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Membrane Expansion and Inhalation Anesthetics

Mean Excess Volume Hypothesis

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SUMMARY

High-precision solution densimetry was used to determine volume parameters for the interaction of inhalation anesthetics with water, nonpolar solvent, and phospholipid vesicles. The precision of the densimeter is mainly limited by the constancy of the temperature during measurement. Therefore, temperature stability was maintained within ±0.0005° and monitored by a microprocessor-controlled Thermistor thermometer with 0.0001° resolution. All values were obtained at 25°. Because volatile anesthetics in liquid form usually contain water, they were purified by passage through activated aluminum oxide columns. The molal volumes of dried preparations at the pure liquid states were: halothane, 106.3_3; isoflurane, 123.6_6; and enflurane, 121.9_9 $cm^3 \cdot mole^{-1}$ at 298.150° K. The mean molal excess volumes of anesthetic-water mixtures were negative at dilute anesthetic concentrations in water and positive at dilute water concentrations in liquid anesthetics. These values were dependent on the mole fractions of each component and showed a minimum in the water-rich region and a maximum in the anesthetic-rich region. In water, the partial molal volumes were halothane 93.7, isoflurane 103.4, and enflurane 98.6 $cm^3 \cdot mole^{-1}$ at infinite dilution, and increased as the anesthetic concentration was increased. The partial molal volumes of water in liquid anesthetics were in halothane 21.7, isoflurane 21.0, and enflurane 20.5 $cm^3 \cdot mole^{-1}$ at infinite dilution, and decreased as the anesthetic concentration was decreased. The mean excess volumes of the anesthetic-decane mixture were positive in the entire mixing range. The partial molal volumes of anesthetics in *n*-decane at infinite dilution were halothane 114.9, isoflurane 135.3, and enflurane 135.2 $cm^3 \cdot mole^{-1}$. The mean specific excess volumes of the mixture of anesthetics and dimyristoylphosphatidylcholine vesicle suspension showed positive values. The partial molal volume was not evaluated because of the theoretical difficulty in estimating it in a dispersed two-phase system. Because the mean excess volume of anesthetics dissolved in water is always negative and that incorporated into phospholipid suspension is positive, anesthetics expand the total volume of the model membrane system when translocated from water to the membrane. Anesthesia occurs when the mean excess volume of the total system exceeds a limiting value, and the bulk membrane size is irrelevant. Although the present result in no way disclaims alternative hypotheses, it demonstrates that the pressure reversal of anesthesia can be explained without assuming any specific receptors for these anesthetics.

INTRODUCTION

In recent disputes about the relevance of "membrane expansion" to the mechanism of anesthesia, two contradictory rationales were used to analyze the experimental data (1, 2).

Bull *et al.* (1) used a glass micropipette and a microscope to measure directly the size of a single erythrocyte membrane, and reported that nonanesthetic long-chain 1-alkanols (i.e., tridecanol, tetradecanol, and hexadecanol) as well as inhalation anesthetics and short-chain 1-alkanols (which have anesthetic potency) expanded the erythrocyte membrane area. The magnitude of the membrane expansion was about equal to the size of these molecules incorporated into the membrane. On the basis

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that *nonanesthetic* molecules expanded the membrane, these authors rejected the idea that anesthesia occurs by the membrane expansion proposed by the critical-volume hypothesis.

Franks and Lieb (2) reached the identical conclusion because *anesthetics* expanded the membrane volumes by the size of the incorporated molecules. They used solution densimetry and reported that the "partial molar volumes" of ethanol and halothane did not change when transferred from water to the erythrocyte or model membranes. This rather strange agreement in conclusion is caused by ambiguity of the concept of membrane expansion. According to Franks and Lieb (2), the membrane is not expanded when the membrane size expands by the size of the incorporated ligand molecules. Bull *et al.* (1), on the other hand, consider that membrane is expanded under the identical situation.

However, the data reported by Franks and Lieb (2)—that the partial molal volume of anesthetics in water is about equal to the molal volumes in their liquid state—are peculiar in light of the well-established fact [see, for instance, Friedman and Scheraga (3)] that the volume of hydrophobic molecules, such as inhalation anesthetics, decreases when dissolved in water. Indeed, Kita *et al.* (4) reported that the partial molal volume of inhalation anesthetics in water is smaller than the molal volume in their liquid state and increased when incorporated into the membrane. Our study was undertaken to reevaluate the volume data by using high-precision solution densimetry in well-defined systems. Because the thermal expansibility of the solution is the main source of error in density measurement, the temperature stability was controlled within 0.0005° K.

The partial molal volume of anesthetics in the membrane phase dispersed in the water phase (three-component two-phase system) is usually an unobtainable quantity by solution densimetry, unless the two phases separate from each other so that each phase can be sampled separately for density measurement. This is because the partial molal volume of anesthetics in the membrane phase is defined as the change in *membrane* volume according to the increase in anesthetic concentration (temperature, pressure, and the concentrations of other components are constant), and not the *total* volume.

In estimating the membrane volume, Franks and Lieb (2) implicitly assumed that phospholipids form membranes without interacting with water molecules (hard-sphere mixing), which is obviously incorrect, because the presence of water is absolutely necessary to membrane formation. Without water, membranes cannot form. Kita and Miller (5) adopted their equation.

The dry weight of membranes is not the mass of the membrane phase in water. The membrane phase consists of hydrated membrane molecules, and the chemical potential of the bound water, therefore the degree of hydration, will change when anesthetics are combined (see Appendix 1). Since partial molal volume is a rigorously defined intensive property, the reported values are anything but the partial molal volume of anesthetics and are contaminated from multiple sources. The multiplicity of factors that affect the system volume is shown in the Appendix 2, and we propose that analysis of each factor contributing to the system volume may be the key to elucidation of the mechanisms of anesthesia. Hence, we refrained from making approximations to derive the "partial molal volume" of anesthetics in the membrane phase. Instead, the mean molal excess volume, which is a clearly defined intensive property (see Results and Discussion), is presented. Partial molal volumes are estimated only in the two-component, one-phase system, where approximations are not needed.

THEORY: MEAN EXCESS-VOLUME HYPOTHESIS

The concept of mean excess volume increase by anesthetics is based on the thermodynamic interpretation on the pressure reversal of anesthesia. Pressure reversal of anesthesia was first demonstrated by Eyring and Magee (6) and Johnson and co-workers (7, 8) in luminous bacteria. The light intensity decreased when the bacteria were doped with anesthetics, and the high pressure of 100–150 atmos reversed the inhibition in the presence of anesthetics. The antagonism was confirmed in tadpoles (9, 10), newts (11, 12), and mice (11).

In thermodynamic terms, the change in an equilibrium constant, K, such as the depth of anesthesia, by pressure, P, expresses the volume change, ΔV, according to the following equation:

$$(\partial \ln K/\partial P)_T = -\Delta V/RT \qquad (1)$$

where R is the gas constant and T is the absolute temperature.

The fact that all general anesthesia induced by inhalation agents is antagonized by the same magnitude of pressure between 100 and 150 atmos indicates that the excess volume expansion (ΔV) is about equal at the same depth of anesthesia, according to Eq. 1. This ΔV is not the volume of anesthetics incorporated into the membrane. It is an excess volume newly created by the interaction between anesthetic molecules and membrane molecules or macromolecules.

Under the principle that propositions in a contraposition pair are equivalent statements, the proposition (contraponend), "all general anesthesia induced by inhalation agents is antagonized by pressure" is equivalent to the contrapositive "not antagonized by pressure is not anesthesia." Restated, anesthesia does not occur without excess volume expansion. The mean excess-volume hypothesis may be stated as follows: anesthesia occurs when the excess volume increase of the total system exceeds a limiting value. The mean excess volume is defined here as the difference in anesthetic volume when transferred from the aqueous phase to the action site by the reasons discussed later under Phospholipid Vesicle Suspension.

The equation does not specify the location of the volume increase: the excess volume may be created anywhere in the total system, not necessarily limited to the membrane proper.

The mean excess-volume hypothesis is in contrast to the critical-volume hypothesis proposed by Miller *et al.* (12), who stated that anesthesia ensues when the volume fraction of anesthetic molecules in the membrane reaches a critical value: the dimension of the membrane deter-

mines the anesthetic depth. The critical-volume hypothesis, however, was not thermodynamically derived. The rigorous derivation according to thermodynamics is presented in Appendix 2. The derivation reveals assumptions implicitly contained in the theory that remain to be justified.

MATERIALS AND METHODS

Enflurane (2-chloro-1,1,2-trifluoroethyldifluoromethyl ether) and isoflurane (1-chloro-2,2,2-trifluoroethyldifluoromethyl ether) were obtained from Ohio Medical Products (Madison, Wisc.), and halothane (2-bromo-2-chloro-1,1,1-trifluoroethane, which contained thymol as a stabilizing agent) was obtained from Ayerst Laboratories (New York, N. Y.). Because these anesthetics and *n*-decane (MCB Reagents, Cincinnati, Ohio) are usually contaminated by water, they were purified by an activated aluminum oxide (Fluka) column several times. The stabilizer contained in halothane was also removed by this procedure. The absence of water from these preparations was confirmed by infrared spectrophotometry (Perkin-Elmer).

Water was first distilled and then purified by a Milli-Q reagent-grade water system (Millipore), and the specific resistivity was maintained above 16 Mohm·cm^{-1}.

Synthetic dimyristoylphosphatidylcholine (1,2-ditetradecanoyl-*sn*-glycero-3-phosphorylcholine) was obtained from Sigma Chemical Company (St. Louis, Mo.). The purity was checked by thin-layer chromatography using chloroform/methanol/water (65:25:4, v/v/v), and a single spot was found. The compound was used without further purification. The phospholipid vesicle suspension was prepared by ultrasonic irradiation above the transition temperature, as previously described (13). The phospholipid concentration was 1 mM.

Water-anesthetic mixtures were prepared in 60-ml glass ampules. Water was pipetted into the ampule, and the mass of the water was measured by weighing the ampule with an analytical semimicrobalance (Mettler) to $5 \cdot 10^{-6}$ g. Dried liquid anesthetics were added to the water, and the added amount was confirmed by weighing. The total volume of the mixture was adjusted to fill the ampule to its neck in order to minimize the escape of anesthetics into the gas phase. The opening of the ampule was flame-closed immediately. The ampules were vigorously shaken and incubated at 298.15° K in a shaking water bath for 3 days. The solvation was a slow process. Liposome-anesthetic mixtures and decane-anesthetic mixtures were prepared similarly, but the incubation time was reduced to 1 day. The concentrations were expressed either by mole fraction or molality because molarity is a temperature-dependent quantity.

Densities of these sample solutions were measured by an oscillation densimeter DMA60/601HT (Mettler-Paar, Hightstown, N. J.). The temperature of the vibration tube was maintained at 298.150 ± 0.0005° K by circulating water from a Hart 5001 constant-temperature water bath (Hart Scientific, Provo, Utah). The temperature of the water bath was monitored by a Hewlett-Packard quartz thermometer 2804 A with 0.0001° K resolution. The temperature of the vibration chamber was monitored by a microprocessor-controlled thermistor thermometer (Micro-Therm 1006, Hart Scientific, Provo, Utah) with 0.0001° K resolution. The densimeter was calibrated daily by water and dry filtered air. All values were obtained at 298.150° K.

The anesthetic mixture was degassed in the ampule by ultrasonic irradiation and warmed to a temperature several degrees above the measuring temperature before introducing it into the vibration tube. This procedure prevented the formation of microbubbles in the test solution during the density measurement. The amount of anesthetic escaping during transfer from the ampule to the densimeter was not determined. But the escaped amount may be negligible because extrapolation of density data to zero anesthetic concentration transected the density coordinate at the identical value of the pure solvent. The density reading was stable for the first 20 min, indicating that escape of the volatile anesthetics from the vibration tube was negligible for this period under the present experimental conditions.

Data reduction, polynomial curve fitting, and calculation of first derivatives were performed with a PDP 11/23 minicomputer.

RESULTS AND DISCUSSION

Liquid anesthetics. The molal volumes of dried anesthetics at 298.150° K were enflurane 121.9_9, isoflurane 123.6_6, and halothane 106.3_3 cm^3·mole^{-1}.

Water solution. Densities of the anesthetic-water mixture are shown in Fig. 1. Anesthetics dissolved in water and water dissolved in anesthetics were studied to the

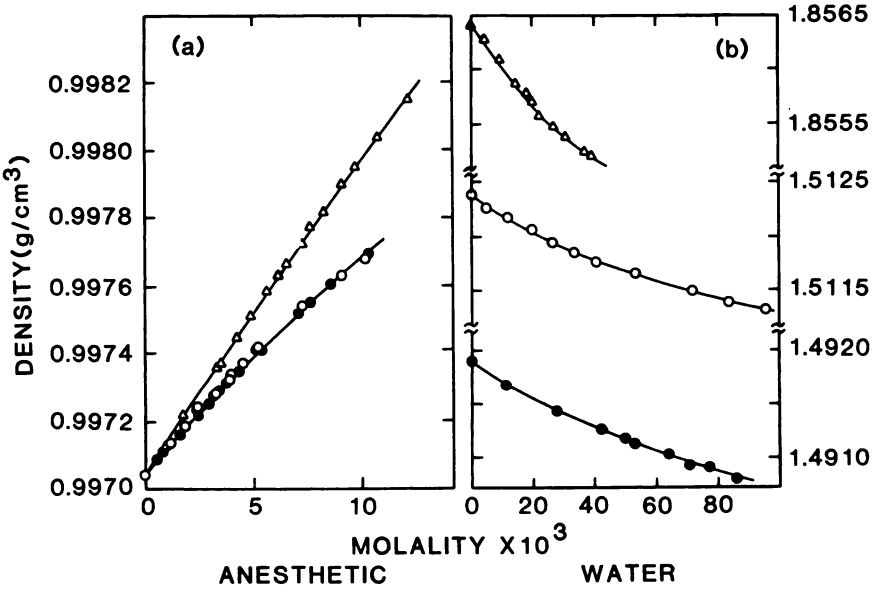

FIG. 1. *Densities of the anesthetic-water mixtures*

Data points represent averages of five observations. Error bars are omitted because the standard errors are within the size of the symbols. a. Dilute concentrations of anesthetics in water. b. Dilute concentrations of water in liquid anesthetics. Enflurane (○), isoflurane (●), and halothane (△).

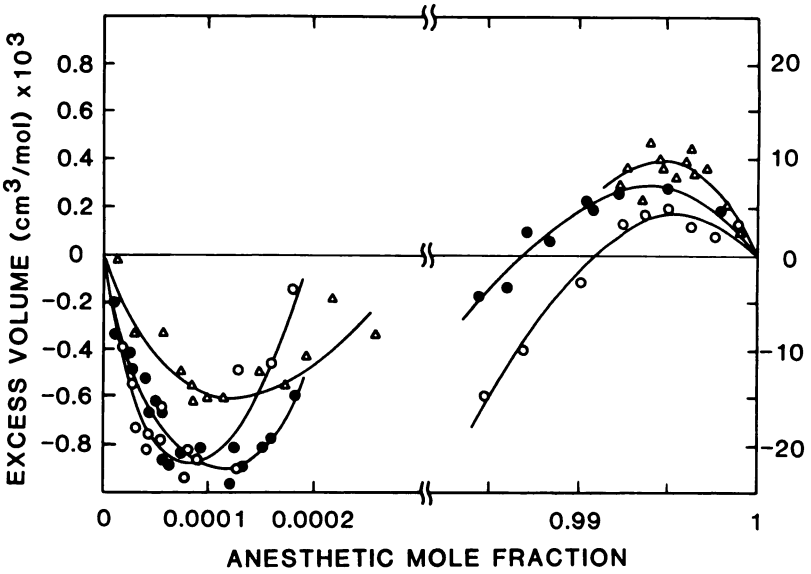

FIG. 2. *Mean molal excess volumes of anesthetic-water mixtures as a function of the mole fraction of anesthetics, estimated from the data shown in Fig. 1*

Enflurane (○), isoflurane (●), and halothane (△).

saturation concentrations. The lines were drawn by the nonlinear polynomial least-squares method.

Properties of the water-anesthetic interaction can be expressed by several volume functions, i.e., partial molal volume of the components, $\bar{v}_i$, and mean molal excess volume, v^E. The mean molal excess volume is defined as

$$v^E = x_A(\bar{v}_A - v_A{}^0) + x_W(\bar{v}_W - v_W{}^0) = v - (x_A v_A{}^0 + x_W v_W{}^0) \quad (2)$$

where x is the mole fraction, $\bar{v}$, is the partial molal volume, and v^0 is the molal volume of the pure liquid form. Subscripts A and W signify anesthetic and water, respectively, and v is the mean molal volume of the system.

The mean molal excess volumes can be obtained from the difference between the reciprocals of the densities of pure liquid and the mixture, and are shown in Fig. 2. The obtained values were not a simple function of the composition of the mixture. When anesthetics were added to bulk water up to saturation, v^E showed negative values with concave curves. When water was added to bulk liquid anesthetics, v^E showed positive values with convex curves.

The mean molal excess volume is a direct expression of the change in total volume of the system. A negative v^E means contraction of system volume and a positive v^E signifies its expansion. The pure liquid state of each component is taken as the standard state, and the values are expressed as a difference from the standard state.

The net negative volume change at dilute anesthetic concentrations was attributed by Kita *et al.* (4) to the possible disruption of the hydrogen-bonded network of water. However, this model does not explain the positive v^E values presently demonstrated in the anesthetic-rich region or the existence of the minimal v^E values in the water-rich region. We consider that the volume change may be caused by contraction of the free volume of the dissolved anesthetic molecules. At dilute concentrations, these anesthetic molecules are surrounded by water molecules, which have stronger intermolecular attractive forces than anesthetics. The distances between the anesthetic molecule and the next neighbor molecules are shorter in aqueous solution than in liquid anesthetic solution; hence, the free volume of an anesthetic molecule is smaller when surrounded by water molecules than when surrounded by anesthetic molecules. A small increase in the number of anesthetic molecules would increase the number of anesthetic molecules surrounded by water molecules, and the numerical values of the negative excess volume would increase. Further increase in the anesthetic concentration would result in the interaction between anesthetic molecules, and the tendency of the increase in the negative excess molal volume would be weakened, resulting in the minimal point in the excess molal volume curve. Similar biphasic response between water and hydrophobic molecules with inflection point has been reported (14). In the anesthetic-rich region in Fig. 2, the volume of water molecules tends to expand rather than contract when surrounded by anesthetic molecules. In this context, system volume represents the strength of the intermolecular forces of the components, and the volume of one molecule has little meaning.

By definition, the partial molal volume of a component in a single-phase system is written

$$\bar{v}_i = (\partial V/\partial n_i)_{T,P,n_j} \quad i \neq j = \phi_i + m_i(\partial\phi_i/\partial m_i)_{T,P} \quad (3)$$

where V is the volume of the system, n is the number of moles of the component, T is the absolute temperature, P is the pressure, m is the molality, and ϕ is the apparent molal volume and is defined as

$$\phi_i = (V - n_1 v_1{}^0)/n_i \quad (4)$$

where n_1 is the number of the solvent molecules, and $v_1{}^0$ is the molal volume of the solvent in the pure liquid state. The apparent molal volume can be estimated from

the solution density data by the equation

$$\phi_i = (1000 + m_i M_i)/m_i d - 1000/m_i d_1^0 \qquad (5)$$

where d is the density of the mixture, d_1^0 is the density of the solvent in the pure liquid state, and M is the molecular weight. The estimated $\bar{v}_A$ and $\bar{v}_w$ are shown in Fig. 3. The partial molal volumes of anesthetics at infinite dilution in water were halothane 93.7, enflurane 98.6, and isoflurane 103.4 $cm^3 \cdot mole^{-1}$. The partial molal volumes of water at infinite dilution in the liquid anesthetics were halothane 21.7, enflurane 20.5, and isoflurane 21.0 $cm^3 \cdot mole^{-1}$ (Table 1).

At infinite dilution, the partial molal volumes of anesthetics in water are smaller than their molal volumes in the pure liquid state, as shown in Table 1. Decreases in volume of 12–19% were observed. These volume contractions are in accordance with the fact that the partial molal volume of hydrophobic solutes at infinite dilution is usually smaller in water than in less polar liquids.

The present partial molal volume of halothane compares favorably with the 89.7 $cm^3 \cdot mole^{-1}$ reported by Kita *et al.* (4) and is in conflict with the 116.7 $cm^3 \cdot mole^{-1}$ reported by Franks and Lieb (2). Our study clearly demonstrates contraction of the volume of anesthetics when dissolved in water and contradicts the conclusion by Franks and Lieb (2) that the volume of halothane in water is equal to the volume in its liquid state. The present result disclaims the possible presence of anesthetic receptors advocated by Franks and Lieb (2), because their claim is based solely on their erroneous experimental data, which showed invariance of anesthetic volume in different phases.

TABLE 1

Volume parameters of anesthetics and water at 298.150° K ($cm^3 \cdot mole^{-1}$)

v^0, Molal volume of pure liquid anesthetics; $\bar{v}_A^0$, Partial molal volume of anesthetics at infinite dilution; $\bar{v}_W^0$, partial molal volume of water at infinite dilution in liquid anesthetics.

	Enflurane	Isoflurane	Halothane
v^0	121.9_9	123.6_6	106.3_3
$\bar{v}_A^0$ in water	98.6	103.4	93.7
$\bar{v}_A^0$ in *n*-decane	135.2	135.3	114.9
$\bar{v}_W^0$	20.5	21.0	21.7

When the anesthetic concentration was increased, the partial molal volume of anesthetics in water was increased. The $\bar{v}_A$ values at nonzero anesthetic concentrations are estimated from the derivative of the apparent molal volume, and therefore generally are not very accurate. Nevertheless, the differences between $\bar{v}_A^0$ and v_A^0 and the tendency of $\bar{v}_A$ to increase with the increase of the anesthetic concentration are quite clear.

In contrast to the dilute solution of anesthetics in water, the dilute solution of water in anesthetics showed $\bar{v}_W^0$ values larger than those in its pure liquid state. Masterson and Seiler (15) reported the volume data of water in several halogenated hydrocarbon solutions. The reported $\bar{v}_W^0$ values in 1,2-dichloroethane, 1,1,1-trichloroethane and carbon tetrachloride were 20.1, 22.3, and 31.6 $cm^3 \cdot mole^{-1}$ at 298.15° K, respectively, which are comparable with the present result.

Nonpolar solvent. The anesthetics were freely miscible with *n*-decane; the densities of the solution with dilute anesthetic concentrations are shown in Fig. 4. The dissolution of anesthetics into the apolar solvent was accompanied by expansion of the volume as expected (Fig. 5). It was similar to the mixing between hydrocarbons and slightly polar organic solvents. The numerical values of $\bar{v}_A^0$ in *n*-decane were enflurane 135.2, isoflurane 135.3,

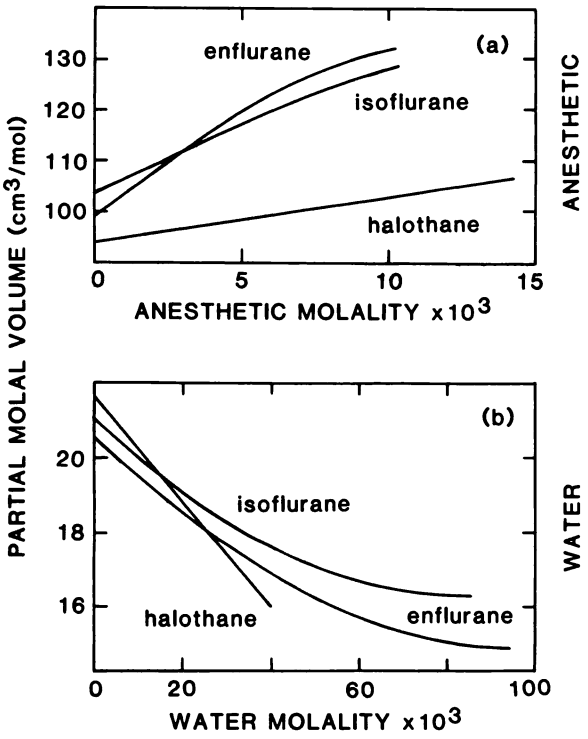

FIG. 3. *Partial molal volumes of anesthetics in water (a) and partial molal volumes of water in liquid anesthetics (b)*

There are no data points in this figure because partial molal volume is estimated from the slope of the curve fitted to the apparent molal volume, according to Eq. 3. The slope at isolated data points does not exist, and data points disappear.

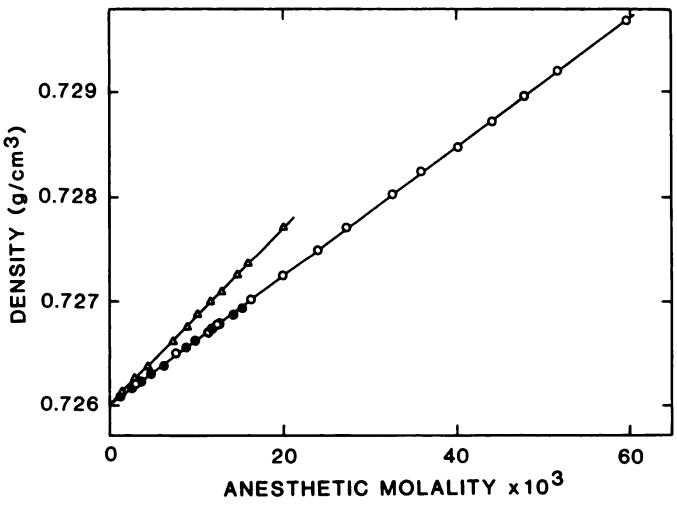

FIG. 4. *Densities of anesthetic solution in n-decane*

Data points represent averages of five observations. Error bars are omitted because the standard errors are within the size of the symbols. Enflurane (○), isoflurane (●), and halothane (△).

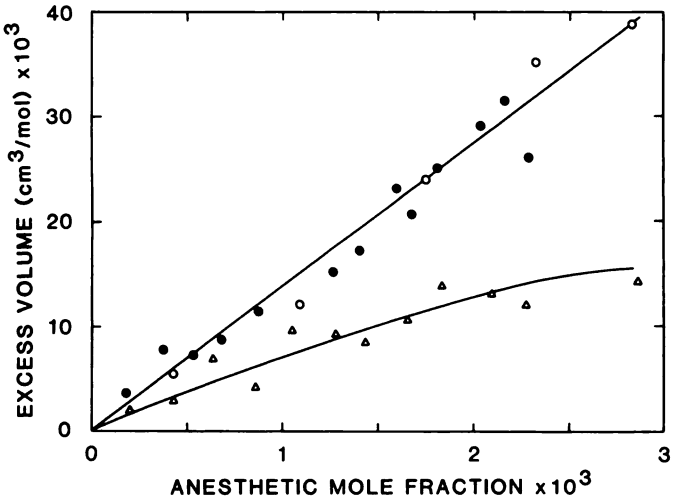

FIG. 5. *Mean molal excess volume of anesthetic solution in n-decane* Enflurane (○), isoflurane (●), and halothane (△).

and halothane 114.9 $cm^3 \cdot mole^{-1}$ (Table 1). The dependence of $\bar{v}_A$ to the anesthetic concentration is illustrated in Fig. 6.

The $\bar{v}_A$ of the ether-type anesthetics, enflurane and isoflurane, was increased when the anesthetic concentration was increased. In contrast, the $\bar{v}_A$ of the halogenated hydrocarbon anesthetic, halothane, was decreased when the anesthetic concentration was increased. The discrepancy was apparently caused by the difference in the interaction forces between the anesthetics and *n*-decane. The magnitude of v^E of halothane was smaller than those of enflurane and isoflurane.

Phospholipid vesicle suspension. Figure 7 represents the densities and the mean specific excess volumes of the mixture of enflurane and dimyristoylphosphatidylcholine liposomes. The partial molal volume of each component in this system was not evaluated because of the aforementioned theoretical difficulties. Instead, we calculated the v^E by mass fraction according to the following equation:

$$v^E = v_S - (w_A v_A^0 + w_S v_S^0) \quad (6)$$

Here, w_A is the mass fraction of anesthetics and w_S is the mass fraction of the lipid suspension, v_S is the specific volume of the lipid suspension with anesthetics, and v_S^0 is the specific volume of the lipid suspension without anesthetics.

The v^E showed a positive sign, and the numerical values increased when the anesthetic concentration was increased. When compared with the negative v^E found in the anesthetic solvation into water, it is clear that the total volume of the lipid suspension increases when the anesthetic molecules are transferred from the aqueous phase into the membrane.

In the mean excess-volume hypothesis, the volume expansion is envisioned by taking anesthetic molecules in the aqueous phase for the reference state. The gas phase is irrelevant because the anesthetic molecules in the gas phase are related to the membrane-water phase by Henry's solubility constant, which is only dependent on temperature and not on pressure in the ideal case. Although nonideality of anesthetic gases may influence the relation to some extent, pressure has essentially no effect upon the distribution of anesthetics between the two phases. The dynamic equilibrium exists between the membrane and water; the gas phase is an isolated entity. This contention is supported by the finding that the

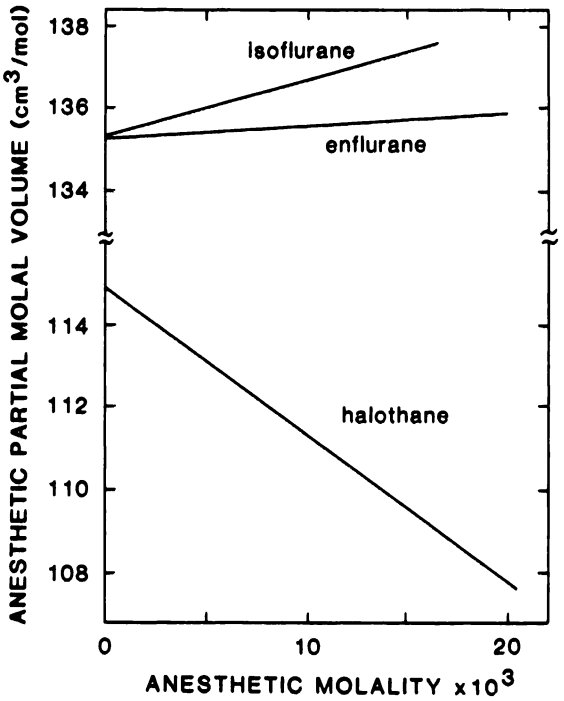

FIG. 6. *Partial molal volumes of anesthetics in n-decane*

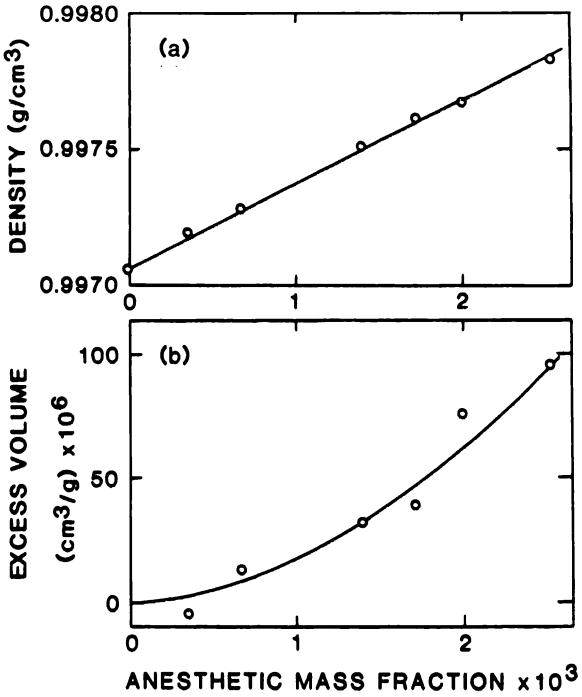

FIG. 7. *Density and mean specific excess volume of enflurane in 1 mM dimyristoylphosphatidylcholine liposome solution*

a. Density. Data points represent averages of five observations. Error bars are omitted because the standard errors are within the size of the symbols. b. Mean specific excess volume estimated from the density data.

effect of hydrostatic pressure upon tadpoles (no gas phase) (9, 10) is essentially the same as that induced by helium pressure in the presence of the gas phase (11, 12). In addition, the pure liquid state of anesthetics is inappropriate for the reference state because the system does not contain pure liquid anesthetics; hence the system is not in equilibrium with liquid anesthetics.

The mean specific excess volume increase in the vesicle suspension appears to be less remarkable than that observed in *n*-decane. This is because the bulk of the vesicle suspension was composed of water molecules: 99.998% by mole fraction or 99.93% by weight. It is obvious that a significant portion of anesthetic molecules is partitioned into the water phase, which would contribute negatively to the mean specific excess volume. Despite this negative effect, the presence of phospholipid vesicles clearly induces positive mean excess volume when compared with the aqueous solution of anesthetics, which is taken as the reference state.

CONCLUSION

There are at least two lines of thought about anesthetic-induced volume changes. In the membrane stabilization-expansion scheme and in the critical-volume hypothesis, the volume occupied by anesthetics in the membrane has a prime importance. Positive excess volume is not a necessary condition for the pressure reversal. The volume fraction of anesthetics in the membrane determines the anesthetic depth. Pressure reversal is solely attributed to the compressibility of the action site. The volume of the membrane proper is assumed to stay constant when anesthetics are bound. This is obviously incorrect when one considers that anesthetics transform the compact solid-gel phase of phospholipid membranes into the expanded liquid-crystalline phase. Furthermore, increase in the partial molal volume of crystalline proteins by anesthetics has been demonstrated (16, 17).

On the other hand, in the mean excess volume concept, the volume fraction of anesthetic molecules in the membrane and the bulk size of the membrane is irrelevant to anesthesia; the magnitude of the increase in the mean excess volume determines the depth of anesthesia. The result of Bull *et al.* (1) that nonanesthetic alkanols expanded erythrocyte membrane to the same extent as seen with anesthetics clearly shows that the membrane size is irrelevant to anesthesia.

Pressure does not reverse anesthesia if anesthetic molecules migrate from the aqueous phase into the membrane, increasing the membrane volume by the volume of the anesthetics in water, without changing the volume of the total system. Even if the partial molal volume of anesthetics expands by association with membranes, high pressure does not reverse anesthesia when the volume of total system is not increased. Pressure reversal occurs not by the compressibility of the membrane but by the reverse reaction of the multiple factors that caused the mean excess volume to increase.

The present result, showing the presence of positive excess volume when anesthetics are bound to phospholipid membranes and negative excess volume when dissolved in water, is direct evidence that these inhalation anesthetics increase the volume of the total system when translocated from the aqueous phase into the membrane. Although this does not disclaim alternative hypotheses in any way, it is sufficient to conclude that the pressure reversal of anesthesia can be explained without assuming any specific receptor sites for inhalation anesthetics.

The excess expansion is caused by the algebraic sum of at least the following interactions: the anesthetic molecules and bulk water, anesthetics and water in the lipid phase, lipid structural change by anesthetics, anesthetic volume change in the lipid phase, and the change in the interaction forces between lipid and water by the presence of anesthetics. The last term occurs because the binding of anesthetic molecules to the lipid phase alters the composition of the lipid phase. The molecular mechanisms of anesthetic interaction with membranes in aqueous media must be elucidated by evaluating each factor contributing to the volume change.

It is true that the observations obtained in model systems may not be directly applicable to the complex natural membranes. On the other hand, the very complexity of natural membranes makes quantitative analysis of anesthetic effect technically difficult. Although model systems represent only part of the natural membranes, they provide quantitative data which can be rigorously examined.

APPENDIX 1

Franks and Lieb (2) estimated the membrane volume from the difference between the density of the membrane suspension (two-phase, three-component system) and the density of the solution without membrane (one-phase, two-component system). The procedure of combining two different systems without justification is a violation of the principles of thermodynamics. Therefore, contrary to their claim, the obtained value is not the partial molal volume of anesthetics in the membrane.

By using superscripts B and L to designate buffer and membrane phases explicitly, and with the usual thermodynamic notations, the Gibbs free energy of the two-phase system is written:

$$\begin{aligned} dG &= dG^B + dG^L \\ &= -S^B\,dT + V^B\,dP + \sum_i \mu_i^B\,dn_i^B - S^L\,dT \\ &\quad + V^L\,dP + \sum_i \mu_i^L\,dn_i^L \end{aligned} \tag{A1}$$

whereas that of the buffer alone is written:

$$dG^{B'} = -S^{B'}\,dT + V^{B'}\,dP + \sum_i \mu_i^{B'}\,dn_i^B \tag{A2}$$

If one assumes that T, P, and n_i^B have identical values in the above two equations, then dG^B can not be equal to $dG^{B'}$ because there is a constraint that μ_i^B must be equal to μ_i^L at equilibrium.

When applied to the volume fraction, the above expression becomes, by using the notations of Franks and Lieb (2):

$$\begin{aligned} V^S = V^L + V^B &= \sum n_i^L \bar{v}_i^L + \sum n_i^B \bar{v}_i^B \quad (i = a, w, l, b) \\ &= v_{mem}(\sum n_i^L M_i) + v_{buf}(\sum n_i^B M_i) \end{aligned} \tag{A3}$$

where the superscript S signifies the membrane suspension; M is the molecular weight; a, w, l, and b, are anesthetic, water, membrane, and buffers, respectively; and $v_{mem} = (\sum n_i^L \bar{v}_i^L)/(\sum n_i^L M_i)$ and $v_{buf} = (\sum n_i^B \bar{v}_i^B)/(\sum n_i^B M_i)$ Eq. A3 is identical Eq. 1 of the report by Franks and Lieb (2).

On the other hand, the volume of the buffer solution obtained in the absence of membrane is expressed as

$$v_{buf} = (\sum n_j^B \bar{v}_j^{B'})/(\sum n_j^B M_j) \qquad (j = a, w, b) \qquad \text{(A4)}$$

Franks and Lieb (2) ignored the differences between $\bar{v}_i^B$ and $\bar{v}_j^{B'}$ (or $\bar{v}_i^E$, volume excess) without justification and the volume of the membrane lost its thermodynamic definition. In order to establish that the differences are small, one must evaluate the contribution of the differences of partial molal volumes of all components between the two systems.

APPENDIX 2

In expressing the state of the system in terms of pressure, the volume, V, of the membrane region containing anesthetic, water, and membrane molecules is explicitly written by the following intensive and extensive variables.

$$V = V(T, P, n_A, n_W, \cdots, n_M, \cdots) \qquad \text{(A5)}$$

where T is the absolute temperature; P is the pressure, n is the number of molecules; and subscripts A, W, and M signify anesthetic, water, and membrane molecules, respectively.

The volume change, dV, is written by taking the total differential.

$$dV = \left(\frac{\partial V}{\partial T}\right)_{P,n_j} \cdot dT + \left(\frac{\partial V}{\partial P}\right)_{T,n_j} \cdot dP + \left(\frac{\partial V}{\partial n_A}\right)_{T,P,n_j} \cdot dn_A + \left(\frac{\partial V}{\partial n_W}\right)_{T,P,n_j} \cdot dn_W + \cdots + \left(\frac{\partial V}{\partial n_M}\right)_{T,P,n_j} \cdot dn_M + \cdots \qquad \text{(A6)}$$

where T, P, n_A, n_W, and n_M are independent variables. The total volume change in the whole system at the half-anesthetized condition (superscript a) is described under the condition that all coefficients remain constant

$$\Delta V = \left(\frac{\partial V}{\partial T}\right)_{P,n_j} (T^a - T^0) + \left(\frac{\partial V}{\partial P}\right)_{T,n_j} (P^a - P^0) + \bar{v}_A(n_A{}^a - n_A{}^0) + \cdots + \bar{v}_W(n_W{}^a - n_W{}^0) + \cdots \qquad \text{(A7)}$$

where $\bar{v}$ signifies the partial molal volume. When this equation is divided by the volume of the system at the half-anesthetized condition, one obtains the fractional expansion, E^a.

$$E^a = \Delta V/V^a = \alpha(T^a - T^0) - \beta(P^a - P^0) + (\bar{v}_A{}^a/V^a)(n_A{}^a - n_A{}^0) + (\bar{v}_W{}^a/V^a)(n_W{}^a - n_W{}^0) + \cdots + (\bar{v}_M{}^a/V^a)(n_M{}^a - n_M{}^0) + \cdots \qquad \text{(A8)}$$

where α is the thermal expansibility and β is the compressibility. When T, n_A, n_W, $\cdots$ n_M, $\cdots$ are constant, E^a becomes

$$E^a = (\bar{v}_A{}^a/V^a)(n_A{}^a - n_A{}^0) - \beta(P^a - P^0) \qquad \text{(A9)}$$

Miller *et al.* (12) defined the fractional expansion as

$$E^a = \frac{\bar{v}_A \cdot x_A \cdot P}{V} - \beta P \qquad \text{(A10)}$$

In Eq. A10, x_A is defined as the fractional solubility of anesthetics and represents the mole fraction of anesthetics in the membrane when the anesthetic partial pressure equals 1 atmos. Because the anesthetic gases obey Henry's law, the equation states that the term $x_A P$ represents the quantity of anesthetics in the membrane transferred from the gas phase. Although the expression for the anesthetic concentration is different, Eq. A9 equals Eq. A10.

In deriving this equation, however, constancy of all differential coefficients in Eq. A6 is assumed. This assumption may not be justified.

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