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Can the Lipid Theories of Anesthesia Account for the Cutoff in Anesthetic Potency in Homologous Series of Alcohols?

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SUMMARY

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The general anesthetic potency of several members of the homologous series of saturated and unsaturated aliphatic alcohols ($C_{n:0}$ and $C_{n:1}$) was determined in tadpoles, using the loss of righting reflex as the criterion of anesthesia. In the $C_{n:0}$ series, anesthetic potency increased with chain length and was maximal for dodecanol (ED_{50} 5.4×10^{-6} M). The cutoff in potency was between $n = 12$ and $n = 14$ such that *n*-tridecanol was a partial anesthetic, whereas *n*-tetradecanol and higher alcohols were totally inactive. However, for the unsaturated alcohols, the anesthetic cutoff point was shifted to a longer chain length, i.e., the Δ^9-tetradecenols are full anesthetics whereas the Δ^9-hexadecenols are partial anesthetics. In each case the *cis*- and *trans*-isomers were equipotent. In the $C_{n:0}$ series in egg lecithin (EPC)-cholesterol (2:1) vesicles spin-labeled with 5-doxyl palmitic acid, the increase in membrane disorder at a fixed alcohol to lipid ratio attenuated progressively, being zero at $n = 20$. This slow decline in disordering ability is not completely consistent with the sharp loss of potency at the cutoff, which is better explained by the limited membrane solubility of the higher alkanols. All of the unsaturated alcohols were more effective disorderers than their saturated analogues when compared at the same membrane concentration. The *cis*- and *trans*-isomers disordered EPC-cholesterol (1:1) bilayers approximately equally in accord with their equal anesthetic potencies; however, at low proportions of cholesterol to phospholipid, *cis*-isomers disordered more than *trans*-isomers. The alternative lipid model based on the lateral-phase separations in mixed DML-DPL bilayers was not supported by data for the *cis*- and *trans*-tetradecenols. Thus, 20 mole per cent in the lipid of the *cis*-isomer lowered the midpoint temperature of the two-phase region, whereas the *trans*-isomer raised this temperature.

INTRODUCTION

The mechanism of action of general anesthetics is ill-defined, but current theories may loosely be divided into two classes. Either anesthetics interact directly with nonpolar sites on an excitable protein, or they dissolve in the lipid bilayer region of a membrane and perturb the lipid environment of an excitable protein. With respect to the protein hypothesis, the enormous structural diversity of the general anesthetics would seem to preclude specific interactions with a *single* binding site as being the primary molecular event. If such specific interactions were to occur with a membrane-bound protein, one might instead expect that they would confer a degree of specificity, such that different structural classes of general anesthetics would require separate binding sites (1–4). On the other hand, the lipid hypothesis is a unitary one, since it correlates the potency of a wide range of anesthetics with their lipid solubility (3, 4). Thus general anesthesia occurs when a critical concentration of any compound is achieved in the lipid. Current work on the lipid hypothesis centers around the question of what is the physiologically important perturbation caused by these anesthetics once they are in the bilayer. The correlation between lipid solubility and anesthetic potency is so good that further progress in this direction can best be obtained by examining apparent exceptions to the lipid solubility correlation. Thus, the observation that

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helium reverses anesthesia is inconsistent with the solubility hypothesis (3) but supports the modified lipid theories based on membrane expansion (3–5), lipid disorder (6), or lipid phase transitions (7).

Another example of such specificity, and the one we are concerned with here, is the so-called anesthetic cutoff phenomenon (4, 8). For example, within the homologous series of saturated aliphatic *n*-alcohols, anesthetic potency (expressed as alcohol concentration in the aqueous phase) increases progressively with chain length and is maximal for $n = 12$, after which point successive alcohols are devoid of anesthetic activity, despite the fact that those physicochemical properties, such as lipid solubility, which are correlated with potency are thought to vary in a continuous and monotonic fashion throughout the series. Thus this cutoff in potency of the higher alcohols is apparently inconsistent with the lipid solubility correlation. Ferguson (8) suggested that as alcohols became more like the components of cell membranes their anesthetic potency (at equal membrane concentrations) would decrease. Today, some 40 years later, two classes of theories based on a lipid bilayer, which echo this sentiment, apparently can account for the cutoff. These theories are based on lipid disorder and on lipid phase transitions.

It was first shown that in the series from ethanol to octanol, perturbations measured by spin probes in lipid bilayers correlate well with physiological potency (9). Subsequently, it was demonstrated that, whereas octanol could cause large changes in order parameter in lipid bilayers, hexadecanol at similar concentrations produced much less disorder (10).

The more recent phase transition theories of Trudell *et al.* (7) and Lee (11) might also account for the cutoff, because short-chain normal alcohols lower the phase transition of saturated lecithins, whereas the long-chain alcohols elevate it (12).

Recently, however, the apparent success of the lipid bilayer theories in accounting for the cutoff has been challenged (13). Thus, in contrast to previous work (10), a variety of spin-labeled phospholipids in a number of different lipid bilayers reported that alkanols with more than 10 carbon atoms decreased, whereas those with fewer than 10 carbon atoms increased, membrane fluidity. (The ratio of the low-field to mid-field peak height was "used as an arbitrary measure of bilayer fluidity" (13); order parameters were not reported.) Similar differential effects were observed with shifts in lipid phase transition temperature, these results being consistent with previous ones (12). The effects of pairs of short- and long-chain alcohols on bilayers were additive, whereas the ability of the short-chain alcohols to block nerves was not reduced in the presence of long-chain alcohols. Also, the long-chain alcohols up to dodecanol produced nerve block, so that there was a poor correlation between the position of the cutoff and the spectroscopic changes.

In this paper we examine more systematically than before the ability of long-chain alcohols to change the order parameter in lipid bilayers and compare this with the position of the cutoff for general anesthesia in the *n*-alkanols, *cis*-alkenols, and *trans*-alkenols. Our data are consistent with those of previous workers (10). No marked ordering effects were found in the bilayers we examined. The difference between this work and that reported in ref. 13 may reflect the different bilayers and labels used. Finally, we found that alcohols just above the cutoff are not partial anesthetics (10), so that the loss in potency is well-defined rather than the gradual decline suggested by our spectroscopic data. The cutoff itself is better explained by a rapid decrease, to below anesthetic levels, of the attainable alcohol concentration in the bilayer than it is by a decline in the longer alcohols' ability per mole to perturb the bilayer.

MATERIALS AND METHODS

Early tadpoles were obtained from Carolina Biological Supply Company, Burlington, N. C. EPC[3] (grade 1) was purchased from Lipid Products, Surrey, United Kingdom, and the synthetic lipids DML and DPL were obtained from Calbiochem, La Jolla, Calif., and Grand Island Biological Company, Grand Island, N. Y., respectively. All of the alcohols used in this study were obtained from Applied Science Laboratories, State College, Pa., and were used without further purification. Cholesterol (Sigma Chemical Company, St. Louis, Mo.) was recrystallized from methanol before use. The spin-labeled fatty acid $CH_3(CH_2)_5C(doxyl)(CH_2)_{10}COOH$:I(5,10) was purchased from Syva, Palo Alto, Calif.; I(10,3) and I(7,6) were synthesized according to the method of Hubbell and McConnell (14). The phospholipid spin label PC(7,6) was synthesized via I(7,6), and lysolecithin by the method of Boss *et al.* (15). TEMPO was prepared by the method of Rozantsev and Neiman (16).

The general anesthetic potency of the alcohols was determined as previously described (17), using the loss of righting reflex of tadpoles as the criterion of anesthesia. For each alcohol, the number of tadpoles anesthetized was determined at different concentrations, and ED_{50} values were calculated from the dose-response data by the statistical treatment of Waud (18).

Lipid-disordering measurements were carried out on a Varian E-109 spectrometer, and order parameters were calculated according to the method of Hubbell and McConnell (14). Samples were prepared by evaporating methanolic solutions of spin label, EPC-cholesterol (either 2:1 or 1:1), and appropriate alcohol [arachidyl alcohol ($C_{20:0}$) was dissolved in acetone]. The resulting thin films were placed under high vacuum for at least 2 hr and then dispersed in 0.9% NaCl solution or Tris buffer (pH 7.4) with vigorous agitation for 2 min followed by bath sonication for 15 min. The dispersions were shaken overnight at room temperature to allow for equilibration. Electron spin resonance spectra were recorded at 21°, and three scans per sample were averaged. Duplicate samples were usually run in each experiment, and at least two separate experiments performed in each case.

[3] The abbreviations used are: EPC, egg lecithin; DML, dimyristoylphosphatidylcholine; DOL, dioleoylphosphatidylcholine; DPL, dipalmitoylphosphatidylcholine; doxyl, 4,4-dimethyloxazolidine-*N*-oxyl; PC, (7,6), 1-acyl-2-(8-doxyl)palmitoylphosphatidylcholine; TEMPO, 2,2,6,6,-tetramethylpiperidine-1-oxyl. I(n,m) is the spin label $CH_3(CH_2)_nC(doxyl)(CH_2)_mCOOH$. $C_{n:0}$ and $C_{n:1}$ denote normal alkanols and alkenols with one double bond, respectively, where n is the number of carbon atoms.

Standard deviations are those between separate samples.

For the phase transition experiments, samples were prepared by evaporating methanolic solutions of DPL-DML (1:1) containing 100 mg of lipid, and either *cis*- or *trans*-Δ^9-tetradecenol. An aliquot of 5 mM TEMPO (200 μl) in distilled water was added, together with 700 μl of 10 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl. Final concentrations were as follows: phospholipid, 10% w/v; TEMPO, 1 mM, and unsaturated alcohol, 20 mole per cent with respect to the lipid mixture. The method of Shimshick and McConnell (19) was used to determine the phase transition temperatures (T_m) of the dispersions; a full description of our experimental procedure has been reported previously (20).

RESULTS

The general anesthetic potencies of all of the alcohols studied are shown in Table 1. Data for the Δ^9-tetradecenols were taken from a previous publication (17). *n*-Dodecanol was the most potent of the alcohols tested, with an ED_{50} of 5.4×10^{-6} M, which is in close agreement with an earlier estimate (21). *n*-Tridecanol was found to be a partial anesthetic with an ED_{50} concentration of 3.7×10^{-5} M; only 70% of the tadpoles were anesthetized even in a saturated solution. *n*-Tetradecanol was totally without anesthetic activity, even at low temperature, and in mixtures it did not alter the ED_{50} concentration of *n*-octanol. Each of the tetradecenols had an ED_{50} of 3×10^{-5} M, which lies between those of octanol and decanol. The Δ^9-hexadecenols were partial anesthetics capable only of anesthetizing one-fourth of the exposed population at saturation. The potency of the *cis*- and *trans*-isomers were identical for both alkenols.

The effects of the long-chain alcohols C_{10}—C_{20} on the order of EPC containing 33 mole per cent of cholesterol were examined in liposomes which were spin-labeled with the fatty acid I(10,3). At 21° the mean value for the control order parameter was 0.666 ± 0.004 S.D. ($n = 8$). Figure 1 is a plot of the order parameter (ΔS) as a function of alcohol chain length. All of the alcohols were incorporated at 25 mole per cent with respect to phospholipid plus cholesterol. Lawrence and Gill (10) reported a change of −0.025 in ΔS for tetradecanol, a result comparable to ours, although they used a DPL-cholesterol (1:1) bilayer and PC (7,6) as label. The ability of the *n*-alkanols to disorder the bilayer decreased, on average, linearly from decanol to arachidyl alcohol ($C_{20:0}$) ($p < 0.05$), which caused insignificant ordering. Although the trend of increasing disorder with decreasing n is not dramatic in this range, it becomes more so when data in the literature for the lower alcohols at the same membrane concentration are considered. Thus, for octanol, Lawrence and Gill (10) found that the order parameter changed −0.09. For shorter-chain alcohols it becomes progressively necessary to correct for the proportion of alcohol not in the bilayer because of the decreasing partition coefficient. When this is done for ethanol and butanol, the order parameter changes at 25 mole per cent are −0.05 and −0.07, respectively (22). These values probably underestimate the size of the change because of uncertainties in the partition coefficients used in the calculations (22). Thus in a general way, as has been noted before (10), the ability of the alkanols to disorder bilayers tends to decrease with increasing chain length, but our results show there is no dramatic change in disordering ability at any chain length as compared with the cutoff (Table 1).

TABLE 1

Anesthetic potency of alcohols in tadpoles at room temperature

Alcohol	C_n	ED_{50} ± SE
		M
Ethanol	2	0.12 ± 0.01
Propanol	3	$5.4 \pm 0.6 \times 10^{-2}$
Butanol	4	$1.2 \pm 0.1 \times 10^{-2}$
Hexanol	6	$7 \pm 1 \times 10^{-4}$
Octanol	8	$6 \pm 0.9 \times 10^{-5}$
Decanol	10	$1.3 \pm 0.2 \times 10^{-5}$
Dodecanol	12	$5.4 \pm 0.9 \times 10^{-6}$
Tridecanol	13	$3.7^a \pm 2 \times 10^{-5}$
Tetradecanol	14	Inactive
Hexadecanol	16	Inactive
Eicosanol	20	Inactive
cis-Δ^9-Tetradecenol	14	$3 \pm 6 \times 10^{-5}$
trans-Δ^9-Tetradecenol	14	$3 \pm 1.5 \times 10^{-5}$
cis-Δ^9-Hexadecenol	16	$4.2^a \times 10^{-5}$ (ED_{25})
trans-Δ^9-Hexadecenol	16	$4.2^a \times 10^{-5}$ (ED_{25})
cis-Δ^9-Octadecenol	18	Inactive
trans-Δ^9-Octadecenol	18	Inactive
cis-Δ^{11}-Eicosenol	20	Inactive

[a] Partial anesthetic.

These results, and those of Lawrence and Gill (10), tend to conflict with those of Richards *et al.* (13), who found that the fluidizing effect of the alcohols changed sign at decanol. It is possible that our data differ because the spin labels of Richards *et al.* (13) were deeper in the bilayer. Therefore we examined the effect of 25 mole per cent of dodecanol on EPC-cholesterol (2:1) bilayers labeled with I(5,10); the alcohol changed the order parameter from 0.477 ± 0.009 to 0.482 ± 0.002 ($p = 0.1$). This compares with a change of −0.024 ($p = 0.006$) when I(10,3) was used. Thus the disordering effect of dodecanol disappears when the spin label is moved deeper into the bilayer. This observation and the different spectral parameter used may explain the discrepancies noted above, but since the data of Richards *et al.* (13) have been given only in a preliminary form, the discrepancies cannot be assessed in detail. However, unlike Richards *et al.* (13) we have seen no significant ordering effects of alcohols in this study, but neither have we used such high alcohol concentrations nor bilayers lacking cholesterol. Other evidence suggests the need to include cholesterol in lipid bilayer models of the anesthetic site (23).

Figure 1 suggests that the *cis*- and *trans*-alkenols should have different anesthetic potencies. This is in contradiction to previous work carried out in EPC-cholesterol (1:1) bilayers with PC (7,6) as label (17), where 11 mole per cent of both tetradecenol isomers caused equal disordering. At 20 mole per cent, however, the *cis*-isomer decreased the order parameter 0.016 ($p = 0.04$) more than the *trans*-isomer, results consistent with those in Fig. 1. A similarly weak difference had previously been observed in a cholesterol-free bilayer (20). If such differences are general they clearly challenge the disordered lipid hypothesis. To proceed further we chose I(7,6)

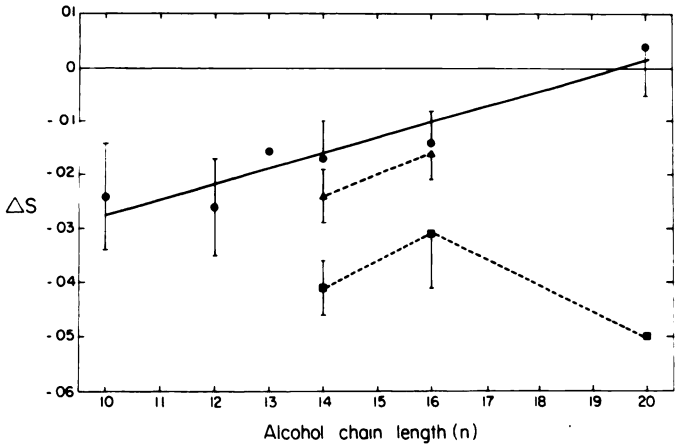

FIG. 1. *The change in order parameter (ΔS) reported by 5-doxyl-palmitic acid, I(10,3), incorporated (1 mole per cent) in EPC-cholesterol (2:1 molar ratio) liposomes in the presence of 25 mole per cent of various alcohols*

Mole per cent of alcohol is given by the equation 100 × [alcohol/(PC + cholesterol + alcohol)]. ●, Alkanols, $C_{n:0}$; ▲ and ■, *trans*- and *cis*-Δ^9-monoalkenols, $C_{n:1}$. The bars denote standard deviations.

because it revealed larger changes than either I(5,10) or I(10,3) when all fatty acids were tested against the disordering induced by *cis*-hexadecenol in EPC-cholesterol (2:1) (compare Fig. 1 and Fig. 2, - - -). Figure 2 shows that, by increasing the cholesterol content to 50 mole per cent, the difference between *cis*- and *trans*-hexadecenol can be suppressed and results consistent with the equal potencies of these isomers are obtained. Thus the rather small *cis-trans* differences observed in some bilayers are not general and do not provide grounds for rejecting the disordered lipid hypothesis.

In addition to accommodating the cutoff for the two isomeric alkenol series at equal values of *n*, the model should also account for the alkenol cutoff's being at a higher *n* than the alkanol's cut-off (Table 1). Consistent with this requirement, 20 mole per cent of hexadecanol change the order parameter by only −0.023 ± 0.010 under the conditions of Fig. 2.

The disordering effect of *cis*-Δ^{11}-eicosenol ($C_{20:1}$) was

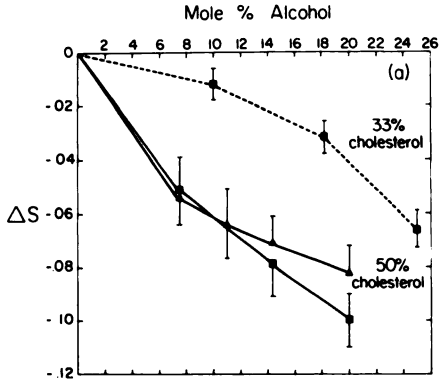

FIG. 2. *The change in order parameter (ΔS) reported by 8-doxyl-palmitic acid, I(7,6), incorporated (1 mole per cent) in EPC-cholesterol liposomes*

▲ and ■ indicate *trans*- and *cis*-Δ^9-hexadecenol. Molar ratio of lecithin to cholesterol: ——, 1:1; - - -, 2:1. The control ΔS value was 0.601 ± 0.009.

very much greater (ΔS = −0.05) than that of any of the other alcohols tested and represented in Fig. 1, although the compound showed no anesthetic activity when administered alone to tadpoles, nor any synergistic activity when administered in conjunction with an ED_{50} concentration of octanol. This anomaly requires an explanation. One attempt is given under Discussion.

The effect of *cis*- and *trans*-tetradecenols on the phase relationships of DML-DPL mixtures was assessed by examining a 1:1 molar mixture of these lipids with 20 mole per cent of alkenol incorporated. In the absence of alcohol, TEMPO reported a broad-phase transition region centered on 30.4°. Previous investigators (7, 19) have identified the upper and lower boundaries of this region as defining the fluidus and solidus regions of a two-phase mixture. In Table 2 we report both the boundaries and the midpoint of this region. *Trans*-tetradecenol raised the midpoint temperature and slightly broadened the two-phase region. On the other hand, the *cis*-isomer lowered the midpoint and markedly broadened the two-phase region. None of the data can be simply related to the equi-anesthetic potency of the isomers, although we did not extend these data to lower alcohol concentrations because in previous work with DPL the direction of the change in phase-transition temperature was independent of concentration in the range 5 to 33 mole per cent of alcohol (20).

DISCUSSION

In general, lipid theories of general anesthesia require that anesthetics both dissolve in and also perturb the lipid region of some membrane. Although the correlation between lipid solubility and potency of many anesthetics is clear, there is no agreement on the nature of the perturbation (24); yet it is at the level of this perturbation, and the coupling between it and protein function, that meaningful mechanisms of anesthetic action can be developed. The object of this study was to use the cutoff in anesthetic potency in the *n*-alkanol and the *cis*- and *trans*-alkenol series to test the lipid theories and to obtain some information on the nature of the above perturbation. We have tested specifically two hypothetical mechanisms of anesthetic action—those based on phase transitions and on lipid disorder.

Prior to this study, Lee (12) had examined the effects of long-chain *n*-alkanols on bilayers containing two saturated phospholipids to represent the lateral-phase separation model of anesthetic action proposed by Trudell *et al.* (7). Short-chain alkanols increased, whereas long-

TABLE 2

Change in boundary temperatures of the two-phase region of a DML:DPL (1:1) mixture produced by 20 moles per cent in the lipid of the geometric isomers of Δ^9*-tetradecenol*

The phase boundaries were defined using the spectral partition coefficient of TEMPO (see Materials and Methods).

	Solidus	Midpoint of region	Fluidus	Shift in midpoint	Change in width
Control	29.6°	30.4°	31.8°	0°	0°
trans-Isomer	29.8	31.5	32.7	+1.1	+0.7
cis-Isomer	22.9	26.5	29.0	−3.9	+3.9

chain alkanols decreased, the proportion of fluid phase lipid. The switchover between these two behaviors, which depended on the lipid phase under study, occurred at $n \simeq 10$–12. Contrary to these positive findings, our data for the *cis*- and *trans*-tetradecanols show that these two alcohols, which are equipotent as anesthetics (Table 1), shift the phase relations in DML-DPL (1:1) mixtures in opposite directions (Table 2). Thus the *cis*-tetradecenol increases the amount of fluid phase whereas *trans*-tetradecenol decreases it. Whether these isomers would exert similar effects on a more complex multiphasic lipid mixture cannot be predicted with certainty, but the model as formulated (7) is not consistent with the data we have presented. A third version of the phase transition theories has recently been proposed by Mountcastle *et al.* (25), who suggested that anesthetics broaden a phase transition region, thereby decreasing the size of the cooperative unit, or cluster, involved in it. In such cases potency would be related to broadening of the transition region, and the direction of the shift in transition temperature is not strictly relevant. Both of the equipotent isomers of tetradecenol did broaden the two-phase region we examined, but they did so by very different magnitudes (Table 2). Thus, although we cannot exclude the possibility that other specific models of these general theories might be more successful, our data highlight the inadequacy of the theories in their present formulation.

When we turn to considering the disordered lipid hypothesis of anesthetic action, the distinctions are not as clear-cut as those discussed above, and the arguments need to be approached in more detail. The ability of a perturber to disorder a membrane in which it dissolves may be expressed as disordering efficacy (23), E, where

$$E = \Delta S/[C]_m \quad (1)$$

and ΔS is the observed change in order parameter when the perturber is present in the membrane at concentration $[C]_m$. The hypothesis supposes that anesthesia occurs when some constant decrease in order parameter, ΔS^{an}, is achieved by dissolution of any agent in the membrane, where

$$\Delta S^{an} = E \cdot [C]_m{}^{an} = -E \cdot \lambda \cdot [C]_w{}^{an} \quad (2)$$

and the membrane-water partition coefficient $\lambda = [C]_m/[C]_w$. Thus a long-chain alcohol could fail to be an anesthetic either because E is small or negative or because an adequate $[C]_m{}^{an}$ cannot be achieved. Our data and those of previous work (10, 22) show that, in general, short-chain alkanols can cause marked disordering, whereas long-chain alkanols cause little or no disordering. Other studies suggest that E may even become negative in long-chain alcohols in some bilayers (20). In spite of this broad trend, which suggests that E decreases as n increases, the spectroscopic data fail to account for the steepness in loss of potency at the cutoff. For example, dodecanol is an anesthetic and tetradecanol is not, yet the ability of these two alcohols to disorder lipid bilayers is equal (Fig. 1). Since E is equal (Eq. 1) for these two alcohols, either the lipid theory must be rejected or the cutoff must be explained by the term $[C]_m$ or, equivalently, $\lambda \cdot [C]_w$.

Table 3 is a compilation of membrane-buffer partition coefficients and aqueous solubility data taken from several sources. Although the partition coefficients in the first column refer to different membrane systems, comparisons between biomembranes are probably correct to within a factor of 2–3 (4, 29), whereas the lipid bilayer values represent a factor approximately 5 higher than the biomembrane values (29). In the biomembranes the partition coefficient rises steadily with chain length up to dodecanol, but then the trend reverses and it falls off at tetradecanol. On the other hand, the saturated aqueous solubility falls steadily with chain length. The result of these two trends is that the maximal obtainable membrane solubility, $[C]_m{}^s$, declines gradually with increasing chain length, but between dodecanol and tetradecanol it suddenly falls dramatically from 120 to 2.9 mM. The last column shows that the membrane concentrations achieved during anesthesia are in the range of 10 to 30 mM, and it is immediately apparent that these concentrations are one order of magnitude higher than the maximal achievable membrane concentrations of the non-anesthetic alkanols above dodecanol. *The dramatic nature of the cutoff is thus adequately accounted for on lipid solubility arguments alone.*

Similar arguments probably apply to the dramatic

TABLE 3

Solubilities and saturated concentrations of alcohols in water and membranes

Alcohol	Membrane-buffer partition coefficient	Saturated aqueous concentration, $[C]_w{}^s$ (32)	Saturated membrane concentration, $[C]_w{}^s$	Membrane concentration at anesthesia $[ED_{50}]w \times \lambda$
	λ	*moles/liter*	*moles/liter*	*moles/liter*
Ethanol	0.14[a]; 0.2[b]	13.8	1.9; 2.8	0.016; 0.023
Butanol	1.5[a]; 3.2[b]	0.96	1.4; 3.1	0.017; 0.037
Hexanol	13[a]; 50[c]	0.061	0.79; 3.1	0.009; 0.035
Octanol	152[a]; 387[c]	4.5×10^{-3}	0.68; 1.7	0.009; 0.023
Decanol	1,222[a]; 1975[d]	3.16×10^{-4}	0.39; 0.62	0.016; 0.025
Dodecanol	5,365[d]	2.19×10^{-5}	0.12	0.029
Tetradecanol	1,975[d]	1.46×10^{-6}	0.0029	Not anesthetic
Hexadecanol	11,500[e]	1.7×10^{-7}	0.0020	Not anesthetic
Mean ± SD				0.022 ± 0.009

[a] Red blood cells (4).
[b] DML (28).
[c] EPC (27).
[d] Intestinal brush border membranes (30).
[e] EPC-cholesterol ratio 2:1 (K. W. Miller and M. J. Pringle, unpublished data).

nature of the cutoff in both the *cis*- and *trans*-alkenol series, but partition coefficients are not available to confirm this hypothesis. However, the facts that both isomeric series have the same cutoff and that this occurs at chain lengths three carbon atoms longer than those for the alkanols also require explanation. Partition coefficients of butanes (29) show that the addition of one double bond raises the bilayer-gas partition coefficient, λ, only slightly, but $[C]_w^s$ is increased substantially. This effect could account for the direction of the shift in cutoff length between the saturated and unsaturated series of alcohols. If λ for *cis*- and *trans*-alkenols is equal, as it is for *cis*- and *trans*-butene, then the equal potency of the isomers is also explained. However, the long-chain alkenols may tend to orient in the membrane, and the latter's anisotropic character could impose different partition coefficients. In such a case, disordering efficacy, E, might assume importance. (The *trans* form might have a greater λ, but a correspondingly smaller E, than the *cis* form). Whatever assumptions one makes about partitioning, E clearly must differ in some circumstances, since equal concentrations of the isomers disorder EPC-cholesterol 1:1 (Fig. 2) equally but EPC-cholesterol 2:1 (Fig. 1) unequally.

The results of Richards *et al.* (13) suggest that a negative value of E does occur for alkanols with $n > 10$. This led to the prediction that alcohols with negative E should reverse the anesthesia. The failure of this prediction led Richards *et al.* (13) to reject the lipid hypothesis. (Strictly speaking, Richards *et al.* presented their arguments in terms of fluidity, not order). The data in Table 3 suggest an alternative explanation of their data. The nonanesthetic long-chain alcohols with negative E cannot achieve a sufficiently high membrane concentration under physiological conditions to reverse, even partially, the effects of anesthetic alcohols.

The membrane-buffer partition coefficients of solutes in lipid bilayers are usually 2 to 5 times higher than those in biomembranes (Table 3; ref. 29). If this fact is taken into account, it is probable that most spectroscopic studies on alkanols for $n \geq 14$ have been carried out under supersaturated conditions. For the mono-unsaturated alkenols, the anesthetic data suggest that this will also be true when $n \geq 17$, but this possibility awaits confirmation. However, it seems certain that for $C_{20:1}$, the solubility limitations provide a sufficient explanation for lack of potency, and the anomalously large disordering effect seen in Fig. 1 must reflect a phase separation rather than a large value of E.

The last column in Table 3 shows that $[C]_m^{an}$ is a few mole per cent. At such concentrations, alcohols disorder bilayers little. This weakness of the disordered lipid hypothesis has been much discussed (see ref. 22 and references therein). Until it can be shown either that some bilayers of different lipid composition are more readily disordered (e.g., compare the two bilayers in Fig. 2) or that proteins very sensitive to small changes in order exist, the disordered lipid hypothesis must be regarded with reservation. Our studies have shown that the hypothesis can clearly be ruled out in bilayers low in cholesterol, where some anesthetics order (23, 26), but not in bilayers high in cholesterol (22). The success of lipid *solubility* alone in explaining the cutoff in potency of the alkenols, the alkanes (31) and the hydantoins (22) means that the cutoff yields no further information on what lipid perturbation is important to anesthetic action. The *cis*- and *trans*-alkenol series may yield some such information, but more detailed work is needed.

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