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THE MEMBRANE CONCENTRATIONS OF ALCOHOL ANESTHETICS

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SUMMARY

1. This work provides evidence for the Overton–Meyer partition theory of anesthesia. This theory states that the cell membrane anesthetizing concentration is of the order of 0.03 molal (moles of anesthetic per kg membrane). The membrane concentrations found experimentally for alcohols ranged from 0.01 molal for decanol to 0.04 Molal for pentanol in hemoglobin-free erythocyte ghost membranes at concentrations which are 25% effective in protecting erythocyte membranes, and which are also effective in stabilizing nerve fibers. Since the membrane concentration decreased linearly with chain length by a factor of about one-third on going from pentanol to decanol, the pharmacological intrinsic efficacy of decanol (in the membrane phase) is 3.2 times greater than that of pentanol.

2. The decrease in membrane concentration ($c_{membrane}$) with increasing chain length of the alcohols indicated that some size-dependent parameter of the anesthetic was important in determining the final amount of membrane stabilization. MULLINS[19] had predicted that such a parameter should exist and suggested that it is the volume of the anesthetic molecule, V_{mol}. The data support this insofar as $V_{mol} \cdot c_{membrane}$ is almost the same for all the alkanols. The data also support, however, a new hypothesis which states that the membrane concentration should be corrected by ΔF, the free energy of binding, since this parameter is possibly the most general index of the "extensive" influence of the anesthetic in the drug–receptor interaction. The value for $\Delta F \cdot c_{membrane}$ was of the order of 60 cal/kg membrane for each alkanol.

3. The mean free energy of alcohol adsorption was -695 ± 81 (S.E.) cal/mole of methylene groups, indicating that the membrane–alcohol interaction was hydrophobic.

4. Assuming an unlimited number of membrane binding sites, the membrane/buffer partition coefficients could be averaged; the mean values were 3.4 for pentanol, 39 for heptanol, 152 for octanol, 582 for nonanol, and 1226 for decanol. Assuming there was only a finite number of binding sites, the mean for the maximum number of alcohol binding sites was computed to be 65.5 mmoles of alkanol per kg of dry membrane.

INTRODUCTION

The classical partition theory of anesthesia[1,2] predicts that the concentration of anesthetic in the cell membrane is of the order of 0.03–0.06 molal (moles of anes-

thetic per kg of membrane). It has recently been found[3] that the membrane concentrations for one local anesthetic overlaps this range, 0.037–0.08 mole of chlorpromazine per kg of dry erythrocyte membrane, at concentrations which anesthetize nerve fibers and stabilize cell membranes. The present paper extends the study to include the alcohol anesthetics.

The erythrocyte membrane is a useful and quantitative model for studying the effects of anesthetics on membranes in general, as discussed previously[3]. One of the main reasons is that anesthetics protect erythrocytes at concentrations virtually identical to those which anesthetize nerve fibers[4,5]. All lipid-soluble anesthetics tested so far have this erythrocyte-protecting effect[6–8]. Anesthetics reduce the membrane viscosity of erythrocyte ghosts, myelin, and synaptosomes at identical concentrations[9,10]. Although there are a number of studies of drug binding to membranes, very little information is available on the binding of anesthetics to membranes[3,9].

The present results indicate that the membrane concentrations of the alcohol anesthetics are also of the order predicted by the Overton–Meyer partition theory. The results indicate, moreover, that the amount of anesthetic in the membrane varies with chain length; the anesthetizing membrane concentration of decanol was found to be one-third that of pentanol. The free energy of interaction was found to be about −695 cal/mole CH_2 groups which is in agreement with a predicted value of −685 cal/mole if the interaction was controlled by hydrophobic interactions[11].

METHODS

Preparation of erythrocyte ghost membranes

Erythrocyte ghost membranes, which were free of hemoglobin, were prepared by the method of DODGE *et al.*[12] with minor modifications, as previously described by KWANT AND SEEMAN[3]. The final ghost suspension consisted of 1–2 g of dry ghost per 100 ml of 10 mM sodium phosphate buffer (pH 7.0); the dry weight of membranes was corrected for the dry weight of the buffer salts[3].

Determination of the membrane concentration of alcohols

The uptake of alcohols by erythrocyte membranes, and the membrane concentrations calculated from these uptake values, were determined as follows:

(1) Using a 200 μl Eppendorf pipette (Brinkmann Instruments, U.S.A.), an aliquot of the alcohol solution was delivered into a tared weighing bottle and the sample weighed to within 0.05 mg on a Stanton unimatic digital CL-5D balance. The alcohol solution contained both radioactive and nonradioactive alcohol molecules and was made up as follows: (a) 100 μC of each ^{14}C-labeled alkanol (specific activities were 2.1 mC/mmole for pentanol, 1.4 mC/mmole for hexanol, 3 mC/mmole for heptanol, 3.5 mC/mmole for octanol, 2 mC/mmole for nonanol and 2.61 mC/mmole for decanol), as received from the supplier (see MATERIALS), was dissolved directly and without any further purification into 4 ml of ethanol. (b) The ethanolic solutions were then diluted down to 1 μC/ml using 10 mM sodium phosphate buffer pH (7.0). (c) 2-ml aliquots (weighed to within 0.05 mg) of the 1 μC/ml solutions were mixed with equal volumes of nonradioactive solutions containing alcohols in varying concentrations in 10 mM sodium phosphate buffer (pH 7.0). These were the final mixtures from which 200 μl was taken (see above). The exact concentrations of radioactive and

nonradioactive alkanol in these final mixtures were calculated precisely using the weights of the 2-ml aliquots. (d) As a result of all the dilutions the final concentration of ethanol in the adsorption test-tube was 0.4%. For three compounds (pentanol, hexanol and octanol) the original radioactive material supplied by the company was also dissolved in 10 mM sodium phosphate buffer (pH 7.0) without using any ethanol. Identical results were obtained.

(2) An aliquot of 0.5 ml of the erythrocyte ghost suspension was now weighed into the weighing bottle. The bottle was closed and rotated by hand approx. 20–30 times. The mixed contents were transferred to a 10 mm × 75 mm pyrex glass test tube using a pasteur pipette (glass). The test tube was covered with "Parafilm" and left at room temperature for a further 10–15 min. Alkanols and related acids are known to diffuse extremely rapidly across the erythrocyte membrane, coming to equilibrium within seconds or a minute at most[13,14].

(3) The 10 mm × 75 mm test tubes with ghosts and appropriate controls (no ghosts) were centrifuged at 36900 × g for 20 min at 22° in a temperature-controlled centrifuge and using rubber adapters to cushion the tubes. The supernatants were removed with glass pasteur pipettes and placed into shell vials (2 cm × 7 mm diameter) which were then stoppered with corks.

(4) Using a 50-μl Eppendorf pipette (Brinkman Instruments) duplicate or triplicate aliquots of the supernatants were added to liquid scintillation counting vials. Each aliquot was weighed to within 0.05 mg. 10 ml of liquid scintillation solution according to BRAY[15] were then added. The samples were counted in a Packard Tri-Carb scintillation spectrometer to a standard deviation of 1% or less.

Method of calculating the membrane concentration of the alcohols

The uptake of alkanol by the membrane was expressed in terms of molality in the membrane phase. These membrane molalities were calculated as follows. An aliquot of 200 μl of each stock solution (of concentration c_{stock}) was put into the tared weighing bottle and weighed as V_{stock}. The number of moles of alcohol which had been added to each weighing bottle was calculated according to Eqn. 1.

$$M_{total} = c_{stock} \cdot V_{stock} \quad (1)$$

The specific activity of each alcohol stock solution, S_{stock}, was obtained by Eqn. 2.

$$S_{stock} = c^*_{stock}/c_{stock} \quad (2)$$

where c^*_{stock} is the radioactive concentration of the alcohol in the stock solution, and obtained by pipetting a weighed aliquot of the stock solution directly into a liquid scintillation vial; the units of c^*_{stock} are in disint./min per ml. The experimental values of the radioactive concentration of alcohol in the supernatant of each adsoption tube was c^*_{free}. The alcohol concentration in the supernatant, c_{free}, was calculated by Eqn.

$$c_{free} = c^*_{free}/S_{stock} \quad (3)$$

The weights of the entire contents in each weighing bottle (V_{total}) and the dry weight (W_{dry}) of the ghost membranes added to each weighing bottle were recorded.

The number of moles of alcohol adsorbed to the combined glassware of the weighing bottle, the pasteur pipette and the adsorption tube, M_{glass}, in the presence of c_{free} was taken from Fig. 1, where the values for M_{glass} at various values of c_{free}

are presented. These values for the glass isotherms of Fig. 1 were obtained by replacing the ghosts with buffer solution. When ghosts were used, the value for M_{glass} (at the c_{free} observed) was taken directly from Fig. 1, using a WANG electronic computer, Model 370.

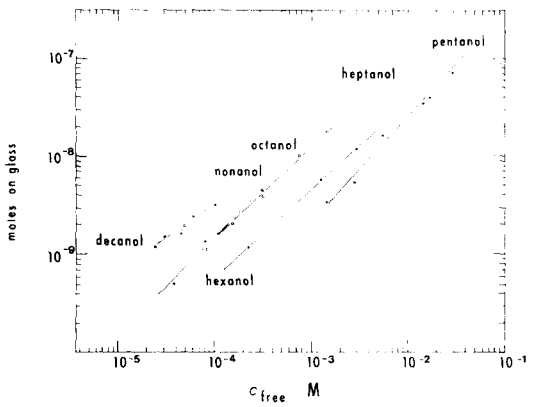

Fig. 1. The adsorption of *n*-alkanols to the glassware used in the experiment.

The number of unadsorbed moles of alcohol remaining free in the supernatant of each adsorption tube, M_{free}, was calculated by Eqn. 4.

$$M_{free} = c_{free} \cdot (V_{total} - W_{dry}/d) \tag{4}$$

where d is the density of the dry membrane. Although the value for d is probably somewhere between 1.1 and 1.2 (see ref. 3 for discussion and references), the value for d was taken as 1.0 in the present study; the error introduced was negligible because the values for W_{dry} were very low.

The number of moles of alcohol adsorbed to the membranes in each tube, $M_{membrane}$, was then calculated by Eqn. 5.

$$M_{membrane} = M_{total} - M_{glass} - M_{free} \tag{5}$$

The molal concentration of alcohol in the membrane, $c_{membrane}$, was then computed by Eqn. 6.

$$c_{membrane} = M_{membrane}/W_{dry} \tag{6}$$

The membrane/buffer partition coefficient for alcohol, P, was derived by Eqn. 7.

$$P = c_{membrane}/c_{free} \tag{7}$$

Every determination was carried out in duplicate or triplicate, agreement being better than 3%.

Evaluation of binding parameters

In evaluating the membrane concentration and confidence limits at a particular anesthetizing alcohol concentration, as well as the free energy of the interaction, it is convenient to have an analytical expression relating bound to free alcohol concentration. Two models for the binding process which yield such expressions were considered. (1) The membranes contain either a finite number of independent sites

(finite site model) or (2) the membrane contains an infinite number of binding sites (partition model). The finite-site model is characterized by an equilibrium constant, K, and a total number of binding sites, n. The partition model is characterized primarily by a partition coefficient, P. Efforts to evaluate K and n were made using the double reciprocal and also the Scatchard method.

In the double reciprocal method the reciprocal of the concentration of bound alcohol is plotted against the reciprocal of the corresponding concentration of free alcohol[3,11,16]. The equilibrium constant and total number of binding sites are then computed from the slope and intercept, according to Eqn. 8 (see ref. 17).

$$\frac{1}{c_{\text{membrane}}} = \frac{1}{K \cdot n}\left(\frac{1}{c_{\text{free}}}\right) + \frac{1}{n} \tag{8}$$

In the Scatchard method the ratio $c_{\text{membrane}}/c_{\text{free}}$ is plotted as a function of c_{membrane}, and K and n evaluated from the slope and intercept using Eqn. 9 (see ref. 17).

$$\frac{c_{\text{membrane}}}{c_{\text{free}}} = K \cdot n - K c_{\text{membrane}} \tag{9}$$

K may be defined as[11]

$$K = \frac{\Theta}{(1-\Theta)c_{\text{free}}} \tag{10}$$

where Θ is the fraction of receptor sites occupied by the alcohol.

Partition coefficients were evaluated in two ways, (i) assuming that they did not vary with c_{free} or c_{membrane} (Eqn. 11), and (ii) assuming that they were a linear function of c_{free} (Eqn. 12).

$$P = \frac{c_{\text{membrane}}}{c_{\text{free}}} = D \tag{11}$$

$$P = A + BC_{\text{free}} \tag{12}$$

where A, B and D are constants.

It is possible to distinguish between the partition model and the finite-number-of-sites model according to the graphical results of the experimental data. If the partition model is correct, the following three graphical points will be observed: (1) The line of the adsorption isotherm extrapolates to the origin when the double reciprocal plot is used. (2) When the Scatchard plot is used, the slope (K) will be zero if Eqn. 11 holds. (3) If P increases with c_{membrane} (*i.e.* $P = E + Fc_{\text{membrane}}$, where E and F are constants and positive), then n will have a negative value.

The regression parameters for the various plots were estimated using the least mean squares method. The variances and confidence limits for parameters computed using these regression parameters were evaluated using standard methods[18].

Evaluation of free energy of binding

The standard free energy of binding was evaluated as

$$\Delta F^\circ = -RT \ln K \tag{13}$$

or as

$$\Delta F^\circ = -RT \ln P \tag{14}$$

The free energy per mole of CH_2 groups was evaluated from the slope of a plot of ΔF *versus* the number of carbon atoms of each alcohol, in accordance with Eqns. 15 and 16.

$$\Delta F^{\circ}_{CH_2} = RT \ln \frac{K_i + 1}{K_i} \tag{15}$$

or

$$\Delta F^{\circ}_{CH_2} = RT \ln \frac{P_i + 1}{P_i} \tag{16}$$

where i and $i + 1$ refer to successive members of a homologous series.

MATERIALS

1-Butanol, 1-pentanol and 1-octanol were obtained from Fisher Scientific Co. 1-Hexanol (practical), 1-heptanol, 1-nonanol (practical), and 1-decanol were from Eastman Organic Chemicals, New York. [1-^{14}C]Butanol, and [1-^{14}C]pentanol were supplied by New England Nuclear Corp., Mass., U.S.A. [1-^{14}C]Hexanol, [1-^{14}C]heptanol, [1-^{14}C]octanol, [1-^{14}C]nonanol and [1-^{14}C]decanol were supplied by Mallinckrodt Nuclear, Orlando, Fla. The specific activities of these compounds are listed in METHODS. All compounds were used as obtained from the supplier. It was later observed by distillation that at least 30% of the hexanol was impure; because of this large impurity all the results for hexanol are omitted except those shown in Fig. 3.

RESULTS

Binding parameters

Binding data for the alcohols are shown in Figs. 2–4 where the data are plotted according to the double reciprocal method. The values of K and n obtained from these plots and from Scatchard plots (not shown) are summarized in Table I.

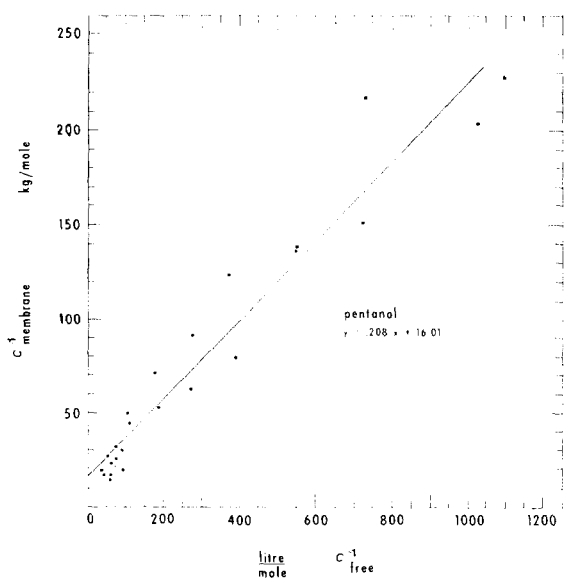

Fig. 2. The adsoption of pentanol to erythrocyte ghost membranes.

It was not possible to distinguish statistically between the finite-number-of-sites model and the partition model from these plots. The 95% confidence limits for K obtained using the Scatchard method included zero for all alcohols except octanol (and it was always negative for octanol). In addition, the 95% confidence limits for n obtained with the Scatchard method included negative values. With the double reciprocal plot, the standard error for the intercept ($1/n$) for two out of the five alcohols

TABLE I

AFFINITY CONSTANT, K, AND TOTAL NUMBER OF BINDING SITES OF ALCOHOL IN THE MEMBRANE, n

Alcohol	*Double reciprocal plot*		*Scatchard plot*	
	K (*l/mole*)	n (*mmoles/kg membrane*)	K (*l/mole*)	n (*mmoles/kg membrane*)
Pentanol	77.18 ± 55.4*	62.5	17.06 ± 20.74*	225 ± 306*
Heptanol	560.9 ± 117.1	80.1	149.5 ± 239.4	275 ± 462
Octanol	1189 ± 2524	127.4	−4729 ± 3308	−301 ± 238
Nonanol	37290 ± 23250	22.3	2772 ± 8835	217 ± 717
Decanol	47790 ± 79300	35.3	3093 ± 46850	406 ± 6.29
	Mean	65.5		

* 95% confidence limits.

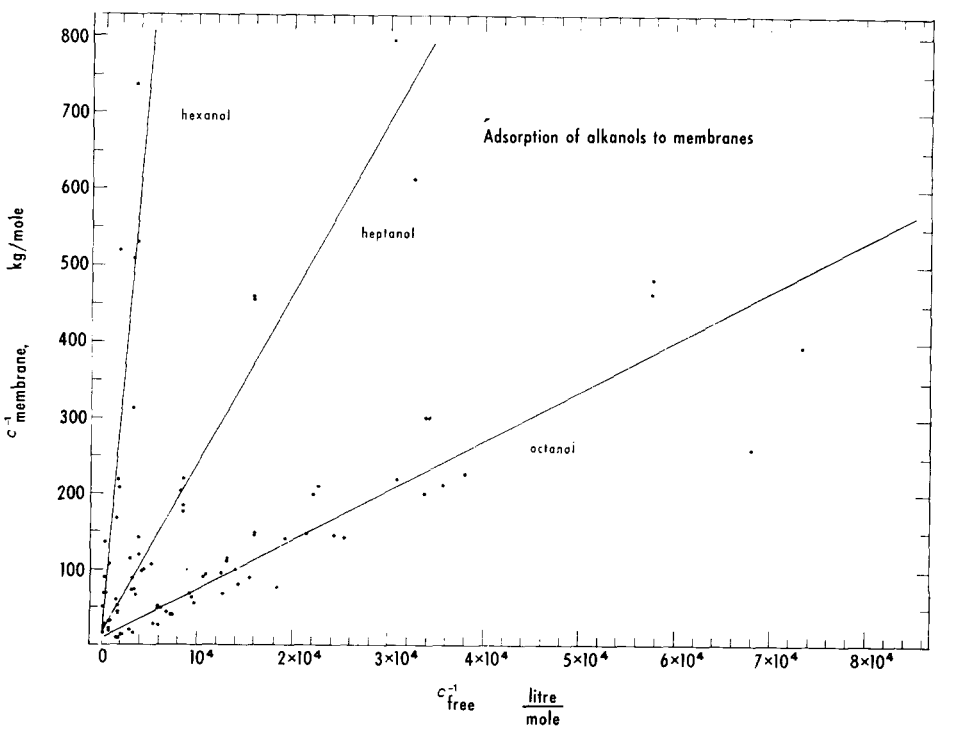

Fig. 3. The adsoption of hexanol, heptanol and octanol to erythrocyte ghost membranes.

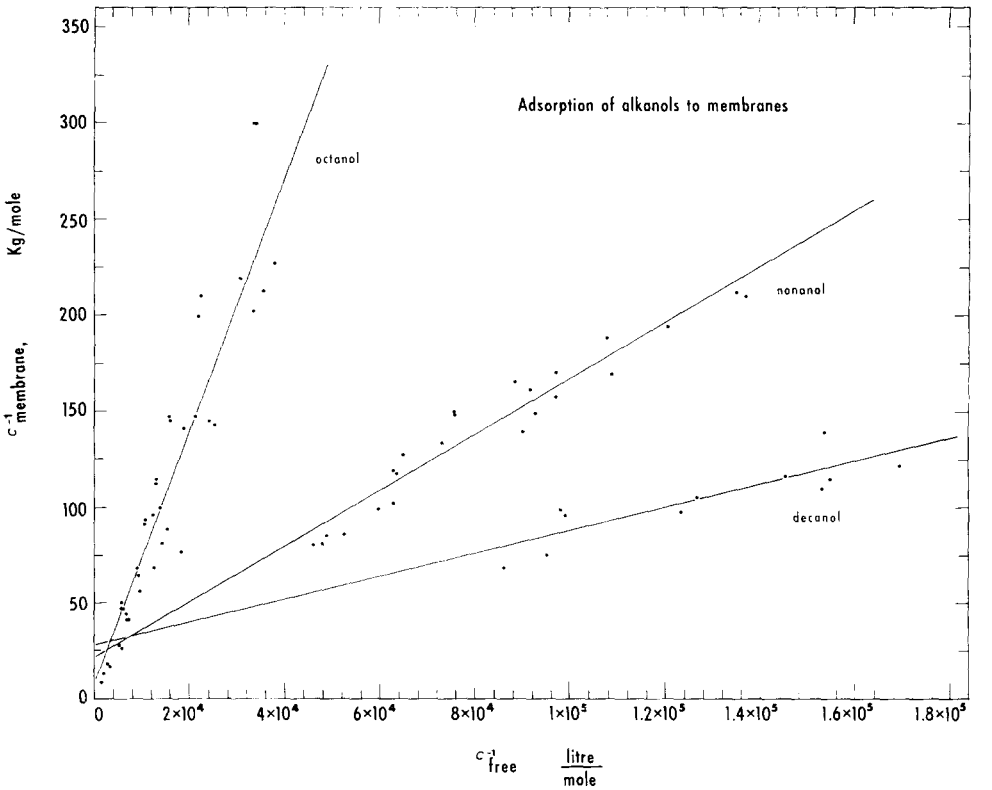

Fig. 4. The adsorption of octanol, nonanol and decanol to erythrocyte ghost membranes.

TABLE II

MEMBRANE/BUFFER PARTITION COEFFICIENTS

Alcohol	c^{E25}_{free} (*moles/l*)	*P* (*at 25% effect*) *when* $P \propto c_{free}$ $\left(\frac{moles/kg\ membrane}{moles/l\ solution}\right)$	*P* (*when P is independent of* c_{free}) $\left(\frac{moles/kg\ membrane}{moles/l\ solution}\right)$
Pentanol	$1.25 \cdot 10^{-2}$	3.118 ± 0.181 *	3.39 ± 0.426 *
Heptanol	$7.8 \cdot 10^{-4}$	35.41 ± 2.01	39.01 ± 2.91
Octanol	$1.83 \cdot 10^{-4}$	156.04 ± 5.03	151.84 ± 9.94
Nonanol	$3.4 \cdot 10^{-5}$	500.78 ± 47.4	582.06 ± 20.07
Decanol	$1.19 \cdot 10^{-5}$	1102.4 ± 80.06	1226.31 ± 92.13

* 95% confidence limits

(heptanol and octanol) included zero. When the corresponding 95% confidence limits were computed, those for heptanol, octanol and decanol included zero.

This behavior is not consistent with the finite site model, but could result if the partition model provided the correct description of the binding process. The partition

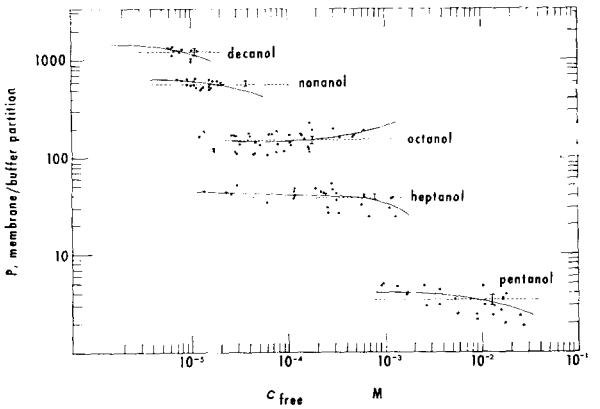

Fig. 5. The membrane/buffer partition coefficients (P) for n-alkanols in erythrocyte ghost membranes. The vertical lines represent the c_{free}^{E25} values which are approximately half the minimal blocking concentrations for the nerve fibers. The dashed line represents the equation $P = Kc_{free}$. The solid line was computed from the equation $P = A + Bc_{free}$.

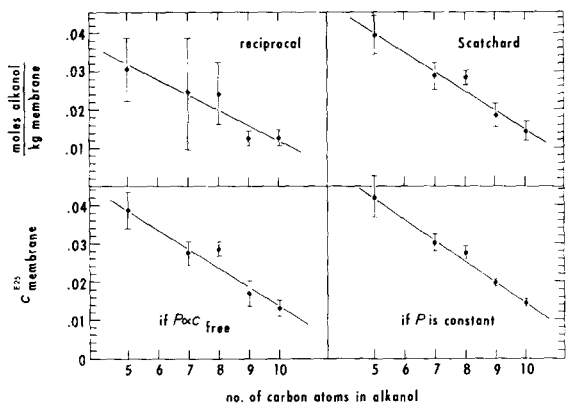

Fig. 6. The concentrations of alkanols in the erythrocyte ghost membranes ($c_{membrane}^{E25}$) at the c_{free}^{E25} for each alkanol. The $c_{membrane}^{E25}$ values are either directly from the isotherms or are calculated from partition coefficients.

coefficients evaluated according the Eqns. 10 and 11 are summarized in Table II. Fig. 5 describes the binding behavior according to the partition model.

Relation between anesthesia and the membrane concentration of alcohol

The Overton–Meyer partition theory of anesthesia predicts that the anesthetizing concentration in the membrane should be of the order of 0.015 molal for 25% effect, 0.030 Molal for 50% effect, and 0.06 molal for 100% effect[1]. This prediction was tested by comparing the membrane concentrations of the alcohols at the c_{free}^{E25} (the alcohol free concentration which reduces osmotic hemolysis from the control value of 60% to 45%; see refs. 11, 4–8).

The membrane concentrations at these equi-active c_{free}^{E25} values were computed from the four analytical expressions for the binding behavior and are presented in Fig. 6 and Table III. The 95% confidence limits are included for each $c_{membrane}^{E25}$

TABLE III

THE MEMBRANE CONCENTRATIONS AT 25% EFFECT, $c^{E25}_{membrane}$ (mmoles/kg MEMBRANE)

Alcohol	*Reciprocal*	*Scatchard*	*If $P \propto c_{free}$*	*If P is constant*
Pentanol	30.7 ± 8.4	39.5 ± 5.1	38.9 ± 4.7	42.4 ± 5.3
Heptanol	24.4 ± 14.6	28.9 ± 3.4	27.6 ± 3.2	30.4 ± 2.3
Octanol	22.8 ± 7.9	28.5 ± 1.9	28.6 ± 1.8	27.7 ± 1.8
Nonanol	12.5 ± 2.1	18.7 ± 3.1	17.0 ± 3.4	19.8 ± 0.7
Decanol	12.8 ± 2.2	14.4 ± 2.6	13.1 ± 2.2	14.6 ± 1.1

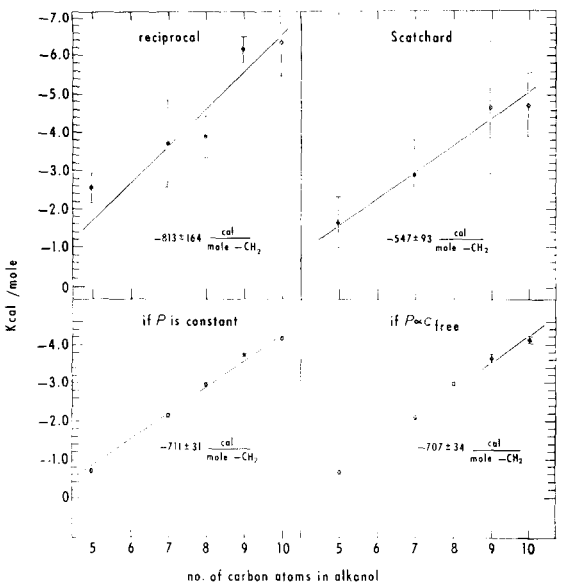

Fig. 7. The free energies of adsorption for *n*-alkanols on erythrocyte ghost membranes.

value. The c^{E25}_{free} values employed are indicated in Fig. 5 by the position of the vertical bars.

The OVERTON–MEYER prediction could not be tested adequately at concentrations above the c^{E25}_{free} (*e.g.* c^{E50}_{free}) because of the difficulty in obtaining binding data in the appropriate region of the isotherms.

Free energy of binding

Values for the free energy of adsorption for each alcohol, and the linear increase in free energy with chain length is shown in Fig. 7. The mean value (± 95% confidence limits) for ΔF was −695 ± 81 cal/mole CH_2.

DISCUSSION

The data for the membrane concentrations of alcohols as a function of chain length (Table III and Fig. 6) clearly show the following two points:

(1) The anesthetizing concentrations in the membrane are of the order of 0.015–0.030 molal, as predicted by the Overton–Meyer theory.

(2) The membrane concentration at c_{free}^{E25} decreases with chain length in a linear manner. The membrane concentration extrapolates to zero at a chain length of 13 carbon atoms.

The conclusions are evident with all of the four methods used to treat the binding behavior. (The second conclusion had been made in a previous study, but only on the basis of indirect data[4].)

MULLINS[19] modified the classical Overton–Meyer theory as follows: equal anesthetic effects should occur at equal volume fractions of the anesthetic in the membrane. According to this view, the product of molar volume and $c_{membrane}^{E25}$ should be a constant. As Fig. 8 shows, using covalent van der Waals volumes for molar volume[20], the product is almost, but not quite constant. It varies by a factor of 1.5

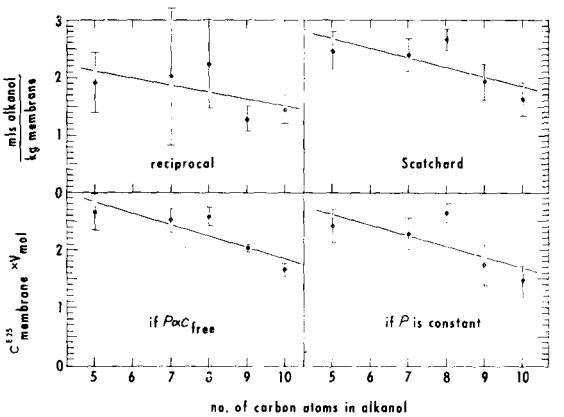

Fig. 8. The volume fraction which each alkanol occupies in the membrane phase. The volume fraction calculated from the isotherms (Figs. 2–4) is equal to $V_{mol} \cdot c_{membrane}^{E25}$.

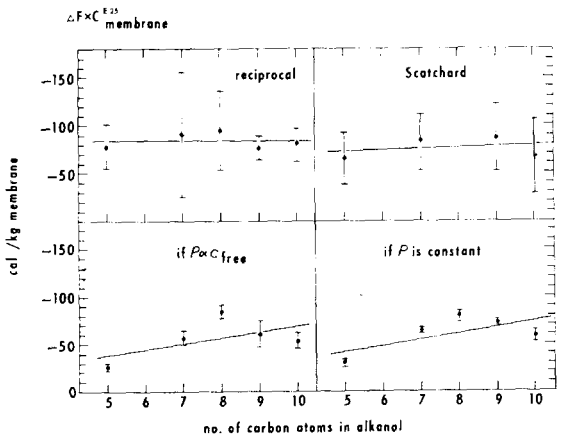

Fig. 9. The variation of $\Delta F^{\circ} \cdot c_{membrane}^{E25}$ with chain length. The error bars represent 95% confidence limits computed assuming ΔF° and $c_{membrane}^{E25}$ are independent.

from pentanol to decanol as compared to a range of 3.2 for $c^{E25}_{membrane}$ alone. It has been proposed by BENSON AND KING[21] and also by PAULING[22] that the molecular polarizability may be important in determining anesthetic potency. Correcting the $c^{E25}_{membrane}$ values for molecular polarizability was found to result in a situation similar to that obtained with the MULLINS[19] correction. Instead of choosing the volume or polarizability of the molecule as an index of the "sphere of influence" of the molecule, one can choose the ΔF as being possibly the most general index of the extensive property of the anesthetic in the receptor–alcohol interaction. The parameter, $\Delta F^{\circ} \cdot c^{E25}_{membrane}$ (Fig. 9) varies somewhat less with chain length than $\Delta F^{\circ} \cdot V_{mol}$, suggesting that $\Delta F^{\circ} \cdot c^{E25}_{membrane}$ is the more general parameter.

The hydrophobic nature of membrane–anesthetic interaction

The mean value for the free energy of adsorption per CH_2 group was −695 cal/mole. In a previous analysis of data on alcohol anesthesia in thirteen systems[11] a mean value of −815 cal/mole was obtained and −823 cal/mole for the hemolysis-inhibition system in particular. These calculations were based on the assumptions that the finite-site model applied and that the extent of anesthesia or protection against hemolysis was directly proportional to $c_{membrane}$. This latter assumption conflicts with the present results which indicate that $c_{membrane}$ decreases by a factor of 3 in going from pentanol to decanol. Application of this factor to the earlier theoretical treatment and assuming that the partition model is valid results in a correction of 130 cal/mole of CH_2 groups. Predicted mean values thus become −693 cal/mole and −685 cal/mole for the hemolysis-inhibition and the thirteen anesthetic systems, respectively. These values agree well with the mean of −695 cal/mole found experimentally.

It has been pointed out that a value of −700 to −800 cal/mole CH_2 is about the same as the free energy of transfer per mole of CH_2 groups from an aqueous phase to a completely non-polar phase (750–880) (see references in ref. 11). This suggests that the CH_2 groups of the alkanol may reside in some hydrophobic region of the plasma membrane. This region may consist of (1) the non-polar portions of lipid molecules, (2) the non-polar interfaces between lipid and protein molecules, or (3) the hydrophobic regions of protein molecules. The $\Delta F/-CH_2$ for proteins is generally in the region between −100 and −560 cal/mole although higher values of −650 to −1100 cal/mole occur with proteins which may undergo conformation changes (see ref. 11 for further references and discussion). Thus, the site is probably the non-polar portion of lipid molecules or involves a protein which undergoes a conformation change on binding the alcohol.

Number af alcohol binding sites

The mean value for n, the total number of binding sites, was found to be 65.5 mmoles/kg dry membrane, using the double reciprocal plot (Table I). This value agrees with that of 80 ± 10 mmoles/kg dry membrane for chlorpromazine[3], and corresponds to about 1/6th the number of phospholipid molecules in the membrane.

The number of alcohol molecules in the membrane at c^{E25}_{free} corresponds to about 1/12th the number of phospholipids for pentanol and to about 1/18th the number for decanol by all four methods of analyzing the data.

Adsorption of anesthetics to membranes, and the membrane expansion caused by anesthetics

Previous studies[23–26] have indicated that the cell membrane expands in area by 1–2% in the presence of low concentrations of anesthetics. The data in Fig. 8 indicate that the volume fraction of anesthetic in the membrane is of the order of 2–5 ml/kg of membrane. If the anesthetic molecules do not change the thickness of the membrane, the membrane area would expand by a corresponding 0.2–0.5%. Since this is smaller than the 1–2% membrane area expansion which is observed, it is apparent that the bulk volume of the anesthetic molecules cannot account for the entire expansion (see ref. 3 for further discussion on this matter). This fact distinguishes drug-induced expansion of the cell membrane from the swelling of rubber by anesthetics[27] or the swelling of olive oil and hexane by gases[28].

Experimental determination of the "intrinsic efficacy" of drugs

The pharmacological "intrinsic efficacy" of a membrane-acting drug may be defined as membrane effect/membrane concentration of drug. (The term "intrinsic efficacy" has informally been adopted recently as a hybrid of "intrinsic activity" according to ARIENS *et al.*[1] and "efficacy" according to STEPHENSON[29]). Although there has been much theory written on the concept of intrinsic efficacy, there have been virtually no experimental determinations of this entity[30].

The present work indicates that it is now possible to determine the intrinsic efficacy on an experimental basis. This is done as follows. It is known that the anesthetics expand the membrane by about 1% in area at the $c_{\text{free}}^{\text{E}25}$ (ref. 23). This membrane expansion of 1% represents about 1.35 μ^2 (or $1.35 \cdot 10^8$ Å^2) in membrane area, and is brought about by the presence of 0.04 molal pentanol in the membrane (or $2.88 \cdot 10^8$ molecules per cell membrane, since each cell membrane weighs about $1.2 \cdot 10^{-12}$ g). The intrinsic efficacy of pentanol in causing membrane expansion, therefore, is 0.5 Å^2/molecule of pentanol. For decanol, the value is 1.5 Å^2/molecule.

These direct results indicate that decanol has a higher intrinsic efficacy than pentanol. Other results, obtained indirectly, had suggested the opposite[31–33]. The overall potency of a drug, however, depends on the number of molecules that are able to get into the membrane. Decanol may partition differently in different membranes such that it may only appear to be less effective than pentanol[31–33]. The present study reveals, however, that once inside the membrane, decanol is intrinsically more effective than pentanol.

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