volume hypothesis in providing a self-consistent explanation of the interaction of anesthetic gases and pressure is rather remarkable. Although it cannot be ruled out that such success arises by chance, the hypothesis provides specific predictions about the sites of action which are accessible to experimental tests at a biophysical level. It seems, at present, most probable that the sites of action are situated in the lipid bilayers of some membranes (*14*). This interaction in itself is probably not directly responsible for the effects observed; rather, one might suppose that the membrane perturbations influence the functions of some membrane proteins in the neurological apparatus. Evidence for such a view may be found in stud-

ies of the red blood cell membrane (*5*), rat phrenic nerve (*15*), and the behaviors of simple antibiotic ionophores, such as valinomycin (*6, 14*) and gramicidin (*16*). Measurement of the appropriate membrane properties should enable this interpretation of the critical volume hypothesis to be examined in more detail.

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Antibody to Leukemia Virus: Widespread Occurrence in Inbred Mice

Abstract. *Mice from a wide variety of inbred strains produce immunoglobulin G antibody against murine leukemia virus. This is contrary to the common view that the mouse is immunologically tolerant to its endogenous leukemia virus.*

Until relatively recently it has been a widely held opinion that the mouse is immunologically tolerant to its endogenous leukemia virus (MuLV) (*1*). The first demonstration of autogenous immunity to MuLV was in NZB mice, where it was thought that the immune response to virus-associated antigens was actually a manifestation of the NZB autoimmune syndrome (*2*). New evidence (*3, 4*) now indicates, however, that autogenous immunity to MuLV exists also in certain other mouse strains (such as AKR and RF), and that immune responsiveness to MuLV-associated antigens might be more prevalent than was originally considered.

We report here studies that substantiate the view that the mouse is immunologically competent in respect to MuLV. With a sensitive radioimmune precipitation (RIP) assay we have found immunoglobulin (IgG) antibody to MuLV in mice of virtually all inbred mouse strains. These findings indicate that immune responsiveness of the mouse to MuLV might be the rule, rather than the exception.

The RIP assay used for the detection of mouse antibody against MuLV was modeled after the technique of Ihle *et al.* (*4*). In brief, MuLV from the

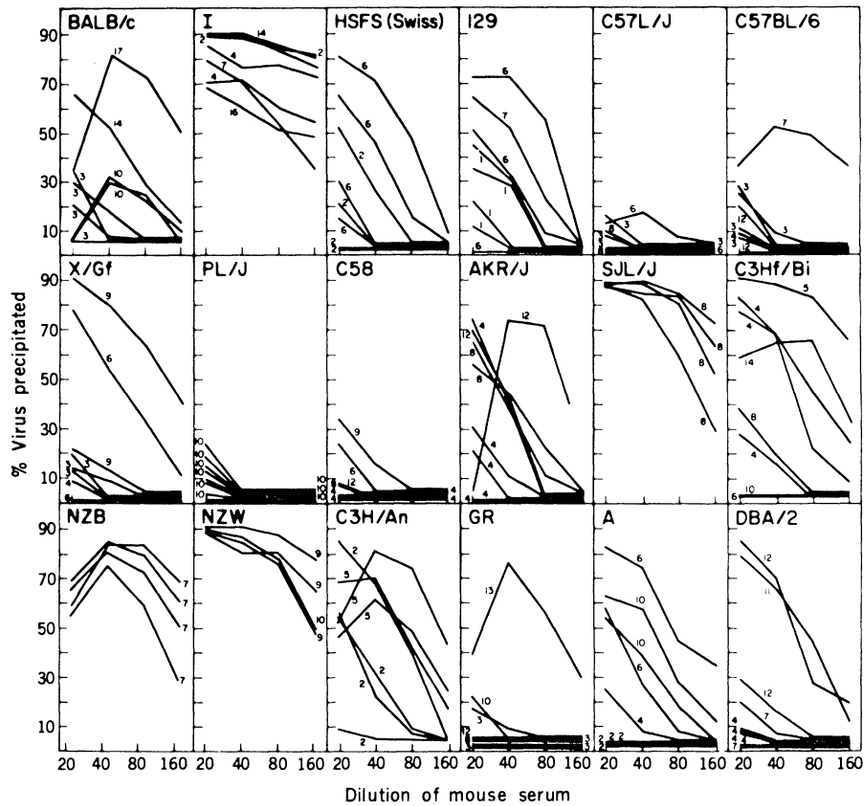

Fig. 1. Radioimmune precipitation (RIP) assay with serum from mice of various inbred strains. Each panel represents the results of mice from a single inbred strain (indicated in the upper left corner of the panel). Each line within a panel represents the titration curve of serum from a single mouse. The age of the mouse (in months) is indicated by the number located immediately next to the titration curve. Prozones of precipitation (for example, as uniformly observed in NZB mice) were considered the result of generally elevated IgG levels in certain individual mice; in support of this hypothesis was the observation that dilution of the antigobulin (goat antiserum to mouse 7*S* gamma globulins) produced analogous prozone effects with all mouse serums.