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Anesthetics Release Unfreezable and Bound Water in Partially Hydrated Phospholipid Lamellar Systems and Elevate Phase Transition Temperature

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SUMMARY

A dimyristoylphosphatidylcholine multilamellar system with varied water content was prepared by dessiccating sonicated vesicles *in vacuo*. The water content in the sample was determined by gas chromatography after dissolving the multilamellar system in water-free benzene. Differential scanning microcalorimetry revealed several endothermic peaks in the heating scan at subzero temperature, ranging from −25 to −3°. The peaks that appeared in the subzero temperature range indicate the existence of water molecules bound to the lipid head groups, differing from free water that freezes at 0°. The difference between the amount of water molecules that froze in calorimetry and the total amount of water detected by gas chromatography indicates the presence of unfreezable, tightly bound water molecules. The relative amount of free, intermediate, and unfreezable water was estimated by comparing the differential scanning microcalorimetry data with gas chromatography measurements. The addition of halothane and 1-hexanol significantly decreased the intermediately bound water peaks. The anesthetics dehydrated the lamellar system. The phase polymorphism of partially hydrated phospholipid multilayers is well known, and the temperature that corresponds to the main phase transition of fully hydrated lipid membranes shifts to a higher temperature. The addition of anesthetics increased the phase transition temperature when the water content was less than 18 wt%. This result is the complete reverse of the depressant action of anesthetics in fully hydrated lipid membranes. The present anesthetic effect upon the elevation of the transition temperature is apparently caused by anesthetic-induced dehydration of the lipid-water interface at the present experimental condition.

The importance of water in biological systems has been amply emphasized. Because lipid membranes cannot be formed without water, water-lipid interaction is of prime importance in the existence of membranes. Membrane structures are supported by a hydrogen-bonded network of water molecules, and anything that weakens the water-lipid interaction tends to expand and disorder the structure. We propose that the primary action site of slightly polar hydrophobic inhalation anesthetics, such as chloroform, diethyl ether, halothane, and other clinically used modern inhalation agents (except nitrous oxide or xenon) is the membrane/water interface, releasing membrane-bound water molecules (1–13). We have shown by proton nuclear magnetic resonance spectrometry that inhalation anesthetics increased the proton spin-spin relaxation time in water-in-oil reversed micelles (13), indicating a release of interfacially bound water.

In fully hydrated phospholipid vesicles, Ladbrooke and Chapman (14) have demonstrated the existence of unfreezable water, even at −100°, by differential scanning calorimetry. They concluded that about 10 water molecules per lecithin molecule do not freeze at 0°. Reports of partially hydrated phospholipids, using a variety of methods including differential scanning microcalorimetry, nuclear magnetic resonance, infrared spectroscopy, X-ray diffraction, etc., have shown that hydrophilic surfaces of lipid membranes interact strongly with water molecules (14–25).

By the use of proton and ^{31}P continuous wave and spin-echo nuclear magnetic resonance spectrometry, Gottlieb *et al.* (15) reported that about six water molecules are bound to a lecithin molecule and up to 11 water molecules can fit into the free volume around each phosphate moiety. Another nuclear magnetic resonance study by Finer and Darke (16) has shown that up to 20 water molecules per lipid molecule are restricted in their motion at the membrane surface. Among those, 11 water molecules are considered to be tightly bound to the membrane. The number of water molecules bound to each lipid molecule is a subject of wide variance, depending on the method of estimation and data interpretation. Thus, other reports include two tightly bound and five weakly bound water molecules (17),

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one tight plus nine weak (18), about five (19), at most six (24), etc.

From these studies, three states of water are generally considered to occur in lipid vesicle suspension (14–25). Free water is the portion that has all the properties of bulk water and freezes at 0°. Tightly bound water is fixed on the lipid surface and is unfreezable to a very low temperature. Intermediately bound water is the portion that has characteristics between free and bound water and freezes below 0°. Water molecules, constrained in their motion at the interface, are not limited to lipid membranes. By infrared spectroscopy of the O—H coupling, three different types of water were identified in water-in-oil reversed micelles (26).

The present study was undertaken to measure the anesthetic action upon water molecules bound to the lipid surface, in a partially hydrated dimyristoylphosphatidylcholine multilamellar system. The term "bound water" may be misleading, because these bound water molecules are exchanging with free water molecules faster than the proton nuclear magnetic resonance resolution. A better description may be that the residence time of water molecules at the surface position is prolonged compared to free water molecules residing at a particular position in the bulk. The changes in the intermediate water peaks in differential scanning microcalorimetry will be reported. The total water content in the multilamellar system is measured by gas chromatography; the tightly bound water molecules, which are invisible in the calorimetry, are estimated from the difference between the total water content and the calorimetry data.

Materials and Methods

Synthetic dimyristoylphosphatidylcholine was obtained from Sigma and recrystallized from chloroform. Reagent grade 1-hexanol was obtained from Eastman, and halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was from Ayerst Laboratories. Benzene was PHOTOREX grade from J. T. Baker and was further dried by passing through an activated aluminum oxide (Fluka) column. Water was triply distilled, once from alkaline potassium permanganate solution.

The vesicle was prepared by sonicating the aqueous suspension of recrystallized dimyristoylphosphatidylcholine in a cup-horn of a Branson (Danbury, CT) Sonifier model 185 at or above the main phase transition temperature. The vesicle suspension was centrifuged at 120,000 × *g* for 2 hr in a Spinco Ultracentrifuge to make a concentrated paste. The vesicle paste was transferred into aluminum pans of a differential scanning microcalorimeter, and dried *in vacuo* to the desired water content in a desiccator. Subsequently, the sample pans were tightly capped and subjected to differential scanning microcalorimetry.

Anesthetics were administered to the partially dried phospholipid vesicles by vapor phase adsorption. A small amount of liquid anesthetics was introduced into a stoppered weighing bottle of about 10 ml capacity, and the open phospholipid sample pan was placed above the liquid anesthetics on a small stand. The bottle was tightly closed by a ground glass top, and the anesthetic vapor was allowed to absorb into the phospholipid samples at room temperature (around 23°). The amount of anesthetics absorbed into the sample was controlled by the length of the exposure time. For 1-hexanol, the exposure time varied between 8 and 24 hr, whereas halothane required shorter periods, typically less than 20 min. After exposure to the anesthetic vapors, the sample pan was tightly capped and calorimetry was started.

After the calorimetry scanning, the sample pans were immediately dropped into water-free benzene in a vial, and the pans were opened. The vial was tightly sealed and the content was sonicated by immersing the vial in a Sonicor (Copiague, NY) ultrasonic sonicator, filled with water to ensure complete dissolution of the sample. The benzene solution was analyzed by gas chromatography for the water and anesthetic content. Total water content in each sample was measured using a Varian 3700 gas chromatograph with a thermal conductivity detector. The column was ⅛ in. × 6 ft. stainless steel, containing Porapak Q 80/100 mesh. Anesthetic concentrations in the sample were determined by a Shimadzu (Columbia, MA) GC-Mini2 gas chromatograph with a flame ionization detector. The column was also ⅛ in. × 6 ft. stainless steel, containing Porapak P 80/100 mesh.

Differential scanning microcalorimetry was performed with a Perkin-Elmer (Norwalk, CT) DSC-2 microcalorimeter with auto-zero. To avoid supercooling, only heating scans (endothermic run) were employed between −53° and 77° at a heating rate of 5°/min. The calorimetry data were stored in an Apple IIe microcomputer interfaced with a PDP 11/23 minicomputer.

To construct a phase diagram in binary systems, the onset temperatures of heating and cooling scans are necessary (27). However, the peak in the calorimetry scan can be identified unambiguously with good reproducibility, and the peak value has often been used for the transition temperature (25, 28). Because the present study only concerns the change in the transition temperature by the additives, the peak position in the endothermic scan was used for the transition temperature.

The excess enthalpy was measured from the area under the transition peak, by comparison with indium and water standards. The number of water molecules that melt at subzero temperatures can be estimated from the measured excess enthalpy if the ΔH of the bound water is known. However, it is difficult, if not impossible, to estimate ΔH values of various species of water molecules that freeze at various subzero temperatures. Although Grabielle-Madelmont and Perron (24) estimated ΔH values of intermediate waters, the values vary according to the assumption on the number of tightly bound unfreezable water molecules. Because of this difficulty, a single value of 5.98 KJ·mol^{-1} for the melting ΔH of ordinary ice (Ice Ih) was used. The enthalpy of ice at 250° K is 4.48 KJ·mol^{-1} and is about 20% lower than the enthalpy of ice at 273° K, 5.38 KJ·mol^{-1} (29, 30). The enthalpy of intermediately bound water is unlikely to differ much from that of ice at comparable temperatures. Then the ΔH at 250° K becomes 6.88 KJ·mol^{-1}, which is only 15% larger than the ΔH at 273 K. Moreover, because the enthalpy of water at 250° K must be smaller than that at 273° K (11.4 KJ·mol^{-1}), the difference further decreases. The purpose of the present study is to investigate whether anesthetics alter the relative amount of various types of bound water molecules in the lipid-water system. We do not attempt to give a quantitative analysis of the exact proportion of various types of water in the system. Therefore, the assumption of a single enthalpy for all species of bound water would not invalidate the conclusion.

Results and Discussion

Differential scanning microcalorimetry thermograms with various amounts of water in dimyristoylphosphatidylcholine are shown in Fig. 1a. The total water, free and intermediately bound water, and tightly bound water contents in each thermogram are expressed in terms of the number of mol of water per mol of the phospholipid, and are shown in Table 1. The amount of free and intermediately bound water was estimated from the thermogram. The amount of bound water was calculated by the difference in the amount of total water estimated from gas chromatography and the amount of free and intermediately bound water estimated from calorimetry.

Control study in the absence of anesthetics. The free water melting peak in thermogram *A* (water content 26.1 wt%) of Fig. 1a is asymmetric. The lower temperature side shows a long tail, indicating overlapping of the free water peak of 0° with lower temperature melting peaks of intermediately bound water molecules. The intermediately bound water does not belong to a single species but consists of water molecules with

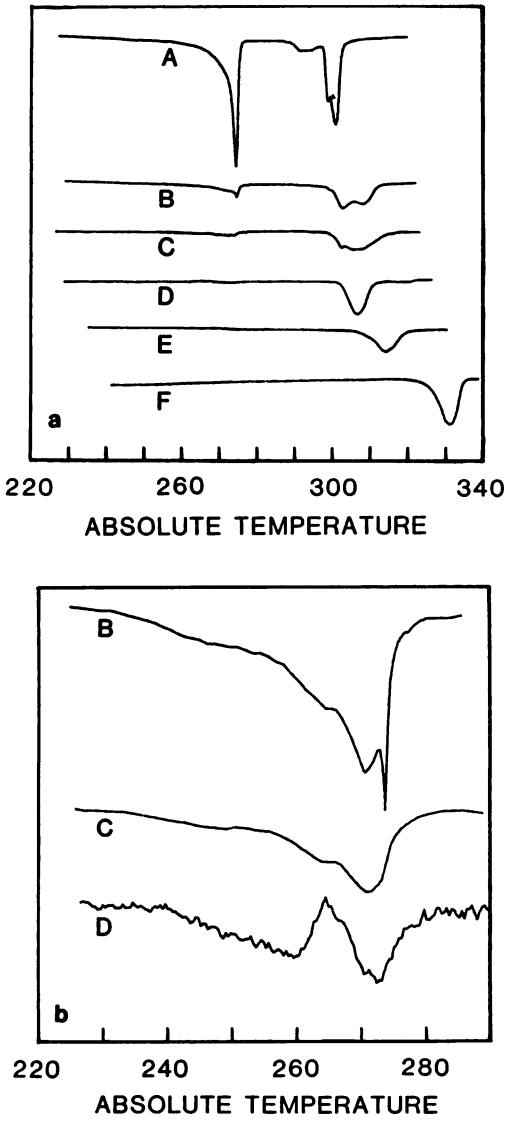

Fig. 1. a. Heating scan of differential scanning microcalorimetry on samples with various amounts of water in dimyristoylphosphatidylcholine vesicle suspensions. The water content in the sample is expressed by wt%. *A*, 26.1; *B*, 17.9; *C*, 15.8; *D*, 12.1; *E*, 6.6, and *F*, 2.8. b. High sensitivity heating scan of samples *B*, *C*, and *D* in a.

TABLE 1

Relative amounts of bound water in dimyristoylphosphatidylcholine-water system

Free and intermediate water and tightly bound water are expressed as a mole ratio against 1 mol of dimyristoylphosphatidylcholine.

	Total wt%	Water mole ratio	Intermediately bound water	Tightly bound water	Transition temperature (K)	
A	26.1	13.3	6.4[a]	6.9	299.0[b]	301.2
B	17.9	8.2	2.0	6.2	302.1[b]	305.0
C	15.8	7.1	1.0	6.1	302.0[b]	305.3
D	12.1	5.2	0.6	4.6		306.0
E	6.6	2.6	0.4	2.2		314.0
F	2.8	1.1	ND[c]	1.1		330.5

[a] This value includes the free water.
[b] The transition temperature splits into two peaks in this water content region as seen in Fig. 1.
[c] ND, not detectable.

various degrees of association. From a study on removing water from lecithin multilayers, Parsegian *et al.* (21) concluded that there are no discrete classes of bound water, and the work of water removal is a continuous function of water content and lattice repeat spacing. Presumably, the first layer of the hydration shell around the hydrophilic parts of phospholipid lattice has the highest affinity. With the increase in the distance between the water molecule and the binding site, the affinity gradually decreases toward that of free water. This lack of a clear difference between bound and free water molecules may be one of the reasons for the scatter in the reported number of binding water molecules.

More detailed information on the multiplicity of the state of interfacial water molecules is shown in Fig. 1b by high sensitivity calorimetry thermograms. Thermogram *B* (water content 17.9 wt%) exhibits a composite spectrum consisting of free water and various states of intermediately bound water. Tightly bound water is unfreezable, hence it is invisible in calorimetry. The sharp peak demonstrated at 0° in thermogram B represents free water. Different states of intermediately bound water appear at lower temperatures ranging between −25 and −3°. The free water peak at 0° disappeared when the water content dropped from 17.9 to 15.8 wt% (*B*–*C*). Nevertheless, the positions of the intermediately bound water peak remained about the same between thermograms *B* and *C*. Further drying the sample to water content 12.1 wt% showed two major peaks at −2 and −14°. The position of these peaks differed from those of thermograms *B* and *C*. It is possible that the intermediately bound water underwent some rearrangement during the drying process. Sample *D* (water content 12.1 wt%) was a relatively stable form. Except by undergoing prolonged drying, lasting several days, these intermediately bound water peaks did not change. For this reason, anesthetic effects on the dimyristoylphosphatidylcholine-water system were studied starting with the sample *D* dryness. Sample *F* is a preparation that underwent 140 hr of drying *in vacuo*, and the water content was 2.8 wt%, in which the intermediately bound water peaks disappeared.

The transition temperature of the dimyristoylphosphatidylcholine-water system increased with the decrease in water content. This result is in agreement with those of dipalmitoylphosphatidylcholine-water mixtures (14–24, 28, 31–33). The main phase transition of fully hydrated phospholipid membranes is the eutectoid phase boundary of water-phospholipid binary systems, where the transition follows $L\beta' + H_2O \rightarrow P\beta' + H_2O \rightarrow L\alpha' + H_2O$ according to the temperature elevation. In the above expression, $L\beta'$, $P\beta'$, and $L\alpha$ signify solid-gel, rippled, and liquid-crystalline phases, respectively, defined by Tardieu *et al.* (31). When the water content decreases below about 20 wt%, the $L\beta' + H_2O$ phase disappears and the $L\beta'$ phase without free water emerges. The splitting of the transition peak seen in the thermograms *A*, *B*, and *C* was presumably caused by the peritectoid reaction, $L\beta' + L\alpha \leftrightarrow P\beta'$ (14, 20, 23, 25, 31, 32). In partially hydrated systems, phase polymorphism is well documented (14, 20, 23, 25, 28, 31, 32). There are experimental and theoretical studies on the quantitative phase diagrams on dipalmitoylphosphatidylcholine-water binary systems at low water content. However, there still remain uncertainties as to the exact location of phase boundaries in partially hydrated phospholipid systems.

The number of bound water molecules appears to be closely

related to the phase behavior in the low water content region. In a water-dipalmitoylphosphatidylcholine system, Grabielle-Madelmont and Perron (32) reported that no water freezing was observed in the $L\beta'$ phase when the water content was less than 14 wt%. Above 14 wt% of water, freezing started coinciding with the peritectoid reaction $L\beta' + L\alpha \leftrightarrow P\beta'$. In the $P\beta'$ phase, part of the water apparently does not interact too strongly with the polar groups. The state of the lipid structure apparently influences the water binding, and vice versa.

The importance of the state of the interfacial water for the integrity of lipid membranes has been amply described. Guildbrand *et al.* (23) concluded that the phase equilibria in the dipalmitoylphosphatidylcholine-water system is to a large extent determined by the difference in the hydration force in the different phases. Kjellander (34) proposed that water and phospholipid hydrophilic parts form a stabilized and highly cross-linked network which constitutes the base in the bilayer membrane. In this framework, the minimum number of basic water molecules that are required to form a membrane was estimated to be 10–12 per phospholipid molecule. Water is an integral part of the lipid membrane, and an inadequacy in the hydration is expected to cause a defect in the membrane structure in fully hydrated phospholipid membranes.

Effect of 1-Hexanol. The differential scanning microcalorimetry spectra of dimyristoylphosphatidylcholine-water system with various amounts of 1-hexanol adsorption are shown in Fig. 2a. Thermogram *G* is a relatively dry control system with a water content of 12.1 wt% in the absence of anesthetics. Thermograms *H* and *I* represent samples exposed to 1-hexanol vapor for 8 and 24 hr, respectively. The mole ratio of 1-hexanol in the phospholipid membrane was 0.31 for 8 hr exposure and 0.43 for 24 hr exposure.

The $L\beta'$ phase transition of the phospholipid membranes shifted to a higher temperature when the sample absorbed 1-hexanol. The magnitude of the high temperature shift was increased with the increase in the 1-hexanol content. The result is in sharp contrast to fully hydrated lipid vesicles where the presence of anesthetics decreases the main phase transition temperature. The increase in the $L\beta'$ transition temperature was associated with the decrease in the intermediately bound water peaks as shown in Fig. 2b. Total water content, 1-hexanol concentration, free and intermediately bound water content, and the phase transition temperature of the dimyristoylphosphatidylcholine-water system are listed in Table 2.

In Fig. 2b, thermogram *H* shows only one intermediately bound water peak. The lower temperature peak at −14° in the control thermogram *G* disappeared in the presence of 1-hexanol. A part of the intermediately bound water, associated with the phospholipid, was apparently released by penetration of 1-hexanol into the interface. A further increase in 1-hexanol concentration resulted in complete disappearance of intermediately bound water peaks as shown in thermogram *I*. The intermediately bound water was totally released by 1-hexanol in this case.

The state of the tightly bound water cannot be assessed by differential scanning microcalorimetry alone. Gas chromatography results revealed that the number of tightly bound water molecules, in terms of number of mol of water per mol of lipid (mole ratio), dropped from 4.6 in sample *G* to 2.6 in sample *I*. The total water content decreased from 12.1 wt% (water/lipid mole ratio 5.2) to 6.1 wt% (water/lipid mole ratio 2.6). The

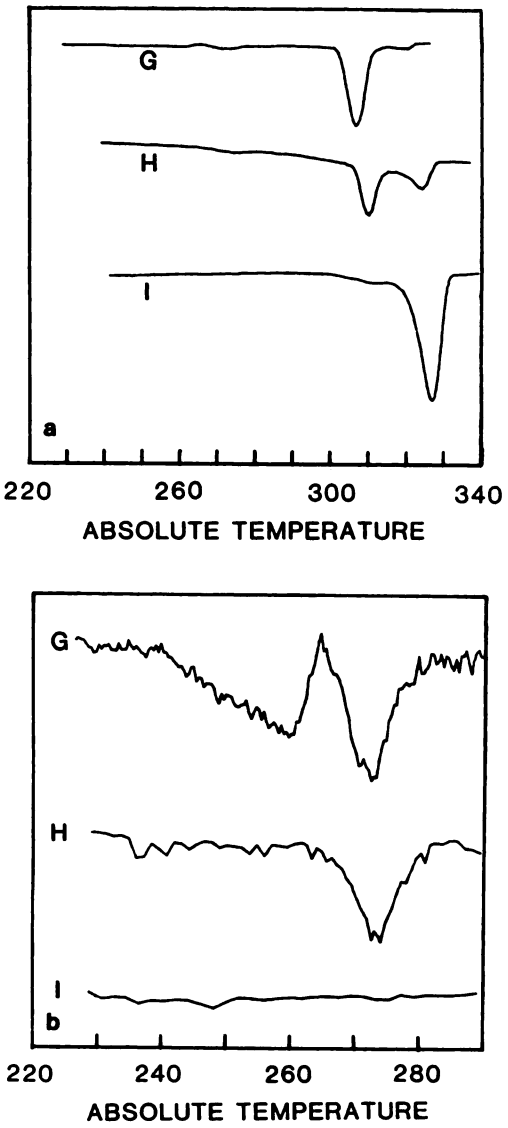

Fig. 2. a. Effects of 1-hexanol on the differential scanning microcalorimetry spectra of dimyristoylphosphatidylcholine vesicle suspension. *G*, Control without 1-hexanol; water content was 12.1 wt%. *H*, After 8 hr exposure to 1-hexanol vapor, water content was decreased to 8.2 wt% without drying. *I*, After 24 hr exposure to 1-hexanol vapor, water content was 6.1 wt%. b. High sensitivity heating scan of samples *G*, *H*, and *I* in a.

TABLE 2

Relative amounts of 1-hexanol and bound water

Water concentrations are expressed as a mole ratio against the phospholipid.

	1-Hexanol	Total water	Intermediately bound water	Tightly bound water	Transition temperature (K)
G	None	5.2	0.6	4.6	306.5
H	0.31	3.5	0.2	3.3	323.7
I	0.43	2.6	ND[a]	2.6	327.0

[a] ND, not detectable.

wt% of water in the presence of anesthetics is expressed by [water]/[water + lipid + anesthetic]. At least part of the tightly bound water was released by 1-hexanol. It is evident that 1-hexanol dehydrated the lipid-water interface.

Effect of halothane. Similar results were obtained when halothane was administered. In Fig. 3a, thermogram *J* is the control that contained 11.7 wt% water in the absence of anes-

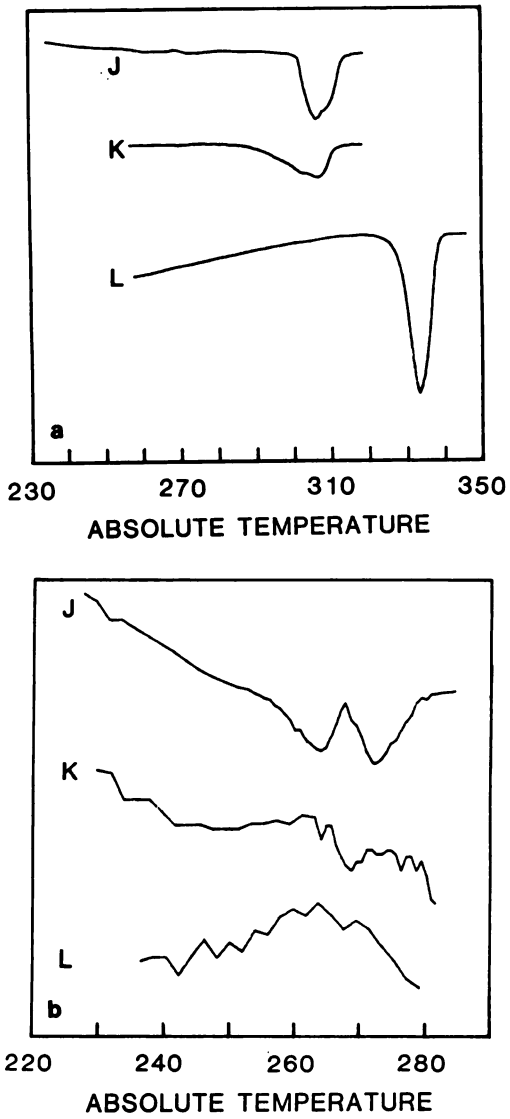

Fig. 3. a. Effects of halothane on the differential scanning microcalorimetry spectra of dimyristolyphosphatidylcholine vesicle suspension. *J*, Control without halothane; water content 11.7 wt%. *K*, After 15 min exposure to halothane vapor; water content 8.9 wt%. *L*, After 20 min exposure to halothane vapor; water content 2.7 wt%. b. High sensitivity heating scan of samples *J*, *K*, and *L* in a.

thetics. The addition of halothane decreased the intermediately bound water peak in thermogram K where the halothane/lipid mole ratio was 0.38. The water/lipid mole ratio was decreased to 4.1, or the total water content was decreased to 8.9 wt%. A further increase in halothane concentration totally eliminated the intermediately bound water peak (thermogram *L*), and the total water content was further decreased to 2.7 wt% (water/lipid mole ratio 1.2). Halothane dehydrated the system to the level that is only attained after prolonged, 140-hr vacuum desiccation. Fig. 3b shows the high sensitivity thermograms of the above samples. Halothane concentration, total water content, free and intermediately bound water, and bound water data obtained by a combination of calorimetry and gas chromatography are summarized in Table 3.

There appears to be some confusion about the potency of anesthetics. A criticism was that it is surprising that halothane and 1-hexanol were approximately equipotent in displacing

TABLE 3

Relative amounts of halothane and bound water

Water concentrations are expressed as a mole ratio against the phospholipid.

	Halothane	Total water	Intermediately bound water	Tightly bound water	Transition temperature (K)
J	None	5.0	0.5	4.6	306.0
K	0.38	4.1	ND[a]	4.1	306.2
L	0.49	1.2	ND	1.2	331.0

[a] ND, not detectable.

water molecules. To clarify the above point, a discussion of anesthetic potency is in order. It is generally accepted [see a critical review by Ueda and Kamaya (1)] that anesthesia commences when a critical number (Overton-Meyer) or a critical volume (Mullins-Miller) of anesthetics are incorporated into lipid membranes. Under the condition of ambient atmospheric pressure, the difference between number and volume is minimal. Hence, 1-hexanol and halothane are equipotent in anesthetic activity at the same mole fraction in the lipid lamellae. The present result that they are also equipotent for releasing bound water demonstrates an excellent correlation between anesthetic potency and water-releasing activity. The difference between the two anesthetics was observed during their administration into the lipid-water mixture; halothane required less than 1 h whereas 1-hexanol required 8 to 24 hr.

Another misconception is the idea that halothane is a nonpolar molecule. On the contrary, halothane was a product of a drug design where dipole moment was intentionally incorporated into the molecule by leaving acidic proton at one end of the halogenated ethane. Polarity in a molecule is known to be indispensable in designing potent anesthetics.

Distribution of water in lipid multilayer. Davenport and Fisher (18) used a benzene solution of purified egg lecithin to investigate the role of initial water molecules in the system by adding a small amount of water. When measured by infrared spectroscopy, the relatively short transverse relaxation time of water and the infrared shift of the P═O stretching band indicated that the first water molecule enters a very restricted environment, probably associating with the phosphate group. Subsequently added water molecules are also associated with the phosphate group, and the observation that the O—H stretching band of the water molecules increased in frequency suggested that the water was indeed forming a "shell" around the phosphate group. The residence times of these water molecules at the phosphate group are much longer than in the bulk state. The water shell can be more than one layer. The inner layer water molecules are tightly bound to the phosphate group, whereas the outer layer water molecules bind less strongly.

The present result is consistent with these findings. In Table 1, sample *F* shows that approximately 1 water molecule per phospholipid molecule still remains after 140 hr of vacuum drying. This remaining water molecule must be located in a very restricted area. We observed with Fourier-transform infrared spectrophotometry that halothane shifted the OH stretching frequency from 3371 cm^{-1} to 3401 cm^{-1} in the partially hydrated dimyristoylphosphatidylcholine-water system, indicating a release of bound water from the phospholipid surface to the free state (to be reported).

Effect of water on phase transition of multilamellar system. Experimentally, it was found that the melting temperature of the lipid-water system increased with a decrease in

the amount of water. When the water content is reduced, the lipid bilayer stacks together to form a multilayer crystal.

The multilamellar system consists of alternating lipid bilayers and water layers. We consider a column perpendicular to the lamellae where all of the lipid layers and water layers are of the same size. To treat the phase transition of a membrane in the presence of water, we consider the free energy expression given by

$$G = G_m + G_{int} \quad (1)$$

where G_m represents the free energy of one unit of lipid systems and G_{int} denotes the interaction free energy between lipid systems due to the presence of water (bound water in this case). Notice that

$$\Delta G = G_l - G_g = \Delta G_m + \Delta G_{int} \quad (2)$$

At phase transition, we have $\Delta G = 0$, hence

$$n(\Delta \bar{H} - T\Delta \bar{S}) + \Delta G_{int} = 0 \quad (3)$$

where $\Delta G_m = n(\Delta \bar{H} - T\Delta \bar{S})$, n denotes the number of lipid molecules, and subscripts l and g denote liquid-crystalline and solid-gel conformations, respectively. T in Eq. 3 is the observed phase transition temperature. Solving for T, we obtain

$$T = Tm\left(1 + \frac{\Delta G_{int}}{n\Delta H}\right) \quad (4)$$

where $Tm = \Delta \bar{H}/\Delta \bar{S}$. We shall assume that ΔG_{int} is due to the bound water molecules between the two adjacent lipid systems. Then, writing $\Delta G_{int} = n_b \Delta \mu_{int}{}^b$ where n_b represents the number of the bound water molecules, we can write Eq. 4 as

$$T = Tm\left(1 + \frac{w_b \Delta \mu_{int}{}^b}{\Delta H}\right) \quad (5)$$

where $w_b = n_b/n$. If $\Delta \mu_{int}{}^b$ is due to the dipole-dipole interaction between the bound water molecules on the two adjacent lipid systems, the $\Delta \mu_{int}{}^b$ is proportional to l^{-3}. Thus we find

$$T = Tm\left[1 + \frac{\alpha w_b}{\Delta H(w_b + w_a)^3}\right] \quad (6)$$

where α is a proportionality constant and w_a represents the unbound portion of water molecules. Eq. 6 indicates that, provided $\alpha > 0$, the melting temperature decreases with increasing water content.

The phase transition in phospholipid membranes is a feature assigned to the order-disorder change in the lipid tail conformation. For this reason, the anesthetic effect upon the main transition temperature in fully hydrated phospholipid vesicle membranes generally is attributed to the direct disordering action of anesthetic molecules upon the lipid tail. However, the present result indicates that the decrease in the main phase transition temperature of fully hydrated phospholipid membranes may not be caused by their direct action upon the state of the lipid chain conformation. If these anesthetics penetrate the lipid core and disorder the lipid chain conformation, they should decrease the transition temperature, regardless of the hydration state of the phospholipid molecules. We postulate that the anesthetic effect upon phospholipid membranes is interfacial, and the depression of the transition temperature is the result of weakening the water-lipid interaction forces that support the membrane structure, as has been discussed previously.

With nonionic surfactant micelles, we have shown that inhalation anesthetics decreased the cloud point (2). An aqueous solution of nonionic surfactants becomes suddenly turbid when heated to a critical temperature, known as the cloud point, and the solution separates into two phases. This phenomenon is caused by the partial release of interfacial water molecules, hydrogen bonded to the hydrophilic polyoxyethylene group. The dehydration is accompanied by an increase in the partial molal volume, because the volume of the interfacially restricted water molecules is smaller than that of the bulk state. The concentration of methoxyflurane, halothane, and enflurane that decreased the cloud point temperature was in the order of clinical potency, and hydrostatic pressure in the range of 150 bar antagonized the anesthetic effect at the clinical concentrations. It was concluded that the anesthetics dehydrated the micelle surface, releasing interfacial water. The present result is in agreement with the surfactant micelle data, indicating that amphipathic anesthetics bind to the macromolecular surface and release interfacial water. The resultant increase in the hydrophobicity of macromolecular surfaces may be related intimately to the state of anesthesia.

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