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The Pressure Reversal of General Anesthesia and the Critical Volume Hypothesis

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SUMMARY

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The anesthetic potencies (ED_{50}) of four gaseous anesthetics and five liquid anesthetics were first determined in newts, using the abolition of righting reflex measured by the rolling response at 20°. The following results were obtained: N_2O, 0.69 atm; N_2, 21.5 atm; SF_6, 1.82 atm; CF_4, 11.0 atm; $CHCl_3$, 0.89 mM; butanol, 16.7 mM; pentobarbitone sodium, 0.85 mM; halothane, 0.39 mM; ether, 25 mM. The ability of elevated pressures to antagonize the effect of these anesthetics was then studied. For the liquid anesthetics, a graded response to pressure was observed and the reversibility of the antagonistic effect was demonstrated. Dose-response curves were obtained for the interaction of pressure with the gaseous anesthetics, and, from these, ED_{50} values at various pressures have been interpolated. The data are used to compare the Meyer-Overton and the critical volume hypotheses; the latter not only is consistent with the data but also provides explanations for the antagonistic phenomenon and the lack of anesthetic effect for helium, neon, and hydrogen. The critical volume hypothesis is developed for three solvent model systems, from which estimates of the compressibility of the site of action are made.

INTRODUCTION

In recent years attempts to infer the mode of action of general anesthetics from correlations of their relative potencies with their physical properties have led to the conclusion that a nonpolar site of action is more probable than an aqueous site (1–3). Whether such a site may be identified as being in the nonpolar region of membrane lipids, or as a hydrophobic region in either a protein or within a lipoprotein complex, is an open question, but the lack of structural specificity among anesthetics, recently re-emphasized by studies on the *d* and *l* forms of halothane (4), and the repeated and remarkable success of the classical Meyer-Overton olive oil correlation draw attention to the first of these areas.

The lipid solubility theories fall into two classes. The first, and earliest, of these has been formulated in modern form: "Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell. This concentration depends on the nature of the animal or cell but is independent of the

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narcotic" (5). The second class looks to the modification of the dimensions of cell membranes by anesthetics for a mechanism of anesthesia. The concept has been advanced in various forms by a number of authors, among whom Mullins has given the most detailed discussion (6). It may be stated in the form 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. In this form we shall refer to it as the critical volume model.

These two hypotheses may be distinguished in principle by examining the correlation of relative potency with mole fraction or volume fraction solubility. In practice, while the two correlations lead to slightly different conclusions on the nature of the site of action, it has not proved possible to decide unequivocally between them, largely because the size range of anesthetic molecules is insufficient (1, 3). To date the best evidence for the critical volume model lies in the observation of the antagonism of general anesthesia by pressure (7), and it has been argued that this arises because the reduction in volume produced by an effective pressure balances the expansion which causes anesthesia. This conclusion is supported by calculations showing that anesthesia occurs when this expansion is of the order of 0.5%, while the pressures required to antagonize anesthesia are of a magnitude sufficient to oppose this degree of expansion (8). In the present paper the results of more detailed experiments on the pressure reversal of anesthesia are used to distinguish between the two versions of the lipid theory.

METHODS

Experiments were carried out on Italian crested newts (*Triturus cristatus carnifex*) about 4½ inches long. Unless otherwise stated, all experiments were carried out at 20° ± 1°.

Abolition of the righting reflexes was used as an end point for anesthesia. To measure the anesthetic effect of the nongaseous anesthetics, concentrated standard solutions were prepared. These were diluted to the required concentration with oxygenated distilled water before being quickly transferred to sealed glass jars, each containing a single newt. After sufficient time had elapsed for equilibration the animals were tested for anesthesia by flipping them on their backs. Those that righted themselves within 10 sec scored 1 point; others scored zero. Each animal was tested at one dose five times and could achieve a score between zero and five out of five. The effect of the gaseous agents was measured by placing single animals in a small, cylindrical, stainless-steel pressure vessel containing soda lime and 1 atm of oxygen, adding the required pressure of anesthetic gas, and testing for anesthesia by rotating the chamber in a manner that has been previously described (8). These two methods of examining the righting reflexes have been shown to yield comparable results (9).

The scores achieved by all animals at each dose of anesthetic were summed and recorded as a percentage. The relation of logarithm of dose to the response was then analyzed by conventional probit techniques to yield the ED_{50} and standard errors (3).

The ability of hydrostatic pressure to antagonize the anesthetics in aqueous solution was studied by completely filling the pressure vessel with the solution and raising the pressure in the absence of a gas phase. For the gaseous anesthetics the method was identical with that outlined above. Thus, after the anesthetic had been added, the pressure was raised with helium, which has previously been shown to be nonanesthetic (8). Pressures above cylinder pressure were achieved by means of an air-driven pressure booster pump. Responses were measured at each pressure, the pressure on each animal being raised in a stepwise manner. Compression was normally carried out over several minutes, and about 15 min were allowed before the response was measured. Unlike mice, these animals appeared insensitive to compression rate (8).

RESULTS

The measurements of the effect of the gaseous anesthetics alone yielded the results presented in Table 1, together with

TABLE 1

Effect of anaesthetics on righting reflexes of newts at 20°

Agent	Dose	No. of animals	Response	ED_{50} [i.e., P_{50} (atm)] ± SE
	atm		%	
N_2O	0.476	11	73	
	0.612	11	65	0.687 ± 0.098
	0.749	11	40	
N_2	20.41	10	50	
	23.82	15	43	21.5 ± 1.97
	27.22	10	26	
	34.02	10	6	
SF_6	1.36	11	68	
	1.77	13	52	
	2.11	12	38	1.82 ± 0.243
	2.79	7	29	
	3.40	8	15	
	4.08	3	20	
CF_4	8.58	9	76	
	9.53	9	58	
	11.15	11	44	11.0 ± 0.89
	12.51	9	49	
	14.55	9	22	
N_2O at 30°	0.681	8	58	
	0.817	11	44	0.747 ± 0.079
	0.953	11	24	
	1.089	8	18	

the calculated ED_{50} values and standard errors. For the liquid anesthetics the following ED_{50} values, expressed as concentration in the bathing fluid, were obtained: $CHCl_3$, 0.89 ± 0.1 mM (SE); butanol, 16.7 ± 2.0 mM; pentobarbitone sodium, 0.85 ± 0.1 mM; halothane, 0.39 ± 0.05 mM; diethyl ether, 25 ± 2.5 mM.

Because of the long equilibration times (1–2 hr) required with the liquid anesthetics, and the difficulties of maintaining the oxygen level of the solutions in the pressure chambers, only qualitative results were sought for the interaction of pressure with these agents. Pressure reversal of anesthesia was successfully demonstrated in the presence of butanol (28 mM), ether (32 mM), halothane (1.15 mM), and sodium pentobarbitone (0.9 mM). Because of the absence of a gas phase, animals could be resuscitated after exposure to pressure (140–200 atm) without suffering from decompression sickness, and all such animals survived. The reversible nature of the effect could also be demonstrated by raising and lowering the pressure successively, the anesthetized animal being awakened by application of pressure and reanesthetized by release of pressure. In one experiment with sodium pentobarbitone a heavy overdose (1.61 mM) was administered to five newts to induce anesthesia rapidly. The rolling responses at 1, 68, 136, and 204 atm were then measured in rapid succession and found to be 4, 36, 56, and 60%, respectively, thus demonstrating the graded nature of the response to pressure even though equilibration was incomplete.

With the gaseous agents N_2O, SF_6, CF_4, and N_2, however, it was possible to obtain quantitative results for the pressure reversal of anesthesia. The results when the applied pressure was increased with helium are given in Table 2. The response vs. pressure curves obtained (see Fig. 1, for example) were analyzed by probit methods to yield the pressure at which the response was restored to 50% in each case (Table 3). [These curves were linear when the probit of the response was plotted with respect to pressure, but deviated significantly from linearity for log (pressure).]

This analysis assumes that the slope of the anesthetic dose-response relationship is not altered by pressure. The technical problems of adding small pressures of anesthetic in the presence of large pressures of helium, however, prevented a test of this assumption, although the remarkable constancy of the slope of the dose-response curve for anesthetics effective at pressures ranging from 0.7 to 22 atm (3) should be noted. In a previous publication (8) it was shown that with helium and neon above about 140 atm response falls off as a result of paralysis alone. With other gas mixtures paralysis was sometimes not observed until higher pressures (see Fig. 1). When paralysis was observed the data at that and higher pressures were excluded from the analysis. It was also found that with hydrogen no

TABLE 2

Response of anesthetised newts at 20° to pressure

The total pressure is composed of the stated pressure of anesthetic gas and the balance of helium.

Anesthetic gas	Partial pressure	Response at total pressure P_T															
		Anesthetic alone	34 atm	41 atm	44 atm	54 atm	68 atm	81 atm	85 atm	95 atm	102 atm	109 atm	122 atm	136 atm	150 atm	170 atm	204 atm
	atm	%	%	%	%	%	%	%	%	%	%	%	%	%			
N_2O	1.09	10 (20)[a]	35 (12)		46 (10)		94 (10)				100 (8)			100 (8)			
	1.36	0 (10)	12 (10)				36 (10)		37 (6)		78 (10)			88 (10)			97 (6)
	1.63	0 (12)					12 (10)				52 (10)			80 (10)		88 (10)	92 (10)
	1.36[b]	0 (9)		33 (9)			60 (9)			84 (9)			82 (9)				
	1.63[b]	0 (9)					49 (9)			64 (9)			73 (9)		71 (9)		
SF_6	3.40	9 (11)	29 (11)				55 (11)				64 (11)			82 (9)			
	4.83	0 (11)					5 (8)				42 (10)			70 (10)		84 (10)	82 (10)
CF_4	23.4	0 (8)				17 (7)		30 (8)				58 (8)			83 (8)		
N_2	34.0	6 (10)					47 (9)							94 (10)			
	68.0	2 (10)												20 (10)			46 (10)

[a] Numbers in parentheses represent number of animals.

[b] Experiments at 30°.

TABLE 3

Relation of anesthetic dose in newts to pressure at which 50% response is obtained

Experiments were performed at 20°, except where noted.

Anesthetic gas	Pressure, P_{50}	Total pressure giving 50% response, P_T
	atm	*atm*
N_2O	0.68	0.68
	1.09	39.9
	1.36	85.1
	1.63	111.0
	0.75[a]	0.75
	1.36[a]	64.1
	1.63[a]	84.0
SF_6	1.82	1.82
	3.40	73.4
	4.83	124.9
CF_4	11.0	11.0
	23.4	103.7
N_2	21.5	21.5
	34.0	77.9
	68.0	208.4

[a] Experiments at 30°.

significant anesthesia was achieved at pressures up to 200 atm, and the onset of paralysis was delayed compared to helium and neon. Results for a group of nine animals were: 102 atm, 100%; 136 atm, 96%; 170 atm, 96%; 204 atm, 80% response.

Comparison of Meyer-Overton and Critical Volume Models

First we may test the two models using the potency data for newts, by comparing the correlations between relative potency and mole fraction or volume fraction solubility.

The critical volume model as formulated in the introduction to this paper assumes that anesthesia occurs at constant fractional volume expansion in some hydrophobic region. If we can treat this region as behaving as a bulk phase, we may write

$$E_a = \frac{\bar{V}_2 \cdot x_2 \cdot P_a}{V_m} \qquad (1)$$

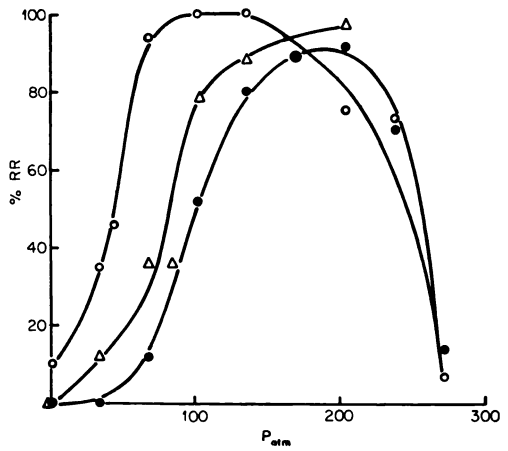

FIG. 1. *Rolling response (RR) of newts as a function of pressure after anesthetization with 1.09 atm (○), 1.36 atm (△), and 1.63 atm (●) of N_2O*

where E_a is the fractional expansion, $\bar{V}_2$ is the partial molar volume of the anesthetic in the hydrophobic region, and x_2 is its corresponding mole fraction solubility. V_m is the molar volume of the region and P_a is the partial pressure of anesthetic. When $P_a = P_{50}$, the partial pressure required to anesthetize 50% of a group of animals (ED_{50}), the fractional expansion for all anesthetics should be constant. Thus a plot of log P_{50} with respect to log $(\bar{V}_2 \cdot x_2)$ should yield a straight line of unit negative slope. The Meyer-Overton model, on the other hand, predicts a linear relation between log P_{50} and log x_2.

These relations are examined for the data given above and in Table 1. Olive oil is used as a model of the site of action, for, although it is not a pure liquid, its composition is reasonably well controlled and its traditional use has furnished solubility data for a wide range of anesthetics. The solubility values used are from a recent compilation (10). Values for $\bar{V}_2$ are not readily available for such a wide class of solutes, but the volume of the anesthetic at the boiling point provides a reasonable approximation (6). Figure 2 demonstrates that the data are broadly consistent with either model.

The data on pressure antagonism provide the opportunity for a more rigorous test, as follows. The phenomenon of pressure rever-

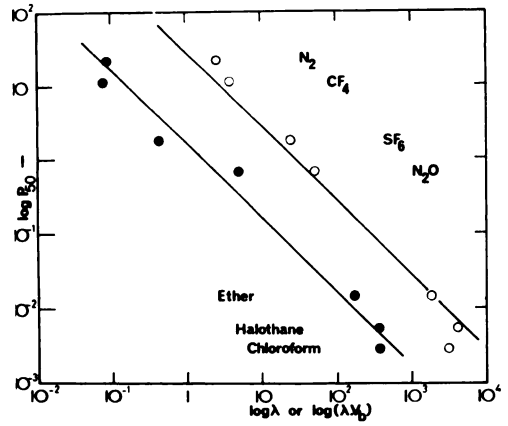

FIG. 2. *Correlation of relative potency of anesthetics for newts* (○) *with olive oil/H_2O partition coefficient* (λ) *at 25° (Meyer-Overton hypothesis) and with expansion volume* ($\lambda \cdot V_b$) *(Mullins hypothesis)* (●)

sal, on the Meyer-Overton model, would arise because anesthetics are believed to cause expansion at their site of action; a rise in pressure would therefore tend to force anesthetics out of solution at the site of action and change the partition between the active and other sites. The thermodynamic equation describing this effect is

$$\frac{\partial \ln (x/P_a)}{\partial P} = -\frac{\bar{V}_2}{RT} \qquad (2)$$

where x is the mole fraction solubility per atmosphere partial pressure, P_a is the partial pressure of the gas (or fugacity if gas imperfections are large), $\bar{V}_2$ is the partial molar volume of the gas, R is the gas constant, and T is the absolute temperature (11). If the ED_{50} partial pressure is P_{50} in the presence of 1 atm of oxygen and the solubility is x_{50} at this pressure, while the ED_{50} partial pressure is P_a at a total ambient pressure P_T when the solubility is x_a, the Meyer-Overton condition yields

$$P_{50} \cdot x_{50} = P_a \cdot x_a \qquad (3)$$

If we integrate Eq. 2 between P_T and P_{50} and eliminate x_{50}/x_a between the two equations, we obtain

$$\frac{1}{\bar{V}_2} \cdot \ln \left(\frac{P_a}{P_{50}}\right) = \frac{1}{2RT} [P_T - P_{50}] \qquad (4)$$

Thus a plot of the left-hand site of Eq. 4 against $[P_T - P_{50}]$ should reduce the data for the four anesthetic gases in Table 3 to a single straight line. (Values of $\bar{V}_2$ for these four gases at the site of action have been approximated using the values for benzene.) Figure 3 clearly demonstrates that the Meyer-Overton prediction is imprecise. Thus the dependence of anesthetic concentration on pressure cannot be explained on the basis of the pressure dependence of solubility. Furthermore, it offers no explanation for the lack of anesthetic effect for helium, neon, and hydrogen.

The critical volume model offers a more direct explanation in terms of expansion and compression. Continuing the previous notation, the fractional expansion E at a pressure P of the anesthetic agent is given by

Fractional expansion

$$E = \frac{\bar{V}_2 x_2 P}{V_m} - \beta P = \left\{\frac{\bar{V}_2 x_2}{V_m} - \beta\right\} P \qquad (5)$$

where β is the coefficient of isothermal compressibility. This is a more exact expression than Eq. 1, the first term representing expansion due to solution of gas and the second term the compression due to pressure on the fluid. For equal pharmacological

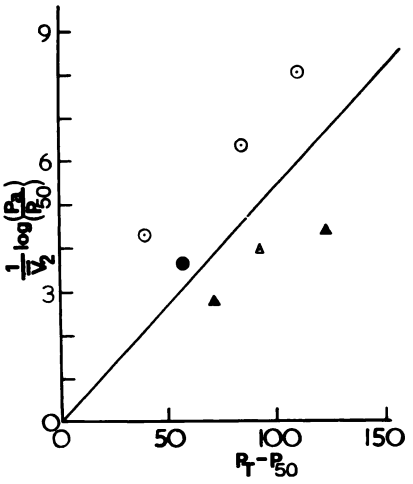

FIG. 3. *Test of Meyer-Overton model for pressure reversal*

⊙, N_2O; ●, N_2; △, CF_4; ▲, SF_6. See the text for experimental details.

effects the further expansion caused by the solution of more anesthetic when its pressure is raised from P_{50} to P_a may be equated to the compression caused by the antagonizing pressure over and above that of the anesthetic itself, i.e., $P_T - P_a$. The fractional compression of the system will be given by

$$\text{Fractional compression} = -\left\{\frac{\bar{V}_{He}\, x_{He}}{V_m} - \beta\right\}\{P_T - P_a\} \quad (6)$$

where $\bar{V}_{He}$ and x_{He} are the partial molar volume and the mole fraction solubility of helium in the hydrophobic region. Therefore, at any combination of P_a and P_T that results in a 50% effect, we have

$$\left\{\frac{\bar{V}_2 x_2}{V_m} - \beta\right\}\{P_a - P_{50}\} = -\left\{\frac{\bar{V}_{He}\, x_{He}}{V_m} - \beta\right\}\{P_T - P_a\} \quad (7)$$

for a given species and temperature. Dividing this by Eq. 5, we obtain

$$\frac{P_a}{P_{50}} = \left\{\frac{\beta}{E_{50}} - \frac{\bar{V}_{He}\, x_{He}}{E_{50}\, V_m}\right\} \cdot\{P_T - P_a\} + 1 \quad (8)$$

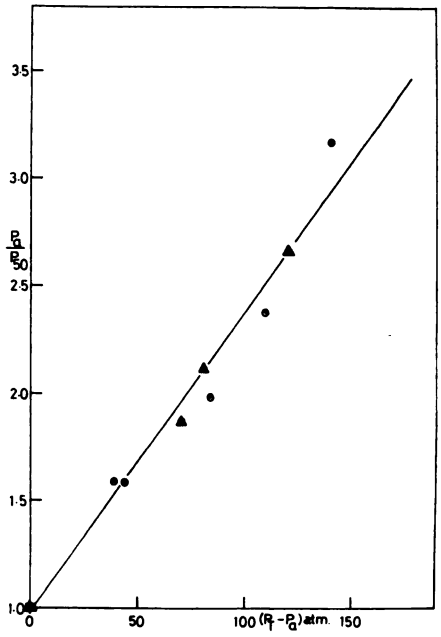

FIG. 4. *Test of critical volume model for pressure reversal*

⊙, N_2O; ●, N_2; △, CF_4; ▲, SF_6. For experimental details, see the text.

Figure 4 shows a plot of P_a/P_{50} against $P_T - P_a$ which demonstrates that the predictions of the critical volume model are consistent with the data. The slope contains the unknown physical parameters for the site of action V_m, β, $\bar{V}_2$, x_2, $\bar{V}_{He}$, and x_{He} in addition to the measurable quantity P_{50}. The data for N_2O at 30° also fit the correlation, suggesting that none of these unknown parameters is particularly sensitive to temperature.

Calculations Based on Model Systems

The above treatment neglects the effects of pressure on deviations from ideality of the gases (fugacity corrections) and, in the latter case, on the solubility of gases. While these effects are small they are by no means negligible, and the fit in Fig. 4 is better than might be expected from such an approximate treatment. A more complete treatment is desirable. To do this, however, some model system must be assumed, since the physical parameters for the actual site of action are not known. In what follows, the site is assumed to have the properties of olive oil, benzene, or carbon disulphide, and the effectiveness of the Meyer-Overton and critical volume approaches are compared in the light of the fuller analysis.

Fugacity correction. The behavior of all real, nonideal gases is given by

$$PV = RT + BP + CP^2 + \cdots$$

where P is pressure, V is volume, T is absolute temperature, R is the gas constant, and B and C are the second and third virial coefficients. Thus for an ideal gas $B = C = 0$. For our purposes CP^2 and higher terms may be neglected, and experimentally determined values of B are obtained from a recent compilation (12) or, where not available, from the application of the principle of corresponding states (13). On the basis of this principle B/V_c is considered as a universal function of T/T_c, where V_c and

T_c are the critical volume and critical temperature (14). The ratio of the fugacity, P^*, to the experimental pressure, P, is given by

$$RT \ln\left(\frac{P^*}{P}\right) = \int_0^P B\, dP \qquad (9)$$

In the case of a gas mixture the fugacity correction for component 1 at mole fraction x_1 is

$$RT \ln \frac{P^*}{P_1} = \int_0^{P_1} [x_1 B_{11} + x_2 B_{12}]\, dP \qquad (10)$$

where B_{11} is the second virial coefficient for pure component 1 and B_{12} is a term that arises from the interaction of the two components of the mixture.

Values for B_{12} are often not available in the literature but may be estimated from the virial coefficients of the pure gases that comprise the mixture (13, 15).

The values of B_{11}, B_{22}, and B_{12} used in making the corrections are summarized in Table 4. Comparison with experimental data, where available, indicates the errors involved in estimating B_{12}. The magnitude of the fugacity corrections for mixtures below 150 atm are small and do not exceed 10%.

Variation of solubility with pressure. The dependence of solubility on pressure has been given in Eq. 2. For exact calculations P_a must be replaced by the appropriate fugacity. $\bar{V}_2$, values for which are given in Table 5, is virtually independent of pressure in the pressure range used in this work (11).

TABLE 4

Second virial coefficients for fugacity corrections

Mixture components		Pure component second virial coefficient (12)		Estimated B_{12}	Experimental B_{12}	Reference
1	2	B_{11}	B_{22}			
He	N_2O	11.7	−133	29		
He	N_2	11.7	−4.5	27	21	16
He	CF_4	11.7	−88	34	26	15
He	SF_6	11.7	−280	38		

TABLE 5

Molar and partial molar volumes

Gas	Molar volume at boiling point (17)	$\overline{V}_2$ in benzene	Reference
	ml	*ml*	
He	32	36[a]	18
Ne	17	33[a]	19
N_2	35	53	20
N_2O	36	47	20
CF_4	54	82	21
SF_6	76	97	21

[a] Estimated.

Application of correction factors. The expansion caused when a gas dissolves in a solvent is ideally proportional to $(\bar{V}_2 \cdot x_2 \cdot P_2)$ (Eq. 1). The deviations from this can be calculated as a correction term by using Eq. 2 with fugacity instead of partial pressure. There are two contributions to expansion in our case, one due to the narcotic gas, the other due to helium. The corrected expansions have been calculated for each gas independently of the other. The correction factor is such as always to reduce the expansion (or concentration, x_2P_2) below the ideal value. The size of the correction term increases with increasing size of the gas and with increasing pressure. At 100 atm it ranges from about 1.2 for nitrogen to 1.6 for sulfur hexafluoride. Using these corrections, the total solubility and expansion can be calculated for our data. The calculations have been carried out using three liquids as models of the site of action: olive oil, benzene, and carbon disulfide. We have recently shown that benzene and carbon disulfide are the best simple solvent models for the site of action of anesthetics in mice (3). Sources of solubility data were given in that paper. Partial molar volumes were taken to be those for benzene in all three solvents. Our conclusions are in fact independent of which of these solvents is considered. Much more data would be required to differentiate unequivocally between them, and when one considers the relatively crude nature of such a model for the active site this is not likely to be a rewarding pursuit.

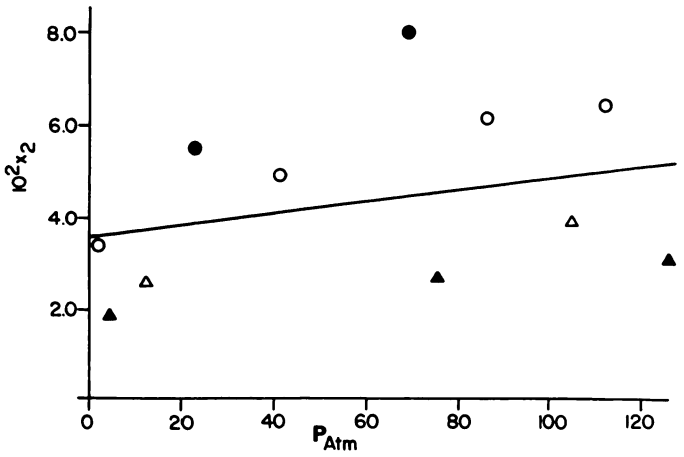

FIG. 5. *Mole fraction concentration (x_2) in olive oil of narcotic gas at ED_{50} values at various pressures*
Correlation coefficient $r = 0.32$. ○, N_2O; ●, N_2; △, CF_4; ▲, SF_6.

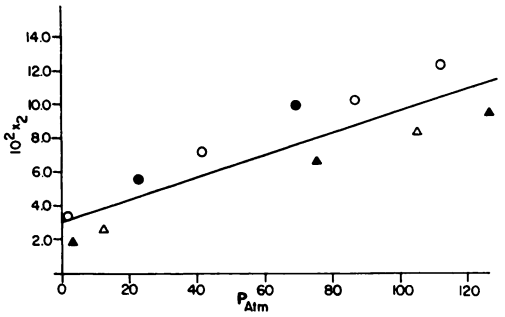

FIG. 6. *Mole fraction concentration (x_2) in olive oil of narcotic gas plus helium at ED_{50} values at various pressures*
Correlation coefficient $r = 0.88$. ○, N_2O; ●, N_2; △, CF_4; ▲, SF_6.

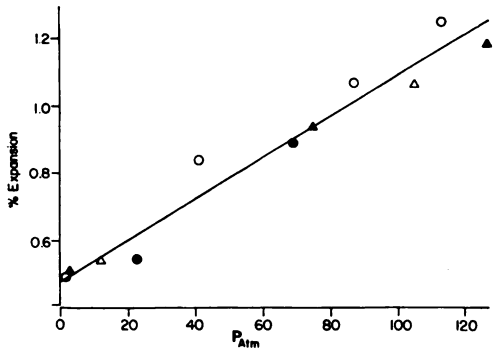

FIG. 7. *Percentage expansion of benzene caused by helium and narcotic gas at ED_{50} values at various pressures*
Correlation coefficient $r = 0.98$. ○, N_2O; ●, N_2; △, CF_4; ▲, SF_6.

Results of analysis. First, on the classical Meyer-Overton hypothesis, the concentration of narcotic gases in the model solvent (for ED_{50} values measured at various pressures) should be constant and independent of pressure. Figure 5 shows that this assumption produces a poor correlation, although the concentrations are not grossly pressure-dependent. If we assume that the helium is also contributing a subliminal anesthetic dose, the total inert gas concentration should be considered (Fig. 6). The data are now more strongly correlated, but the anesthetic concentration at the ED_{50} has become pressure-dependent, and the model is incapable of offering an explanation for this without further ad hoc assumptions.

The critical volume model was tested by correlating the expansion due to the dissolution of gas with pressure. This should lead to a linear relation between expansion and pressure, where the slope of the line yields the compressibility of the site of action. The expansion caused by helium is included. Figure 7 illustrates the situation when benzene is chosen as the model solvent. This model is highly successful and yields an estimate of the compressibility of 6×10^{-5} atm^{-1}. (The experimental value for benzene is 9×10^{-5} at 25°.) Furthermore,

the model is consistent with helium and neon not being anesthetics. The expansion they cause when dissolving is smaller than the compression due to the effect of the pressure per se, so that the net effect is compression and the critical volume for anesthesia is never attained. With hydrogen, on the other hand, the two effects nearly balance and there is a very small net expansion. In newts this is insufficient for the critical volume to be achieved below pressures where other effects become important (approximately 140 atm). In mice, on the other hand, it appears that the critical volume may be achieved at 130 atm (22). If one accepts this figure, then either the hydrogen solubility is sufficiently enhanced at the site of action on going from 20° in newts to 37° in mice or the compressibility of the site in mice is less than that in newts. Calculations for mice, cited in Table 6 suggest that the former is the case.

The conclusions for the critical volume model are not strongly dependent on the choice of solvent. All three solvents give very good correlations, though the predicted degree of expansion at 1 atm (intercept) and the compressibility (slope) of the site of action vary slightly (Table 6). Such small variations between the models of the anesthetic site of action do not, however, seem significant. Indeed, what is striking is how successful the critical volume model is at providing a self-consistent explanation of the pressure dependence of the anesthetic potencies of inert gases.

TABLE 6

Comparison of model solvents

Solvent	Expansion at ED_{50} dose at 1 atm	β values	Experimental β values for pure solvent (23, 24)	Correlation coefficient, r
	%	10^{-5} atm^{-1}		
Newts				
Olive oil	0.20	3	6	0.96
Benzene	0.48	6	9	0.98
Carbon disulfide	0.29	4	7	0.94
Mice (25)				
Carbon disulfide	0.58	3	7	0.99

Results of similar calculations using carbon disulfide as model solvent for the data recently obtained (25) for mice in nitrous oxide–helium mixtures are also shown in Table 6. [Using the degree of deviation of fluorinated anesthetics from relative potency data as the criterion for the correlation, the best fit was found to be with carbon disulfide for mice (3).] The higher expansion for an ED_{50} dose at 1 atm reflects the higher value for the ED_{50} (1.5 atm for mice, 0.7 atm for newts).

DISCUSSION

The success of a nonpolar fluid, such as benzene, in serving as an analogue of the site of action of anesthetics suggests that this site too must be both nonpolar and fluid. There are three possible sites that may fulfill these criteria: (a) nonpolar sites within proteins, (b) the cell lipids, or (c) associations of lipid and protein.

Nonpolar (or hydrophobic) regions within proteins are common, but the need for "fluidity" suggests that only sites associated with a flexible or labile structure would be compatible with the critical volume model. Thus, whereas xenon and cyclopropane can occupy nonpolar sites in myoglobin, the rigidity of the protein prevents other anesthetics from interacting at this site (26). Although the inhibition of luminescence in luminous bacteria by anesthetics may be antagonized by pressure (27, 28), it is not yet clear whether this arises from direct interaction with the luciferase or not. There is thus at present no unequivocal evidence for the direct action of anesthetics at clinical doses on protein structure in a manner consistent with the relative potency data and the critical volume model. Although such interactions cannot be entirely ruled out, a relatively good case may, on the other hand, be made for interaction with membrane lipids.

Recent spectroscopic studies (29, 30) have revealed that the ends of the hydrocarbon chains remote from the polar head groups of lipids in bilayer membranes are in a highly fluid state, and it would not be too surprising if three-dimensional nonpolar fluids provide a good analogue of the be-

havior of such a region toward small, relatively nonpolar solutes such as general anesthetics. Such spectroscopic studies also show that the action of anesthetics, for example benzyl and other alcohols, is to increase the membrane "fluidity" (19, 31). Although these substances are hardly typical general anesthetics, equivalent effects have been demonstrated for clinical and inert gas anesthetics in the membranes of phospholipid vesicles (liposomes) by studying ion permeability (32). Again, monolayers of lipoprotein (33) and of phospholipid (34) are expanded by anesthetic but not by helium. The effects of anesthetics on liposome ion permeability are reversed by pressures comparable to those required in animals, and it has been suggested that the expansion, and consequent increase in freedom of molecular motion, within a lipid bilayer is involved in both processes (35). Recently reported work in which the effects of general anesthetics and of pressure on membrane "fluidity" were studied is consistent with this prediction (36).

Whether in a bilayer membrane the expansion would be isotropic (as in benzene) or anisotropic is not certain. Available evidence suggests the latter. Thus studies in which actual membrane concentrations of alcohols were determined revealed the anomalous result that only about one-third of the observed increase in area could be attributed to the dissolution of the drug molecules (37). We have calculated that this unaccountable increase in membrane area would result if the membrane decreased its thickness by about 10 nm while increasing its area at constant volume. [General anesthesia occurs at lower doses than these, and the effect would be smaller (38).] Such an effect seems quite plausible, for, as the polar head group area of the lipids increases, so does the "fluidity" of the hydrocarbon tails, which now move through a wider arc exhibiting even less anisotropy, as is observed spectroscopically (19, 31). At sufficiently high concentrations this effect would lead to lysis. A similar effect of decreasing thickness and increasing head group area has been discussed by Fettiplace, Andrews, and Haydon (39) with respect to nonpolar interactions between bilayers and proteins. With long-chain hydrocarbons, on the other hand, thickening at constant head group area may be occurring (39).

The mechanism by which this increase in "fluidity" and/or volume in the lipid leads to anesthesia is not clear. Is the action on the lipids themselves important, or is the disturbance in the lipid transmitted to some membrane protein? The small effects that anesthetic doses have on pure phospholipid membranes (e.g., K^+ permeability in liposomes increased by about 20% at clinical doses) and the variety of effects that anesthetics may produce in cells (40) seem to favor the latter hypothesis. Such a second-order involvement of lipids has been discussed in a recent review (41) and speculations on the role of anesthetics in a hypothetical lipid-protein interacting system for nerve propagation have been published (42). A suggestive experimental study employing spin-labeled fatty acids in sarcoplasmic vesicles demonstrated that the activity of calcium-dependent ATPase was directly related to membrane "fluidity" and that the enzymatic activity of a lipid-deficient membrane could be restored by the addition of oleic acid (43). If such effects can be shown to be more general, then the dependence of membrane protein function on membrane fluidity could provide a mechanism of action for anesthetics. It should be noted, however, that the latter experiments involved much greater changes in fluidity than would be produced by anesthetic doses alone.

If the interpretation of the pressure reversal of anesthesia in terms of expansion and compression of a membrane (and consequent changes in fluidity) is correct, several interesting corollaries may be noted. By addition of anesthetics and pressure it is possible to vary membrane fluidity and volume at constant temperature, and in this way anesthetics may be used as simple probes of the behavior of membranes. A knowledge of the dependence of the effective dose on pressure may provide an insight into the mechanism by which drugs act. The convulsions and hyperexcitability observed in primates at 60–80 atm in O_2-He breathing mixtures occur when membrane compression is of the order of 0.25%, and

it is interesting to note that the presence of anesthetic gases elevates the pressure threshold for convulsions (44).

CONCLUSION

The critical volume hypothesis provides a much more self-consistent explanation of the relative potency of anesthetics and of the pressure reversal of anesthesia than does the classical Meyer-Overton lipid solubility hypothesis. The degree of self-consistency and the prediction of a realistic compressibility coefficient are most satisfactory. No other current hypothesis of the mode of action of anesthetics [e.g., the microtubule model (45)] is as successful. The critical volume model makes predictions that are accessible to direct experimental test; for example, the dimensions and "fluidity" of cell membranes under anesthetic and under pressure may be determined. The critical volume model, unlike the lipid solubility hypothesis, provides an indication of the mechanism of action of anesthetics.

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