MOLECULAR PHARMACOLOGY, 14, 950-959

# Pressure Resolves Two Sites of Action of Inert Gases

KEITH W. MILLER,[1] MICHAEL W. WILSON, AND RAYMOND A. SMITH

*Departments of Pharmacology and Anaesthesia, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114*

(Received January 27, 1978)
(Accepted April 14, 1978)

SUMMARY

MILLER, KEITH W., WILSON, MICHAEL W. & SMITH, RAYMOND A. (1978) Pressure resolves two sites of action for inert gases. *Mol. Pharmacol.*, 14, 950-959.

The effect of pressure upon the potency of two pharmacological effects of inert gases has been studied in mice. In one series of experiments the effect of high pressures of helium on the anesthetic potency of nitrogen, argon, nitrous oxide, carbon tetrafluoride and sulfur hexafluoride was studied up to pressures of 183 atm. Pressure increased the $ED_{50}$ for loss of righting reflexes by 36% at 100 atm on average. In the other experiments we measured the ability of these inert gases to raise the $ED_{50}$ pressure at which pressure-induced hyperexcitability (spasms) was observed. Subanesthetic partial pressures of all the gases raised the $ED_{50}$ pressure for spasms significantly. These data were used to test the two hypotheses that anesthesia results when anesthetics expand some hydrophobic phase by a critical amount, while the hyperexcitability occurs when pressure reduces the volume of some hydrophobic phase by a critical amount (the critical volume hypothesis). Theoretical calculations show that both sets of data are consistent with their respective hypotheses. The site at which the inert gases exert their anti-hyperexcitability effect is much more compressible and has a slightly lower solubility parameter than the site for anesthesia.

## INTRODUCTION

The traditional lipid solubility theories of general anesthetic action (1, 2) have had to be modified in recent years to account for the remarkable antagonism of general anesthesia by pressure per se (3, 4). The currently accepted form of the lipid theory is the critical volume hypothesis, which states that anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical volume by the absorption of molecules of an inert substance. Pressure opposes this volume change and so reverses the anesthesia (5). This hypothesis has been shown to be quantitatively consistent with pressure reversal data for a number of anesthetics in newts (5). Pressure reversal also has been demonstrated for a variety of agents in tadpoles (6) and for conduction block in the squid giant axon (7, 8). The question then arises whether the critical volume hypothesis describes a general mechanism of anesthetic action which may occur at different sites of action within neural tissue, and if so whether the physical parameters required to describe such sites will vary from site to site. In this paper we have examined the interaction between unreactive gases

This research was supported in part by a National Institute of General Medical Sciences Research Center Grant (GM-15904-10) and in part by an Office of Naval Research Contract (N00014-75-C-0727) with funds provided by the Naval Medical Research and Development Command.

[1] Keith W. Miller is a Research Career Development Awardee of N.I.G.M.S. (GM 00199).



0026-895X/78/0145-0950$02.00/0

and pressure in mice when the gases act either as general anesthetics or as agents which ameliorate the high pressure neurological syndrome (9). By a suitable choice of gases, specifically the inclusion of fully fluorinated compounds (10), we show that these two pharmacological properties of the gases are mediated by a hydrophobic site or sites with rather similar solvent properties. However, the isothermal compressibility parameter, which describes the variation of pharmacological potency with pressure, differs by half an order of magnitude for these two sites. Thus, we have differentiated two pharmacological sites of action of inert gases in a single species of mammal on the basis of the effects of pressure. A preliminary analysis of available data had previously shown this to be the case when different species of mice were involved (11), although this conclusion was questionable because insufficient data were then available to define the solvent properties of the site of action. In a number of other studies sites of action of volatile agents have been distinguished on the basis of solvent properties (2, 12).

The application of the critical volume hypothesis to the high pressure neurological syndrome has been proposed previously (11). It states that the high pressure neurological syndrome occurs when some hydrophobic region has been compressed beyond a critical amount by the application of pressure. Absorption of an inert gas will cause an expansion which compensates for such compression and raises the threshold pressure for symptoms. The high pressure neurological syndrome is a complex of symptoms that are observed when mammals are compressed either hydraulically (13, 14) or in helium-oxygen atmospheres (15). At moderate pressure it manifests itself as tremor of the limbs but as the pressure is further raised rhythmic spasms, clonic, and then tonic convulsions occur. In this paper we consider the effect of inert gases on the threshold pressure for spasms, which is one of the earliest objectively definable end points. (The tremors which occur at lower pressure were too difficult to distinguish from the fasciculation caused by moderate doses of anesthetics.)

## METHODS

Male CD-1 mice (Charles River) weighing 20–30 g were used in all experiments. Measurements of anesthetic potency were performed in a 34 liter steel hyperbaric chamber equipped with two large windows, a temperature control system and moisture and carbon dioxide scrubbers. Complete details have been published previously (16).

The level of anesthesia was determined by testing the ability of each mouse to remain upright when its cylindrical wire mesh cage was rotated at 4 rpm for five complete revolutions; a score between 0 and 5 was assigned (16). A group of seven mice was exposed at each window. Two additional mice with rectal thermistors were placed between these groups, and the chamber temperature adjusted to maintain rectal temperature at 37 ± 1°C. The partial pressure of oxygen was maintained in the range of 0.3 to 1.0 atm.

Mice were examined for the high pressure neurological syndrome in separate experiments. Two groups of five mice were used in each experiment, together with two additional rectal temperature controls. After addition of the anesthetic gas, helium was admitted to the chamber at a constant compression rate of 60 atm per hour and the animals continuously monitored for the symptoms of the high pressure neurological syndrome. Complete spasms were defined as rhythmic tensing and relaxing of all muscle groups but not of sufficient severity to cause the animal to lose its upright posture (17). These spasms generally were observed to appear after the onset of coarse whole body tremors but before generalized clonic convulsions involving loss of upright posture (15, 17).

During the anesthesia experiments the number of mice responding was determined as a function of anesthetic partial pressure at a series of given total pressures. At each total pressure a dose response curve was obtained and analyzed on a digital calculator using the method of Waud for quantal responses (18). Since each animal was used at several doses on each curve, the number of animals at each dose was weighted so that the sum for all doses equalled the

number of animals actually employed. Pressure-response curves for the high pressure neurological syndrome were obtained for each gas mixture and analyzed as above, but since each animal responded once only weighting was unnecessary. In about half of these experiments complete spasms were not observed in all animals, but all the animals were included in the cumulative pressure-response curve in order to avoid biasing the analysis with the most susceptible animals.

Helium (99.995% pure) and nitrous oxide (98%) were obtained from Ohio Medical Products, Wisconsin; nitrogen (99.9%) and oxygen (99.6%) were from Medical Technical Gases, Massachusetts; argon (99.995%) and sulfur hexafluoride (99.8%) were from Matheson Gas Products, New Jersey; and hexafluoroethane (99.6%) and carbon tetrafluoride (99.7%) were from Dupont-de-Nemours EI and Co., Delaware.

## RESULTS

### *Experimental*

$ED_{50}$ values for the loss of righting reflex in mice are listed in Table 1 with their standard errors and scale parameters which provide a measure of the slope of the dose response curves (18). Some of the data for nitrogen and argon have been published previously by us but are reproduced here for completeness (16). The $ED_{50}$'s in the absence of helium agree well with earlier data (19, 20). The value for carbon tetrafluoride is higher than that obtained under less well controlled conditions (19), while that for nitrous oxide is lower than most (19–21), but not all (22), literature values. Sulfur hexafluoride exhibited a low therapeutic index and it was impossible to obtain meaningful results unless some helium was added. However, the value obtained agrees closely with previous work (20). Similarly, hexafluoroethane caused marked respiratory distress. By increasing the partial pressure of oxygen to two atmospheres and lowering the rectal temperature by 0.5°C, we determined an $ED_{50}$ close to that reported by Miller et al. (20).

There were no consistent and significant trends in the slopes of the dose-response curves with increasing pressure (Table 1). This contrasts with intravenous anesthetics, where a small but significant increase in slope with pressure has been reported (16). Comparison of the $ED_{50}$ at one atmosphere (extrapolated where necessary) to that at 100 atm reveals the following percentage increases: $N_2$ 33; $CF_4$ 34; Ar 33; $SF_6$ 36; $N_2O$ 42. These values agree closely with those obtained independently for $N_2O$, $N_2$ and Ar (21, 23). For each agent plots of $ED_{50}$ as a function of pressure were linear, and introduction of a quadratic term in the regression gave no significant improvement in fit ($F$-test).

Pressure per se causes marked effects above about 140 atm (13–15); consequently most of our measurements were obtained at lower pressures. With the densest gases

TABLE 1

*Variation of $ED_{50}$ values for the loss of righting reflex in mice as a function of pressure*

| Anesthetic | Total pressure | $ED_{50}$ ± SE | Scale parameter ± SE | N |
|---|---|---|---|---|
| | *atm* | *atm* | | |
| $N_2O$ | 2.2 | 1.22 ± 0.048 | −9 ± 4.0 | 56 |
| | 30 | 1.32 ± 0.033 | −17 ± 6.6 | 56 |
| | 40 | 1.35 ± 0.022 | −22 ± 6.3 | 70 |
| | 50 | 1.30 ± 0.074 | −9 ± 4.3 | 70 |
| | 80 | 1.61 ± 0.059 | −12 ± 5.3 | 41 |
| | 90 | 1.61 ± 0.038 | −17 ± 5.8 | 41 |
| $N_2$ | 40[a] | 38.9 ± 0.94 | −12 ± 3.8 | 63 |
| | 81[a] | 45.8 ± 0.68 | −30 ± 14 | 26 |
| | 101 | 47 ± 1.1 | −20 ± 11 | 26 |
| | 121[a] | 48.9 ± 0.52 | −50 ± 20 | 26 |
| | 141 | 50.4 ± 0.93 | −26 ± 9.7 | 26 |
| Ar | 19[a] | 18.1 ± 0.67 | −11 ± 4.6 | 35 |
| | 81[a] | 21.4 ± 0.78 | −14 ± 8.7 | 17 |
| | 124[a] | 24.2 ± 0.59 | −32 ± 16 | 14 |
| | 128 | 26 ± 1.6 | −11 ± 7 | 14 |
| | 131 | 23.7 ± 0.77 | −20 ± 11 | 17 |
| | 183 | 27.1 ± 0.67 | −25 ± 15 | 14 |
| $CF_4$ | 27 | 26 ± 1.0 | −12 ± 5.2 | 26 |
| | 60 | 30 ± 1.4 | −10 ± 6.7 | 26 |
| | 99 | 33 ± 1.2 | −20 ± 11 | 14 |
| | 140 | 35 ± 2.9 | −11 ± 13.3 | 12 |
| $SF_6$ | 12 | 5.58 ± 0.086 | −16 ± 3.1 | 119 |
| | 20 | 6.08 ± 0.072 | −29 ± 6.5 | 80 |
| | 40 | 6.42 ± 0.11 | −19 ± 6.8 | 47 |
| | 50 | 6.6 ± 0.08 | −28 ± 10.4 | 48 |
| | 60 | 6.5 ± 0.16 | −15 ± 19.3 | 33 |
| | 70 | 6.8 ± 0.11 | −21 ± 8.5 | 43 |
| $C_2F_6$ | 23 | 17.1 ± 0.39 | −11 ± 3.2 | 84 |

[a] Data previously reported (16).

studied ($SF_6$ and $C_2F_6$), the stress of the hyperbaric environment further limited the accessible pressure range (Table 1). In the case of argon, however, by using slow compression rates we were able to demonstrate pressure reversal at pressures up to 183 atm. Thus, although the hyperbaric environment undoubtedly introduces significant problems in mammalian studies, necessitating close control of environmental conditions, satisfactory quantitative data are obtainable and reproducibility between laboratories is fair (21, 23). The complete spasms threshold pressures are given in Table 2. The threshold pressure in helium was found to be 83 ± 2.5 (SE) atm, and all the other gases elevated this threshold significantly at doses which were well below those required to cause anesthesia at these pressures. In control experiments without anesthetic, 17% of the animals exhibited the more severe symptoms of the high pressure neurological syndrome, such as clonic and tonic convulsions, without proceeding through a complete spasm phase. This partly reflects the small pressure increments occurring between successive phases of the syndrome; for example, the $ED_{50}$'s for clonic and tonic convulsions were 88 ± 1.4 and 96 ± 3.4 atm. The proportion of animals with complete spasms is indicated in Table 2.

Our data for clonic convulsions agree well with a value of 89 atm reported by Brauer (24) for CD-1 mice compressed at 60 atm per hour. This threshold pressure is somewhat dependent on compression rate, varying from 103 atm at 10 atm $hr^{-1}$ to 86 atm at 100 atm $hr^{-1}$ (24). In our work, therefore, the compression rate was kept constant within ±2%. Only one complete spasm threshold has been reported in CD-1 mice: this value of 87 atm was obtained at a compression rate of 60 atm $hr^{-1}$ (17).

TABLE 2

*Median pressures for the appearance of complete body spasms in mice breathing helium-oxygen and helium-oxygen-anesthetic gas mixtures*

| Anesthetic gas | $ED_{50}$ ± SE | Scale parameter ± SE | No. responding/total no. |
|---|---|---|---|
| | *atm* | | |
| None | 83 ± 2.5 | 12 ± 3.5 | 33/40 |
| 0.1 atm $N_2O$ | 90 ± 6.3 | 11 ± 7.5 | 9/10 |
| 0.2 atm $N_2O$ | 103 ± 8.3 | 8 ± 8.0 | 6/10 |
| 0.4 atm $N_2O$ | 99 ± 2.0 | 9 ± 6.9 | 9/10 |
| 0.8 atm $N_2O$ | 100 ± 1.5 | 23 ± 18.0 | 10/10 |
| 1.5 atm $N_2O$ | 120 ± 1.3 | 13 ± 8.4 | 10/10 |
| 3 atm $N_2$ | 88 ± 4.0 | 17 ± 16.0 | 8/10 |
| 15 atm $N_2$ | 110 ± 3.0 | 30 ± 21.0 | 10/10 |
| 30 atm $N_2$ | 112 ± 6.6 | 13 ± 9.4 | 10/10 |
| 45 atm $N_2$ | 120 ± 5.1 | 20 ± 13.0 | 9/9 |
| 3 atm Ar | 95 ± 4.0 | 20 ± 12.0 | 10/10 |
| 8 atm Ar | 108 ± 6.9 | 12 ± 9.1 | 8/10 |
| 18 atm Ar | 129 ± 3.6 | 29 ± 23.0 | 10/10 |
| 3 atm $CF_4$ | 86 ± 3.3 | 21 ± 16.0 | 10/10 |
| 10 atm $CF_4$ | 88 ± 3.8 | 19 ± 13.0 | 10/10 |
| 17.2 atm $CF_4$ | 137 ± 10.6 | 10 ± 8.5 | 7/10 |
| 25 atm $CF_4$ | 115 ± 6.2 | 17 ± 18.0 | 6/10 |
| 0.2 atm $SF_6$ | 91 ± 6.5 | 11 ± 9.1 | 7/10 |
| 0.4 atm $SF_6$ | 89 ± 6.7 | 10 ± 7.8 | 9/10 |
| 1.0 atm $SF_6$ | 83 ± 9.9 | 9 ± 7.2 | 10/10 |
| 2.5 atm $SF_6$ | 87 ± 8.5 | 8 ± 5.2 | 10/10 |

### *Theoretical Analysis of Data*

It is not possible to account fully for pressure reversal of anesthesia by addition to either the Meyer-Overton (1) or Mullins (2) hypothesis of a term which takes into account the dependence of the lipid solubility of the anesthetic on pressure (5). The original Mullins formulation was stated in terms of volume occlusion of free space in membranes. Although such a mechanism is now known to occur in a number of cases of cytoplasmic protein-anesthetic interactions (25, 26), it is not clear how it can account for the pressure reversal of anesthesia (2). If the formulation is restated in terms of the membrane expansion which has been shown more recently (27–29) to be caused by small hydrophobic molecules, then pressure reversal is readily accounted for by adding a term for the isothermal compressibility ($\beta$) of the membrane (5). This critical volume hypothesis of anesthetic action, which we have defined previously (5), may be written down in its most general form as

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

Where $\Delta V_c$ is the volume change which is associated with anesthesia (or any other end point), $\Delta V_i$ is the expansion caused when the $i$th anesthetic dissolves in the hydrophobic region and $P_T$ is the total mechanical pressure.

Equation (1) may also be used to formulate the critical volume hypothesis applied to the high pressure neurological syndrome (11). In our case $\Delta V_c$ will be the volume change at which complete spasms are observed. Since these may be produced by hydraulic compression of mice in oxygenated fluorinated hydrocarbon solvents (13, 14) (when $\Delta V_i = 0$), it is evident that this syndrome is accompanied by a negative volume change since under these conditions equation (1) reduces to

$$\Delta V_c = -\beta P_T \qquad (2)$$

Since all gases, including helium, contribute positively to $\Delta V_i$, it follows that for helium the term $(\Delta V_{He} - \beta P_{He})$ must be less than zero both at the anesthetic site and at the site mediating complete spasms. If this were not so the model would predict helium to be anesthetic and the high pressure neurological syndrome would not be observed in helium-oxygen atmospheres.

In order to perform a quantitative test of these two versions of the critical volume hypothesis it is necessary to know the physical parameters of the sites of action which are required to solve equation (1) in each case. Since the sites of action are not identified this is not possible, but it is well known that certain apolar solvents provide good models of the anesthetic site. It is possible that such solvents might also provide a suitable model for the site associated with complete spasms (11).

*Choice of Solvent Models for Anesthesia and Complete Spasms*

It is possible to choose objectively the best solvent model of a site of action of inert gases if pharmacological data are available for some fully fluorinated gases (10, 19, 20). This procedure is based on the observation that when a set of nonpolar solvents of graded solvent power are compared it is found that fully fluorinated gases deviate systematically, depending on solvent power, from the correlations always observed for the nonfluorinated gases. By minimizing these deviations the solvent power of the site of action may be defined objectively.

Hildebrand (30) defined the solvent power of a solvent in terms of a solubility parameter, $\delta$, which is defined as

$$\delta^2 = \frac{\Delta E_v}{V_m} \qquad (3)$$

where $\Delta E_v$ is the heat of vaporization of the solvent at constant volume and $V_m$ is its molar volume. Strictly speaking, equation (3) applies only to nonpolar solvents and to solutes and solvents of equal molecular size. However, in practice semiquantitative agreement is found over a wider range of situations (30). Sufficient solubility data were available to carry out such calculations for six solvents, including water which was not included in the final analysis because of its high polarity. The analysis of the deviations of the fluorocarbons, which is described below, differs somewhat from that used before (20) since we wished to apply it to our data obtained at all pressures. For each $ED_{50}$ at a given pressure ($P_T$) in Tables 1 and 2, $\Delta V_i$ may be calculated for each gas at a partial pressure, $P_i$, in each solvent since (5)

$$\Delta V_i = \frac{\bar{V}_i \cdot X_i \cdot P_i}{V_m} \qquad (4)$$

where, $\bar{V}_i$ is the partial molar volume of the gas in the solvent, $X_i$ is its mole fraction solubility at a partial pressure of one atmosphere, and $V_m$ is the molar volume of the solvent. Corrections must be made to equation (4) to account for the non-ideal behavior of gases and for the dependence of $X_i$ upon total pressure (5). These corrections reduced the value of $\Delta V_i$ calculated by equation (4). In general such corrections lay in the range of 10–20%, but in a few cases are in the range 50–60%. Sources of the physical parameters used in these calculations are given in the appendix.

$\Sigma\Delta V_i$ in equation (1) was calculated for each datum in Tables 1 and 2 and plotted against $P_T$ (equation 2). A solvent which ideally represented one of the sites of action should yield a linear plot with a slope of $\beta$, the compressibility, and an intercept of $\Delta V_c$. Each gas gave such a line; those for nitrous oxide, nitrogen and argon were essentially co-linear in all solvents, while those for sulfur hexafluoride and carbon tetrafluoride were above the other gases at low solubility

parameter and below at high solubility parameter (Fig. 1). Hexafluoroethane was not included in this analysis because there are

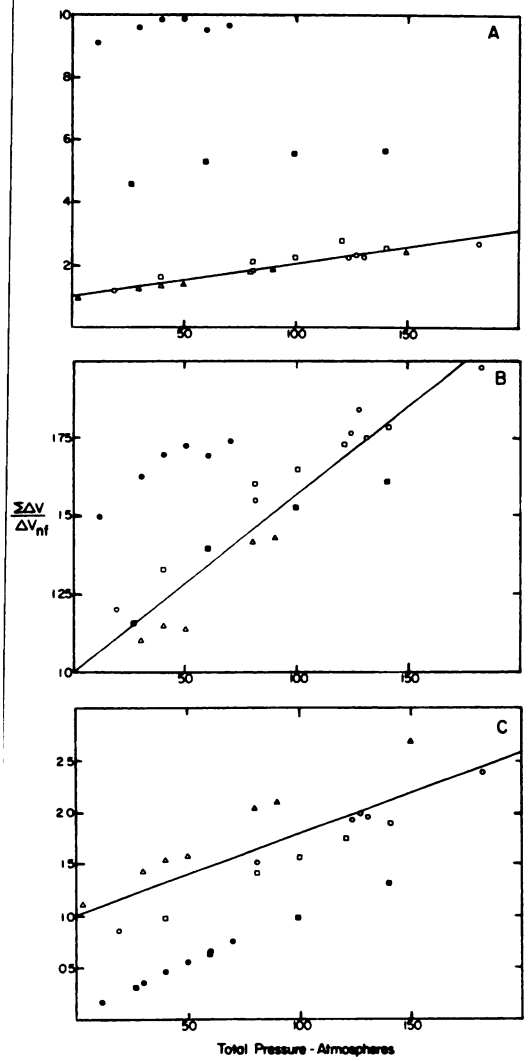


FIG. 1. *Plots of the calculated expansion caused by $ED_{50}$ doses of anesthetics with helium ($\Sigma\Delta V$) at various pressures*

The expansion has been normalized to the intercept for the inert gases, ($\Delta \bar{V}_{nf}$), for comparative purposes. Key: $N_2$ □; Ar ○; $N_2O$ △; $CF_4$ ■ and $SF_6$ ●. For the fluorinated solvents (A) $\delta = 6$, for carbon disulfide (B) $\delta = 10.0$ and for water (C) $\delta \simeq 23$. Note that the ordinates of each figure are plotted with a different scale. Values of $(\Delta \bar{V}_f - \bar{V}_{nf}/|\Delta \bar{V}_f + \Delta \bar{V}_{nf}|)$ (see text) in each solvent for anesthesia and complete spasms respectively were: fluorinated solvents 0.75 and 0.76; cyclohexane ($\delta = 8.2$) 0.39 and 0.09; benzene ($\delta = 9.2$) 0.24 and 0.05; carbon disulfide 0.08 and 0.01 and octanol ($\delta = 10.3$) 0.10 and 0.02.

insufficient solubility data. To quantify these deviations of the fluorinated gases a straight line was fitted through the nonfluorinated gases to yield an intercept ($\Delta \bar{V}_{nf}$). A parallel line was fitted to the data for the fluorinated gases to yield a second intercept ($\Delta \bar{V}_f$). For each solvent the expression $(\Delta \bar{V}_f - \Delta \bar{V}_{nf})/|\Delta \bar{V}_f + \Delta \bar{V}_{nf}|$ was plotted against the solubility parameter, $\delta$. Linear regression yielded zero deviation at $\delta$ (anesthesia) = 10.8 ± 0.15 (SD) and $\delta$ (complete spasm) = 9.8 ± 0.40 ($p = 0.001$) $(Cal/cm^3)^{1/2}$. The complete spasms site is thus a slightly better solvent (lower $\delta$) than the anesthetic site. However, such small differences in solubility parameter should be regarded with reservation because the analysis is critically dependent on the fluorinated gases with which the experimental difficulties are greatest. Thus a previous analysis with poorer data for carbon tetrafluoride yielded $\delta$ (anesthesia) = 10 (20).

### *Results of the Critical Volume Hypothesis Analysis*

The actual solvents which most closely represent the solubility parameters defined above are octanol ($\delta = 10.3$) and carbon disulfide ($\delta = 10.0$). We have chosen the latter for detailed consideration since the requisite physical properties have been better defined. We have also considered olive oil both because of its historical importance and because its molar volume is closer to that of membrane components such as phospholipids. The absolute values of $\Delta V_c$ given by it should thus be more realistic than those given by carbon disulfide. The results obtained by calculating $\Sigma\Delta V_i$ (equation (4)) for all the data in Tables 1 and 2 and analyzing them according to equation (1) are given in Table 3 and in Fig. 2. The parameters obtained for the two sites of action are not grossly dependent on the solvent model, although as expected the volume changes predicted by olive oil are less marked than those predicted by carbon disulfide. In fact the success of olive oil at modeling these sites of action would seem to vindicate a long tradition.

Two major conclusions stand out. First, increases or decreases in volume or density in hydrophobic sites in the central nervous system may lead to profound effects, such

TABLE 3

*Results of calculations to test the critical volume hypothesis*

| Model solvent | Effect | Critical volume change (%) ± SD | Compressibility ($\times 10^{-5}$ atm$^{-1}$) ± SD | Correlation coefficient |
|---|---|---|---|---|
| Carbon disulfide | anesthesia | +0.63 ± 0.036 | 2.1 ± 0.39 | 0.72 |
| | complete spasms | −0.6 ± 0.17 | 11 ± 1.6 | 0.83 |
| Olive oil | anesthesia | +0.43 ± 0.029 | 2.4 ± 0.32 | 0.83 |
| | complete spasms | −0.5 ± 0.12 | 8.4 ± 0.12 | 0.85 |

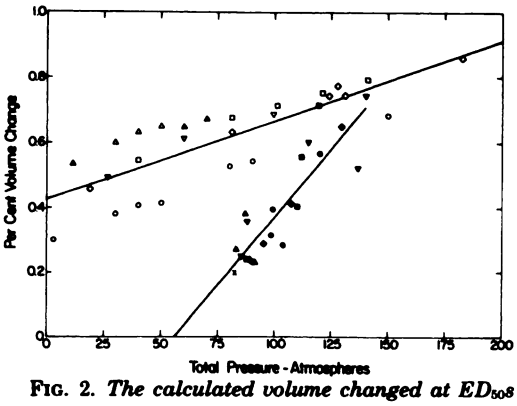


FIG. 2. *The calculated volume changed at $ED_{50}$s for anesthesia (open symbols) and pressure-induced spasms (closed symbols) using olive oil as a model solvent*

Key: $N_2$ □, ■; Ar ◇, ◆; $N_2O$ ○, ●; $CF_4$ ▽, ▼; $SF_6$ △, ▲ and helium ×.

as the depression and excitation studied here. The magnitude of these changes seems to be in the range of ½–1% although this magnitude reflects somewhat the choice of the model to represent the hydrophobic site. Second, more than one site of action is involved in the two phenomena studied here, the site mediating complete spasms being four to six times more compressible than that mediating anesthesia. These conclusions confirm a preliminary test of these two applications of the critical volume hypothesis which was based on scant data derived from heterogeneous sources (11). A difference in the pressure sensitivity of anesthesia and convulsions has also been noted when barbiturates are used (31).

A number of interesting corollaries follow from these calculations. Equation (2) predicts that mice compressed hydraulically would experience complete spasms at 56 atm (olive oil model), a prediction which could be checked experimentally. For tonic convulsions this threshold is found to be 62 atm (14).

Figure 2 illustrates clearly that subanesthetic doses are required initially to prevent complete spasms. However, since the compressibility of the spasm site is greater, then as the pressure is progressively raised, relatively higher doses of inert gas are required to prevent spasms until above 148 atm more than an anesthetic dose is required. In fact two reports of convulsing anesthetized mice at extreme pressures have been published (31, 32).

An important point is that the theory does not predict that helium will compress all hydrophobic sites. Equations (1) and (4) show that a negative volume change only occurs when $(V_{He} \cdot X_{He}/V_m) < \beta$. If, for example, $X_{He}$ is larger (i.e., in a solvent of lower $\delta$) net expansion might occur. Thus, synergism between helium and an anesthetic cannot be taken as *prima facie* evidence that a mechanism inconsistent with the critical volume hypothesis is involved, unless quantitative studies are performed to derive $\delta$ and $\beta$ or hydraulic pressure is used as an added criterion. Synergism between helium and nitrogen has been reported in some, but not all, behavioral tests in rats (33), while synergism between hydraulic pressure and anesthetics has occasionally been noted (34).

Similarly, if we consider gases just a little more soluble than helium at the anesthetic site of action, we find that neon, which is 1.2 times more soluble than helium, causes a volume change at 100 atm partial pressure of only 0.035% (olive oil model). This is consistent with the finding that high pressures of neon do not change the potency of nitrous oxide (21).

It is also possible to make an estimate from this data of the thermal expansion

coefficients, $\alpha$, of the sites of action. For a simple nonpolar solvent one may write (30, 35)

$$\delta^2 = \left(\frac{\partial E}{\partial V}\right)_T = T\left(\frac{\partial P}{\partial T}\right)_V \simeq T\frac{\alpha}{\beta} \quad (5)$$

Using the parameters in Table 3 for carbon disulfide, equation (5) yields an $\alpha$ of $8 \times 10^{-4}$ for the anesthetic site and $4 \times 10^{-3}$ $°C^{-1}$ for the spasm site. The actual $\alpha$ coefficient for carbon disulfide itself is $1.2 \times 10^{-3}$, and for oleic acid is $9.6 \times 10^{-4}$ (36), so these predictions are not unreasonable in spite of the assumptions inherent in their derivation.

## DISCUSSION

The success of the critical volume hypothesis in providing a unified description of the pressure reversal of anesthesia and of the amelioration of the high pressure neurological syndrome by inert gases is remarkable. When data for the fluorinated gases are available so that an objective choice of model solvent may be made, the only remaining adjustable parameter is the compressibility of each site of action. Physically realistic values of this parameter must lie within a fairly narrow range of values spanning little more than an order of magnitude. Thus, the actual values for olive oil and for carbon disulfide are 6 and $7 \times 10^{-5}$ $atm^{-1}$, respectively. For hexadecane and pentane the values are 7 and $17 \times 10^{-5}$ $atm^{-1}$ (36). On this basis the values of compressibility found in Table 3 are seen to be quite plausible.

A putative site of action for anesthetics is the lipid bilayer region of membranes. In principle we should be able to test this model, but the data are currently inadequate. Some data (37, 38) suggest that gas solubility in olive oil is less than twice that in bilayers. Thus we would expect lipid bilayers to yield slightly smaller critical volumes and compressibilities than those shown for olive oil in Table 3. The only available bilayer compressibility is for the liquid crystalline phase of dipalmitoyl lecithin and it is $10^{-4}$ $atm^{-1}$ (39). Thus lipid bilayers will probably provide about as good a model as the simple solvents used here. Moreover the anisotropy of bilayers may need to be considered (5).

Although anesthetics are relatively nonspecific when compared to receptor directed drugs, they do in fact exhibit considerable selectivity at low doses when studied in well defined systems (40–42). Attempts have been made to explain some of this specificity on the grounds of differential solubility in target membranes (2, 43). This is not the case in our study where the two sites have barely distinguishable solubility properties. The spasm site is most clearly differentiated from the anesthetic site by its greater sensitivity to pressure, which results from a fivefold higher compressibility. Thus we have characterized two separate sites of action which reach a critical point and cause a physiological response, one at a positive volume change and the other at a negative volume change. Under ambient pressure conditions both will be almost equally expanded by an anesthetic but whether such expansion would prove critical to the HPNS site, and if so what the result would be, cannot be defined. It would seem quite reasonable to suppose, however, that a sufficient volume change in any direction could have profound effects (44). Only studies in simpler systems will answer such questions.

The demonstration of two distinct sites in the central nervous system where anesthetics and pressure interact antagonistically raises questions about the simplifying assumptions in our treatment of the critical volume hypothesis. We have assumed that the anesthetic-pressure interaction which mediates a given physiological response, such as righting reflex, occurs at a single class of site which has uniform physical properties. Thus, even when the assumption of direct pressure-anesthetic interaction is retained, our results might merely reflect the average physical properties of a number of distinct sites, all of which are sensitive to both anesthetics and pressure. In isolated ganglia (42) the lowest effective doses of anesthetic block only one pathway, but at higher doses others are blocked. In the central nervous system a number of distinct pathways might be blocked over a very narrow dose range and our idea of

distinct sites might then need to be replaced by a continuum of sites. Our results cannot fully resolve this problem, but the linearity observed in each case in Fig. 3 does suggest that for a given physiological response there are no strong interactions between two or more sites with different physical properties. Furthermore the anti-HPNS and the anesthetic sites are distinct and independent, since when both have supra-critical volume changes (e.g., above 150 atm, see Fig. 3) we observe signs of the HPNS in anesthetized animals (31, 32).

With the above reservations the critical volume hypothesis provides a description of the thermodynamic properties of a hydrophobic region at which anesthetics appear to act. To proceed further requires thermodynamic measurements of the properties of actual putative sites. At least three mechanisms can be distinguished. The anesthetic might interact directly with hydrophobic regions in an excitable protein (27), or it might affect such a protein by a perturbation of its lipid environment, either by expanding and thereby fluidizing it (45) or by changing the phase relationships in it (46). These three models are qualitatively consistent with the predictions of the critical volume hypothesis. To distinguish between them with quantitative calculations requires thermodynamic measurements that are not yet available for gaseous anesthetics, although the solubility parameter of erythrocytes is close to those predicted here (47). Even when such data are available a successful model can only be shown to have properties consistent with those of the site of action. It then provides a powerful predictive and rather exclusive thermodynamic framework without specifying the details of mechanisms. The deductive elucidation of the latter requires a different approach.

## APPENDIX

Physical quantities used in the calculations were generally available in the literature. Solvent molar volumes, solubility parameters and gas solubilities were taken from a number of useful reviews (29, 30, 48–51). Partial molar volumes of gases in solvents are not all available, but since they vary little with solvent we used the values for benzene in all cases. Sources have been given in a previous publication (5), except the values for argon (44.6 ml/mole) and hexafluoroethane (110 ml/mole) which were taken from references 52 and 53. Virial coefficients for pure gases and for gas mixtures have also been given previously (5) except for B (Ar-He) for which +24 ml/mole was estimated (54).

## REFERENCES


1. Meyer, H. (1906) in *The Harvey Lectures*, pp. 11–17, Lippincott, Philadelphia.
2. Mullins, L. J. (1954) *Chem. Rev.*, **54**, 289–323.
3. Johnson, F. H. & Flagler, E. A. (1950) *Science*, **112**, 91–92.
4. Lever, M. J., Miller, K. W., Paton, W. D. M., Streett, W. B. & Smith, E. B. (1971) in *Proc. Fourth International Symposium on Underwater Physiology*, **2969**, pp. 101–108, Academic Press, New York.
5. Miller, K. W., Paton, W. D. M., Smith, R. A. & Smith, E. B. (1973) *Mol. Pharmacol.*, **9**, 42–57.
6. Halsey, M. J. & Wardley-Smith, B. (1975) *Nature*, **251**, 811–813.
7. Spyropoulos, C. (1957) *J. Gen. Physiol.*, **40**, 848–857.
8. Parmentier, J. L., Shrivastav, B. B., Bennett, P. B. & Narahashi, T. (in press) *Science*.
9. Brauer, R. W., Dimoo, S., Fructus, X., Gosset, A. & Naquet, R. (1969) *Rev. Neurol.*, **121**, 264–265.
10. Miller, K. W., Paton, W. D. M. & Smith, E. B. (1965) *Nature*, **206**, 574–577.
11. Miller, K. W. (1974) *Science*, **185**, 867–869.
12. Cohen, S., Goldschmid, A., Shtacher, G., Srebrenik, S. & Gitter, S. (1975) *Mol. Pharmacol.*, **11**, 379–385.
13. Kylstra, J. A., Nantz, R., Crowe, J., Wagner, W. & Saltzman, H. A. (1967) *Science*, **158**, 793–794.
14. Lundgren, C. E. G. & Ornhagen, H. C. (1976) in *Underwater Physiology V*, pp. 397–404, (Lambertsen, C. J., ed.) Fed. Amer. Soc. Exper. Biol., Bethesda.
15. Brauer, R. W., Goldman, S. M., Beaver, R. W. & Sheehan, M. E. (1974) *Undersea Biomed. Res.*, **1**, 59–72.
16. Miller, K. W. & Wilson, M. W. (1978) *Anesthesiology*, **48**, 104–110.
17. Rowland-James, P. (1976) *Chemistry Part II Thesis*, Oxford.
18. Waud, D. R. (1972) *J. Pharmacol. Exper. Therap.*, **183**, 577–607.
19. Miller, K. W., Paton, W. D. M. & Smith, E. B. (1967) *Brit. J. Anaesth.*, **39**, 910–918.
20. Miller, K. W., Paton, W. D. M., Smith, R. A. & Smith, E. B. (1972) *Anesthesiology*, **36**, 339–351.
21. Halsey, M. J., Eger, E. I., Kent, D. W. & Warne,

P. J. in *Molecular Mechanisms of Anesthesia*, (Fink, B. R., ed.), Vol. 1, pp. 353–361, Progress in Anesthesiology, Raven Press, New York.
22. Brauer, R. W. & Way, R. O. (1970) *J. Applied Physiology*, **29**, 23–31.
23. Smith, R. A., Winter, P. M., Halsey, M. J. & Eger, E. I., II (1975) *Abstracts Am. Soc. Anesthesiol.*, 217–218.
24. Brauer, R. W., Beaver, R. W., Mansfield, W. M., O'Connor, F. & White, L. W. (1975) *J. Appl. Physiol.*, **38**, 220–227.
25. Schoenborn, B. P., Watson, H. C. & Kendrew, J. C. (1965) *Nature*, **207**, 28–30.
26. Sachsenheimer, W., Pai, E. F., Schulz, G. E. & Schirmer, R. H. (1977) *FEBS Letters*, **79**, 310–312.
27. Seeman, P. (1972) *Pharmacol. Rev.*, **24**, 583–655.
28. Roth, S. H. (1975) in *Molecular Mechanisms of Anesthesia*, (Fink, B. R., ed.), Vol. 1, pp. 405–420, Progress in Anesthesiology, Raven Press, New York.
29. Seeman, P. (1974) *Experientia*, **30**, 759–760.
30. Hildebrand, J. H., Prausnitz, J. M. & Scott, R. L. (1970) in *Regular and Related Solutions*, pp. 201–203, Van Nostrand Reinhold, New York.
31. Beaver, R. W., Brauer, R. W. & Lahser, S. (1977) *J. Appl. Physiol.*, **43**, 221–229.
32. Miller, K. W. (1977) *Fed. Proc.*, **36**, 1663–1667.
33. Thomas, J. R. & Burch, L. S. (1975) *Perceptual and Motor Skill*, **41**, 797–798.
34. Youngson, A. F. & MacDonald, A. G. (1970) *Br. J. Anaesth.*, **42**, 801–806.
35. Lever, M. J., Miller, K. W., Paton, W. D. M. & Smith, E. B. (1971) *Nature*, **231**, 368–371.
36. International Critical Tables, National Research Council U.S. (1926) McGraw-Hill, New York.
37. Power, G. C. & Stegall, H. (1970) *J. Appl. Physiol.*, **29**, 145–149.
38. Miller, K. W., Hammond, L. & Porter, E. G. (1977) *Chem. Phys. Lipids*, **20**, 229–241.
39. Liu, N-I., & Kay, R. L. (1977) *Biochemistry*, **16**, 3484–3486.
40. Barker, J. L. & Gainer, H. (1973) *Science*, **182**, 720–722.
41. Nicoll, R. A. (1975) *Proc. Natl. Acad. Sci.*, **72**, 1460–1463.
42. Larrbee, M. G. & Posternak, J. M. (1952) *J. Neurophysiol.*, **15**, 91–114.
43. Richter, J., Landau, M. & Cohen, S. (1977) *Molecular Pharmacology*, **13**, 548–559.
44. Metcalfe, J. C., Hoult, J. R. S. & Colley, C. M. (1974) in *Molecular Mechanisms of General Anaesthesia*, (Halsey, M. J., Miller, R. A. & Sutton, J. A., eds.), pp. 145–163, Churchill Livingstone, Edinburgh.
45. Johnson, S. M., Miller, K. W. & Bangham, A. D. (1973) *Biochim. Biophys. Acta*, **307**, 42–57.
46. Trudell, J. R., Pagan, D. G., Chin, J. H. & Cohen, E. N. (1975) *Proc. Natl. Acad. Sci.*, **72**, 210–213.
47. Bennett, L. J. & Miller, K. W. (1974) *J. Med. Chem.*, **17**, 1124–1125.
48. Wilhelm, E., Baltino, R. & Wilcock, R. J. (1977) *Chem. Rev.*, **77**, 219–262.
49. Wilhelm, E. & Baltino, R. (1973) *Chem. Rev.*, **73**, 1–9.
50. Hansch, C., Wiltoria, A., Silipo, C. & Jow, P. Y. C. (1975) *J. Med. Chem.*, **18**, 546–548.
51. Martin, R. A. (1975) *Tables of Solubility Parameters*, Union Carbide Corp., Tarrytown.
52. Walkley, J. & Jenkins, W. I. (1968) *Trans. Faraday Soc.*, **64**, 19–22.
53. Linford, R. G. & Hildebrand, J. H. (1969) *Trans. Faraday Soc.*, **65**, 1470–1472.
54. Hirschfelder, J. O., Curtis, C. F. & Bird, R. B. (1954) in *Molecular Theory of Gases and Liquids*, Wiley, New York.