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THE MEMBRANE CONCENTRATIONS OF NEUTRAL AND POSITIVE ANESTHETICS (ALCOHOLS, CHLORPROMAZINE, MORPHINE) FIT THE MEYER–OVERTON RULE OF ANESTHESIA; NEGATIVE NARCOTICS DO NOT

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SUMMARY

1. The anti-hemolytic action of 44 anesthetics, and the adsorption of most of these anesthetics to erythrocyte membranes, synaptosomes and sarcoplasmic reticulum were compared. Neutral, positive and negative anesthetics all reduced hypotonic hemolysis at concentrations known to cause local anesthesia.

2. The membrane/buffer partition coefficients of anesthetics in the nerve or muscle membranes were the same as those for the erythrocyte membranes.

3. The membrane/buffer partition coefficients for the neutral anesthetics were independent of the free drug concentration or the ionic strength; the coefficients for the positive anesthetics decreased at higher free concentrations and higher ionic strength; the coefficients of the negative anesthetics were independent of the free concentration but increased with ionic strength.

4. The membrane/buffer partition coefficients for the neutral anesthetics were invariably one-fifth of the octanol/water partition coefficients, indicating that the membrane is much less hydrophobic than octanol. The good correlation justifies and facilitates the calculation of the membrane/buffer partition coefficients for neutral molecules directly from the octanol/water system. There was no correlation between the two sets of coefficients for the positive anesthetics.

5. For all the neutral and positively charged anesthetics, the concentrations in the membrane phase (for equi-effective anti-hemolytic action) fell in the range predicted by the Meyer–Overton rule of anesthesia, all values being of the order of 0.04 mole anesthetic per kg dry membrane. The membrane concentrations of the negatively charged anesthetics (barbiturates and fatty acids) were 20– to 40–fold lower than the Meyer–Overton range.

6. The anesthetic molecular volume occupying the membrane (at equi-effective anti-hemolytic action) was calculated as 2–3 ml/kg dry membrane for all the neutral and positive anesthetics; the negative molecules occupied around 0.1 ml/kg dry

Abbreviations: G 3428, *N*-methyl-5-*n*-butylbarbituric acid; RAC 109, *N*-(γ-diethylaminopropyl)-1,2,3,4-tetrahydronaphthalin-1-spirosuccinimide.

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membrane. It is concluded that the negative molecules have a 20- to 40-fold higher intrinsic efficacy in the membrane phase.

INTRODUCTION

The MEYER–OVERTON rule of anesthesia[1–3] states essentially that the anesthetizing concentration of narcotic in the membrane phase of the cell is always of the order of 0.03 molal (moles of narcotic per kg membrane). This rule is supported by direct evidence[3,4] for chlorpromazine and the alcohols.

It was necessary to extend this earlier work[3,4] to other types of anesthetics such as phenols, barbiturates, morphine, procaine, fatty acids, *etc.* in order to test whether the Meyer–Overton rule was more generally applicable. Since the previous studies had measured the anesthetic binding to erythrocyte membranes, it was further necessary to compare this binding to that which occurs to electrically excitable membranes such as muscle or nerve.

METHODS

Preparation of membranes

Membranes were isolated and purified from erythrocytes, brain and muscle. Human erythrocyte membranes were prepared from human stored blood[5,3], wherein the hemolysing solution contained 15 mM Tris–HCl, pH 7.4, and 1 mM EDTA instead of 20 mosM sodium phosphate buffer[5].

Synaptosome membranes were prepared from guinea-pig brain by the method of GRAY AND WHITTAKER[6]. The synaptosome fraction was checked by thin-section electron microscopy using a Phillips EM 300 electron microscope. At least 60–70 % of the membrane profiles could be recognized as nerve endings or synaptosomes, according to their content of 500-Å-wide vesicles and the presence of pre- and post-synaptic electron-dense material.

Sarcoplasmic reticulum membranes were isolated and purified from rabbit gastrocnemius muscle, the details of which, as well as the electron microscopic appearance, has been described previously[7].

After purification all membranes were resuspended and washed 3 times with 15 mM Tris–HCl, pH 7, and finally resuspended in this medium at a concentration of approximately 0.4–1.0 % dry weight; dry weights were obtained by drying overnight at 80° and correcting for the weight of buffer salts present.

The adsorption of drugs to membranes

The adsorption of [^{35}S]chlorpromazine (specific activity 19 mC/mmole; Amersham/Searle), [^{14}C]procaine (2.84 mC/mmole), [2-^{14}C]pentobarbital (3.23 mC/mmole; New England Nuclear Corp., Mass. U.S.A.), [^{14}C]morphine (57 mC/mmole), [^{14}C]-benzyl alcohol (2.0 mC/mmole; Amersham/Searle), and sodium *n*-[1-^{14}C]valerate (11.3 mC/mmole; Amersham/Searle) to the various membrane fractions was carried out by a method previously described[4]. All experimental steps, including centrifugation of the membranes, were done at room temperature, 23 ± 1°. Appropriate corrections were made for any adsorption of the drug to glass, as previously outlined[4].

Determination of the anti-hemolytic effects of the anesthetics

The anesthetic concentration which reduces osmotic hemolysis by 50 % is referred to as the AH_{50} % and the procedure for determining this value has been outlined in a companion paper[8]. Briefly, human fresh erythrocytes were incubated for 5 min at room temperature in different concentrations of anesthetic in isotonic solution (0.9 % NaCl, 15 mM Tris–HCl buffer, pH 7). The cells were then subjected to hypotonic hemolysis by the rapid injection of 15 mM Tris–HCl buffer containing the same concentration of anesthetic. After a further 10 min the cells were centrifuged and the optical density of the supernatant was measured at 540 nm in a Zeiss spectrophotometer. All drug solutions were checked for pH; if necessary, the pH was adjusted to 7 by extremely small amounts of HCl or NaOH.

MATERIALS

Chlorpromazine–HCl was a gift from Poulenc Labs., Montreal, Canada. Procaine–HCl was obtained from Sigma Chemical Co., St. Louis, U.S.A., procainamide–HCl from K and K Laboratories, Inc., New York, U.S.A., butyric, valeric and hexanoic acids from Eastman Organic Chemicals, New York., sodium pentobarbital (pK_a = 7.97), Ingram and Bell, Toronto, sodium barbital (pK_a = 7.43; British Drug Houses) and benzyl alcohol from Fisher Scientific Co., Toronto. G3428 is *N*-methyl-5-*n*-butylbarbituric acid and was generously donated by Dr. Karl Thomae, GmbH, Biberach, West Germany, through the courtesy of Dr. H. Machleidt; G3428 has a pK_a of 3.83. The octanol/water partition coefficient for sodium pentobarbital is 89.13, for sodium barbital 4.47, and for G3428 12.6; these values were kindly determined by Dr. A. Reuter of Dr. Karl Thomae, GbH, Biberach.

RESULTS

In order to determine the membrane concentrations of a wide variety of anesthetics, a selection of positively charged anesthetics (chlorpromazine, procaine, procainamide and morphine), negatively charged anesthetics (pentobarbital, barbital, butyric acid, valeric acid, hexanoic acid, heptanoic acid and G3428) and neutral anesthetics (benzyl alcohol, chloroform, alcohols and phenols) were chosen for study. The membrane concentrations (at equal drug effect) were obtained experimentally in two steps: (1) the anti-hemolytic effect of the anesthetic was measured at different concentrations; (2) the adsorption isotherm of each drug was obtained on erythrocyte ghost membranes and in some cases sarcoplasmic reticulum membranes and synaptosome membranes. The membrane concentration of the drug could then be obtained directly from the isotherm at a particular drug effect.

Anti-hemolysis by anesthetics

The AH_{50} % (or the anesthetic concentration which reduces osmotic hemolysis by 50 %) is known to be a simple and reliable index of the extracellular anesthetizing concentration[9]. A few examples of typical dose–response curves for anti-hemolysis are shown in Fig. 1 for procaine, chloroform, procainamide, butyric acid, valeric acid, sodium pentobarbital and chlorpromazine–HCl. Each point in this figure represents the average of 6 individual test tubes at least, and in the majority of cases 12 test

tubes. A complete dose–response curve for each drug was repeated at least 3 times.

The drug concentrations in Fig. 1 represent the concentrations if no adsorption occurred to the erythrocytes. Since the final hematocrit was only 0.6 %, the actual amount of drug adsorbed by the small number of cells was 3 % or less of the number

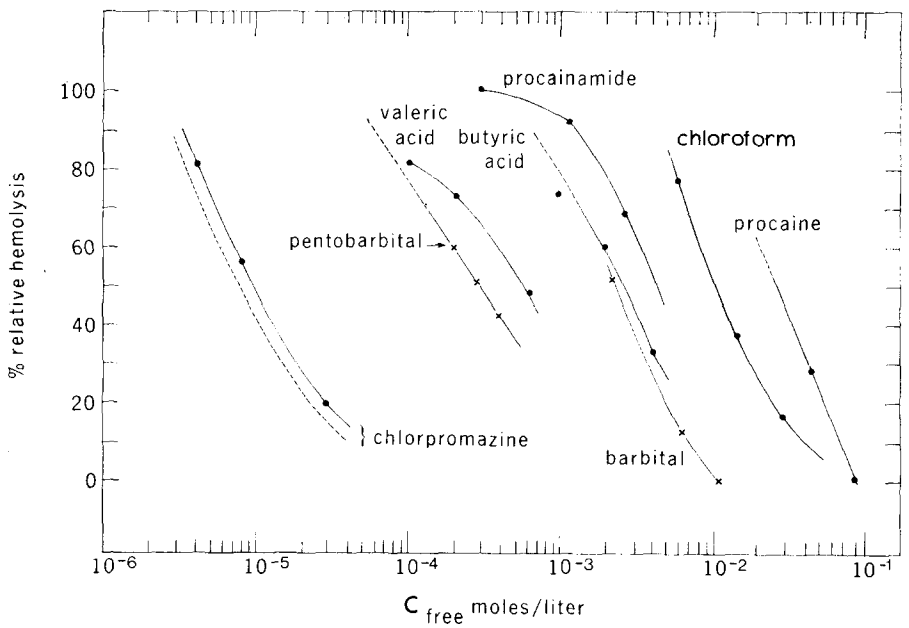

Fig. 1. A variety of anesthetics, including neutral, positive and negative compounds, reduced hypotonic hemolysis of human erythrocytes at drug concentrations which are known to cause local anesthesia. The chlorpromazine dose–response curve which is interrupted has been corrected for the drop in c_{free} which occurred when about 16% of the molecules were adsorbed by the intact cells. A relative hemolysis of 100% represents 25% hemoglobin release. Hematocrit is 0.6%

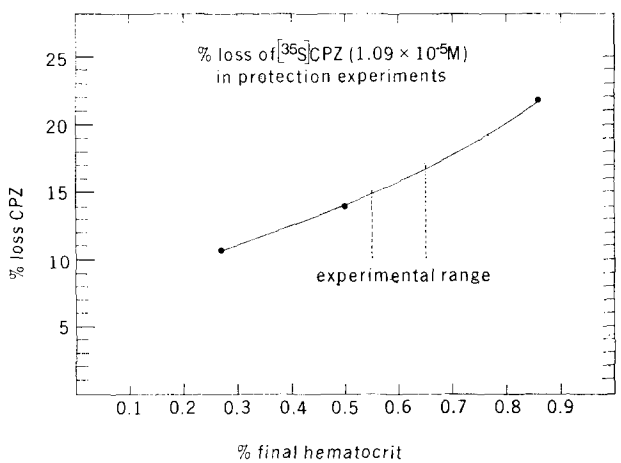

Fig. 2. Showing the fractional loss of chlorpromazine (CPZ) molecules which occurs at different hematocrits in the presence of $1.09 \cdot 10^{-5}$ M [^{35}S]chlorpromazine–HCl. The experimental range for the hematocrits was usually 0.55–0.65%.

of molecules present, with the exception of chlorpromazine–HCl. The fractional loss of chlorpromazine, as percentage of the number of molecules added to the final test tube, is shown in Fig. 2; in the experimental range of 0.6 % hematocrit, the loss of chlorpromazine was about 16 %. The dose-response curve for chlorpromazine (interrupted line in Fig. 1) has been corrected for this 16 % loss.

From data of the type presented in Fig. 1, the concentrations for 50 % antihemolysis were obtained. These values of $AH_{50\ \%}$ are listed in column 2 of table I.

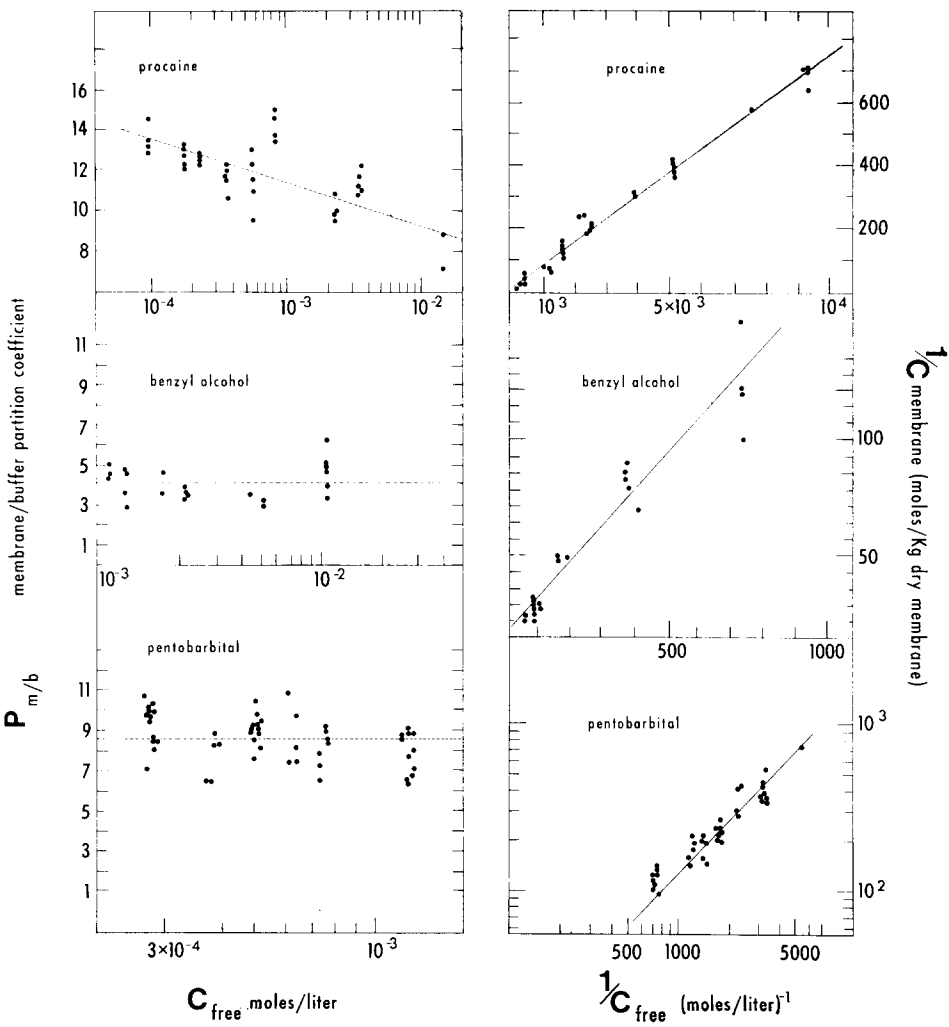

Fig. 3. The erythrocyte membrane/buffer partition coefficients and the adsorption isotherms for a positively charged anesthetic (top), a neutral one (middle), and a negatively charged compound (bottom). The isotherms are graphed as double reciprocal plots. The membrane/buffer partition coefficients for the neutral and negative anesthetics were independent of the free drug concentration; those for the positive anesthetics varied inversely with c_{free}.

TABLE I

THE MEMBRANE CONCENTRATIONS OF ANESTHETICS

The anesthetic concentration in the membrane phase ($c_{membrane}$) was calculated by multiplying the AH_{50} % by the membrane/buffer partition coefficient (at 0.4% NaCl). The $c_{membrane}$ values for all the neutral and positive anesthetics fall in the range predicted by the Meyer–Overton rule of anesthesia; the negative molecules (bottom group) have much lower membrane concentrations.

	AH_{50} % (*moles/l*)	$P_{m/b}$ at AH_{50} % (*in 0.4% NaCl*)	$c_{membrane}$ (*moles/kg dry membrane*)	$V_{molecule}$ (*ref. 12*) (*ml/mole*)	$c_{membrane} \cdot V_{molecule}$ (*ml/kg dry membrane*)
Benzyl alcohol	$2.2 \cdot 10^{-2}$	4.0	0.089	64	5.7
Butanol	$4.2 \cdot 10^{-2}$	1.5 *	0.062	52	3.3
Pentanol	$1.45 \cdot 10^{-2}$	3.6	0.053	63	3.3
Hexanol	$1.75 \cdot 10^{-3}$	13.0 *	0.023	73	1.7
Heptanol	$6.18 \cdot 10^{-4}$	39.6	0.025	83	2.0
Octanol	$2.35 \cdot 10^{-4}$	151.8	0.036	93	3.3
Nonanol	$4.10 \cdot 10^{-5}$	582	0.024	104	2.5
Decanol	$1.0 \cdot 10^{-5}$	1226	0.012	114	1.4
Chlorpromazine	$8.1 \cdot 10^{-6}$	1600	0.013	164	2.1
Procaine	$2.6 \cdot 10^{-2}$	4.5	0.117	142	16.6
Procainamide	$5.0 \cdot 10^{-2}$			146	
RAC I	$2.73 \cdot 10^{-3}$	8	0.022	204	4.5
RAC II	$2.37 \cdot 10^{-3}$	8	0.019	204	3.9
Morphine	$5.2 \cdot 10^{-3}$	7.1	0.037	145	5.4
Phenol	$7.8 \cdot 10^{-3}$	8.5	0.066	46	3.0
4-OCH_3-phenol	$6.0 \cdot 10^{-3}$	5.4 *	0.033	61	2.0
4-F-phenol	$3.0 \cdot 10^{-3}$	13.1 *	0.039	49	1.9
3-$N(CH_3)_2$-phenol	$2.8 \cdot 10^{-3}$	8.0 *	0.022	75	1.7
4-CO_2CH_3-phenol	$2.4 \cdot 10^{-3}$	18.1 *	0.044	75	3.3
3-NO_2-phenol	$2.2 \cdot 10^{-3}$	20 *	0.045	60	2.7
3-CH_3-phenol	$1.7 \cdot 10^{-3}$	17.7 *	0.030	57	1.7
4-OC_2H_5-phenol	$1.6 \cdot 10^{-3}$	13.1 *	0.021	71	1.5
4-CH_3-phenol	$1.4 \cdot 10^{-3}$	17.7 *	0.025	57	1.4
2-Cl-phenol	$1.15 \cdot 10^{-3}$	28.8 *	0.033	55	1.8
2,6-$(CH_3)_2$-phenol	$1.05 \cdot 10^{-3}$	44 *	0.046	68	3.2
3,5-$(CH_3)_2$-phenol	$9.5 \cdot 10^{-4}$	43 *	0.041	68	2.8
4-Cl-phenol	$7.4 \cdot 10^{-4}$	43 *	0.032	55	1.8
2-CF_3-phenol	$5.5 \cdot 10^{-4}$	119 *	0.066	65	4.3
4-Br-phenol	$3.6 \cdot 10^{-4}$	92	0.033	58	1.9
2,4-Cl_2-phenol	$2.0 \cdot 10^{-4}$	375 *	0.075	65	4.9
3-I-phenol	$1.9 \cdot 10^{-4}$	188 *	0.036	63	2.3
4-*t*-C_4H_9-phenol	$1.27 \cdot 10^{-4}$	164 *	0.021	88	1.8
3-CH_3, 4-Cl-phenol	$1.13 \cdot 10^{-4}$	131	0.015	67	0.99
Chloroform	$1.05 \cdot 10^{-2}$	18.0 *	0.189 **	44	8.2 **
Ether	$1.2 \cdot 10^{-1}$ (ref. 24)	1.3 *	0.156 **	52	8.1 **
Ethanol	$8.5 \cdot 10^{-1}$ ***	0.14 *	0.119 **	32	3.8 **
Pentobarbital	$2.95 \cdot 10^{-4}$	8.5	0.0025	133	0.33
Barbital	$2.25 \cdot 10^{-3}$	0.39 *	0.0009	102	0.090
G 3428	$1.05 \cdot 10^{-3}$	0.96 *	0.0010	112	0.11
Butyric acid	$2.7 \cdot 10^{-3}$	0.64	0.0017	54	0.093
Valeric acid	$5.8 \cdot 10^{-4}$	1.9	0.0011	64	0.070
Hexanoic acid	$4.3 \cdot 10^{-4}$	6.0 *	0.0026	74	0.191
Heptanoic acid	$2.2 \cdot 10^{-3}$	18.0 *	0.0396	84	3.34

* Extrapolated from Fig. 5.
** Not corrected for amount lost *via* evaporation and not graphed, therefore, in Figs. 7 and 8.
*** Extrapolated from other alkanols.

The adsorption of anesthetics to membranes of erythrocytes, muscle and brain

Adsorption isotherms. The adsorption characteristics of a positively charged anesthetic ([^{14}C]procaine), a neutral anesthetic ([^{14}C]benzyl alcohol) and a negatively charged anesthetic sodium ([^{14}C]pentobarbital) to erythrocyte ghost membranes are shown in Fig. 3. The results are graphed in the form of a double reciprocal plot, where the reciprocal of the bound drug is plotted against the reciprocal of the free drug concentration (see ref. 3). The results are also presented in the form of partition coefficients, where the partition coefficient is the ratio of the membrane concentrations (in moles of anesthetic per kg of dry membrane) over the free concentration of anesthetic. The partition coefficients for the neutral and negatively charged anesthetics were independent of the free concentrations, as shown in Fig. 3 for benzyl alcohol and sodium pentobarbital. The partition coefficients for the positively charged anesthetics, however, were not constant and varied inversely with the free concentration as in the example shown in Fig. 3 for procaine.

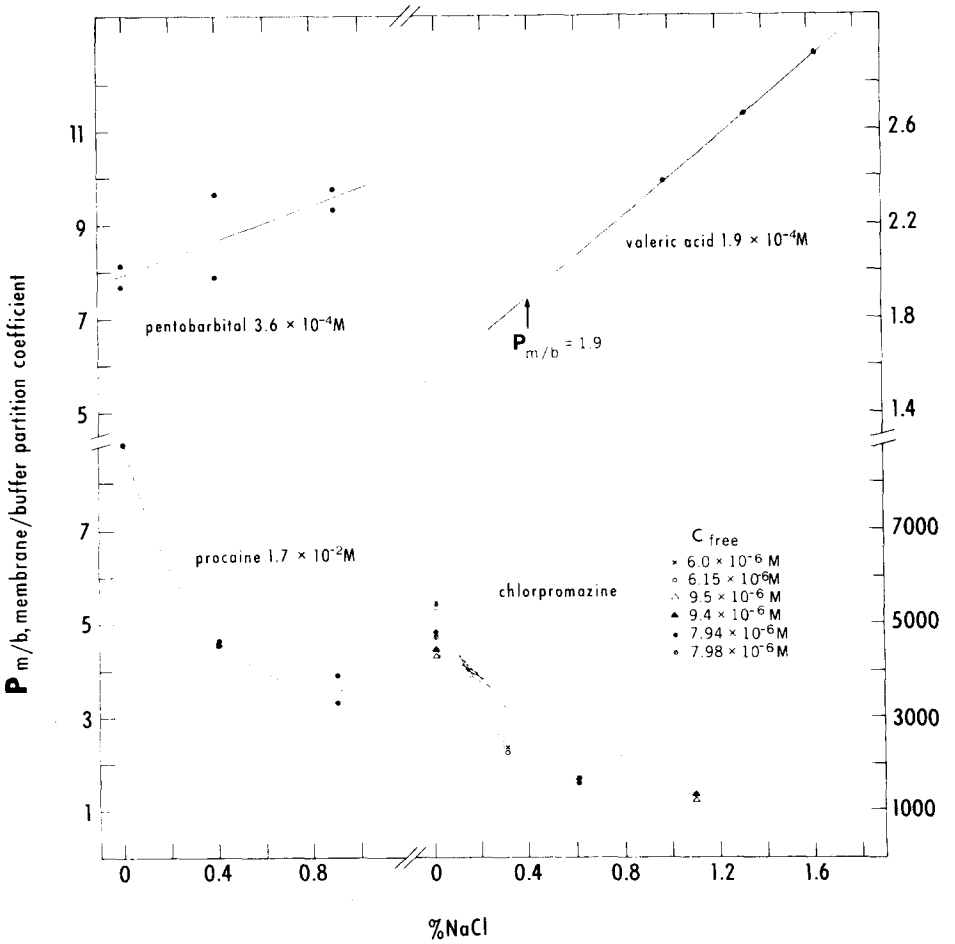

Fig. 4. Raising the ionic strength increased the erythrocyte membrane/buffer partition coefficients of the negatively charged narcotics (top: pentobarbital and valeric acid) but reduced the coefficients for the positively charged anesthetics (bottom).

The effect of ionic strength on anesthetic adsorption to membranes. The partition coefficients of the neutral anesthetics were also independent of the ionic strength. The partition coefficients of the charged anesthetics, however, depended on the ionic strength, the coefficients for the positive drugs decreasing with ionic strength, the coefficients for the negative drugs increasing with ionic strength; this is shown in Fig. 4 for procaine, chlorpromazine, pentobarbital and valeric acid.

Correlation between membrane/buffer and octanol/water partition coefficients. The membrane/buffer partition coefficients of the neutral drugs correlated very well with the octanol/buffer partition coefficients of these anesthetics[10] and this is shown in Fig. 5. The interrupted line in Fig. 5 is for the negative molecules. For the positive anesthetics, there was no correlation at all between the membrane/buffer partition coefficients and the octanol/water partition coefficients; these values are not shown in Fig. 5.

Because of the excellent correlation in Fig. 5 for the neutral molecules, it was possible to extrapolate the membrane/buffer partition coefficients for a number of phenols, knowing the octanol/buffer partition coefficients[10], and these $P_{m/b}$ values are also presented in column 3 of Table I.

Comparison between nerve, muscle membranes with erythrocyte membranes. The membrane/buffer partition coefficients of anesthetics in nerve and muscle membranes were the same as those for erythrocyte membranes. This is shown in Fig. 6 for various

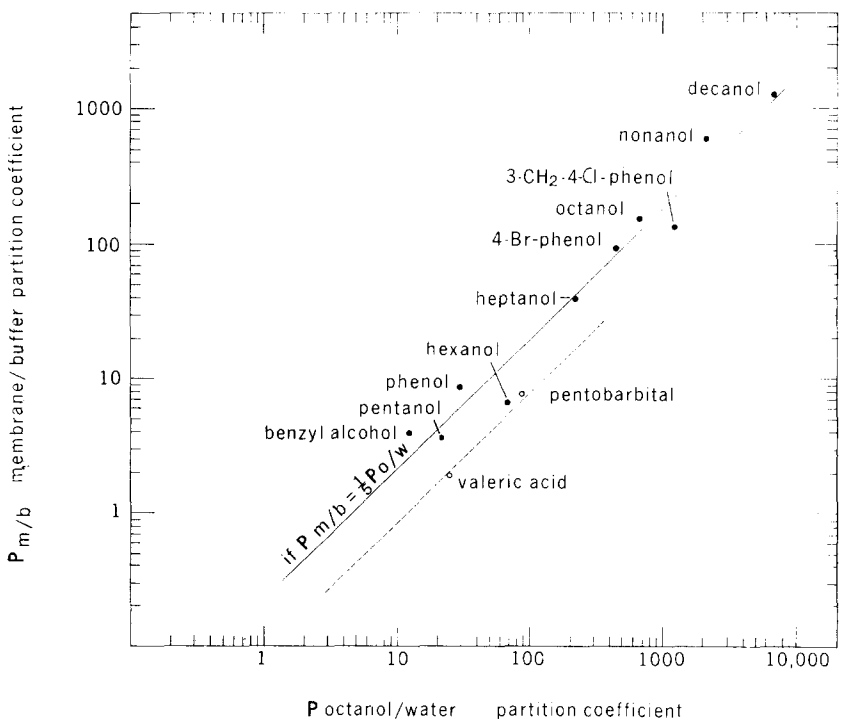

Fig. 5. Correlation between the erythrocyte membrane/buffer partition coefficients and the octanol/water partition coefficients for a variety of neutral anesthetics and 2 negative molecules. The solid line represents the relation if the membrane/buffer coefficient was exactly one-fifth the octanol/water coefficient.

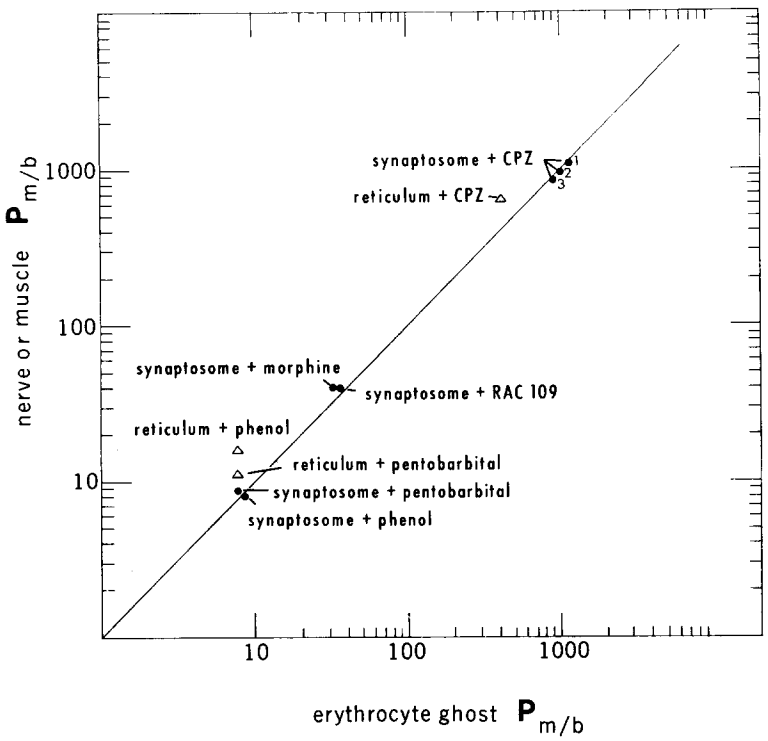

Fig. 6. Results indicating that the membrane/buffer partition coefficients of anesthetics in nerve and muscle membranes are the same as those for erythrocyte membranes. The chlorpromazine (CPZ) concentrations used with synaptosomes were 0.076, 0.09 and 0.10 mM for the points indicated by 1,2,3, respectively; for reticulum the CPZ concentration was 0.18 mM. The phenol and pentobarbital concentrations were 6.5 and 0.18 mM, respectively. The morphine and RAC 109 concentrations were 5.2 and 2.7 mM, respectively.

concentrations of chlorpromazine, morphine, phenol, the local anesthetic RAC 109 (*N*-(γ-diethylaminopropyl)-1,2,3,4-tetrahydronaphthalin-1-spirosuccinimide) and pentobarbital.

The membrane concentrations of the anesthetics

The membrane concentration of each anesthetic was calculated by multiplying $P_{m/b}$ (in column 3 of Table I) by AH_{50} % (in column 2). These membrane concentrations of the anesthetics at 0.4 % NaCl are listed in column 4 and graphed in Fig. 7.

It can be seen that the membrane concentrations of the neutral and positively charged anesthetics all fall into the range predicted by the Meyer–Overton rule of anesthesia, except those which are negatively charged, namely the barbiturates and the fatty acids. Heptanoic acid stood out as an exception in the fatty acids; this is not surprising since the fatty acids with 7 or more carbon atoms are ineffective anesthetics in most systems; octanoic acid, for example, had no effect on erythrocytes.

MULLINS[11] modified the Meyer–Overton rule of anesthesia by postulating a rule that equal anesthetic effects should occur at equal volume fractions of the anesthetic in the membrane. MULLINS rule, therefore, predicts that the product of $C_{membrane}$

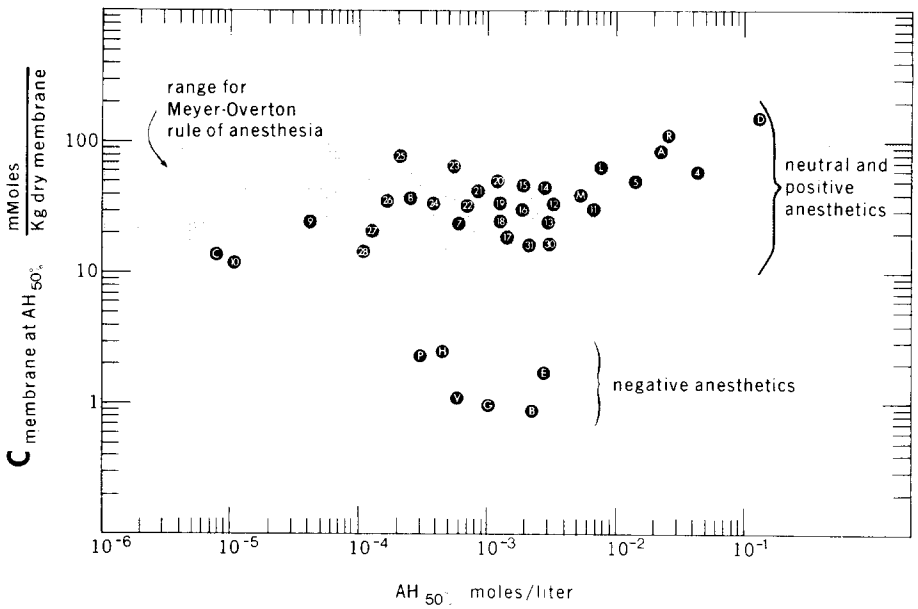

Fig. 7. Showing that the membrane concentrations (for equi-effective anti-hemolytic action) for all the neutral and positive anesthetics fall into the range predicted by the Meyer–Overton rule of anesthesia. The membrane concentrations of the negative anesthetics are 20–40-fold lower than the Meyer–Overton range; consequently, these drugs have a 20- to 40-fold higher intrinsic efficacy in the membrane phase. A, benzyl alcohol; B, sodium barbital; C, chlorpromazine; D, ether; E, butyric acid; G, barbiturate G-3428; H, hexanoic acid; L, phenol; M, morphine; P, sodium pentobarbital; R, procaine–HCl; V, valeric acid; 4, butanol; 5, pentanol; 6, hexanol; 7, heptanol; 8, octanol; 9, nonanol; 10, decanol; 11, 4-OCH_3-phenol; 12, 4-F-phenol; 13, 3-$N(CH_3)_2$-phenol; 14, 4-$COOCH_3$-phenol; 15, 3-NO_2-phenol; 16, 3-CH_3-phenol; 17, 4-OC_2H_5-phenol; 18, 4-CH_3-phenol; 19, 2-Cl-phenol; 20, 2,6-$(CH_3)_2$-phenol; 21, 3,5-$(CH_3)_2$-phenol; 22, 4-Cl-phenol; 23, 2-CF_3-phenol; 24, 4-Br-phenol; 25, 2,4-Cl_2-phenol; 26, 3-I-phenol; 27, 4-*tert.*-butylphenol; 28, 3-CH_3-4-Cl-phenol; 30, RAC 109 I; 31, RAC 109 II.

(in column 4) and $V_{molecule}$ (molecular volume calculated according to BONDI[12], and listed in column 5) should be a constant. This product is tabulated in column 6 of the Table and graphed in Fig. 8, where it can be readily observed that the product $C_{membrane} \cdot V_{molecule}$ is not the same value for all the anesthetic molecules.

DISCUSSION

The two major findings in this work are that: (1) The membrane concentrations of the neutral and positively charged anesthetics fit the Meyer–Overton rule of anesthesia, but the membrane concentrations of the negatively charged anesthetics are lower than would be predicted by the rule by a factor of around 20-fold; (2) The membrane/buffer partition coefficients of excitable membranes are quantitatively similar to those of erythrocyte membranes.

The extracellular anesthetizing concentrations of the anesthetics compared with the anti-hemolytic concentrations

The extracellular anesthetizing concentrations reported in the literature are similar to those which protect erythrocytes from osmotic hemolysis. Sodium pento-

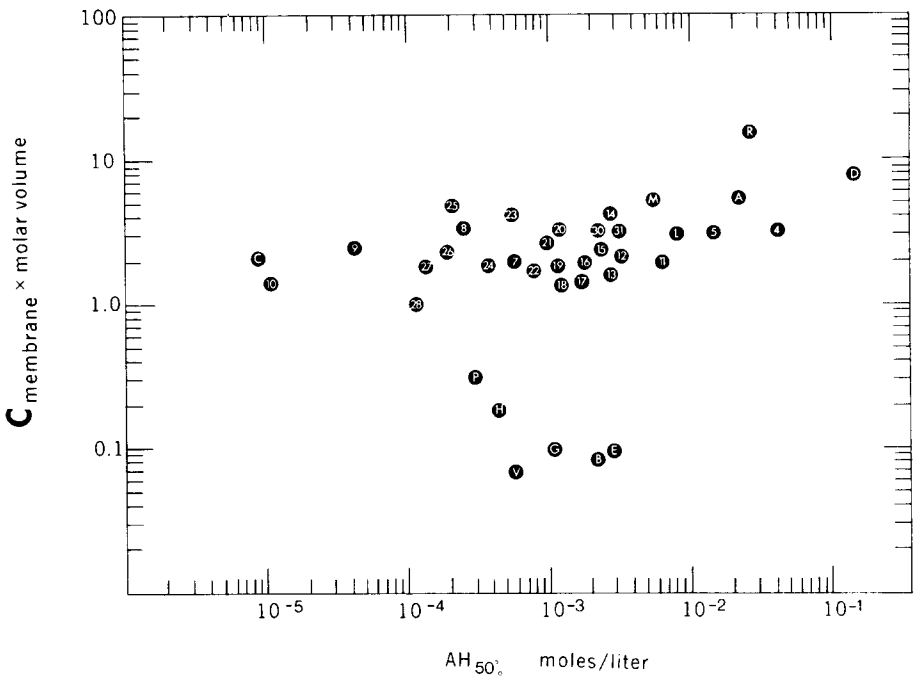

Fig. 8. The ordinate represents the molecular volume of the anesthetic (in ml drug per kg dry membrane) which occupies the membrane phase at the $AH_{50}\%$. It can be seen that although most of the molecules occupy about 2–3 ml/kg dry membrane, the negative molecules occupy around 0.1 ml/kg dry membrane. (See Fig. 7 legend for code.)

barbital, for example, causes local anesthesia[13] of just under 50 % of muscle fibers at 1 mM, a concentration which causes around 75 % protection of erythrocytes (see Fig. 1). Local anesthesia of perfused ganglia occurs at 0.24–0.4 mM sodium pentobarbital[14], a concentration range which causes about 50 % antihemolysis (Fig. 1). It has been discussed previously[15] that *in vivo* extracellular concentrations of anesthetics which cause general anesthesia are similar or identical to the extracellular concentrations which cause 8 % anti-hemolysis. The barbiturates fit in with this general observation, since it is known that pentobarbital concentration in brain fluid during anesthesia is around $6 \cdot 10^{-5}$ M (ref. 16), and it can be seen in Fig. 1 that this concentration of pentobarbital causes about 8 % anti-hemolysis. There is no direct information on the concentration of butyric acid anesthetic in the brain, although some estimates have been made[17]. It is known that the closely related anesthetic, γ-hydroxybutyrate, has a critical concentration in brain tissue of $8 \cdot 10^{-4}$ M for inducing anesthesia in brain tissue[18]; butyric acid at this concentration caused 15 % anti-hemolysis (Fig. 1).

It appears, therefore, that the extracellular anesthetizing concentrations of the negatively charged anesthetics are similar to those which protect erythrocytes from osmotic hemolysis, a conclusion made previously for neutral and positively charged anesthetics[9].

The membrane concentrations of anesthetics

At equal membrane effect, the membrane concentrations of the barbiturates

and narcotic fatty acids were about 20-fold lower than any of the neutral or positively charged anesthetics (Fig. 7). It appears, therefore, that the negative charge of the molecule is important in determining the 'efficacy' of the anesthetic. This observation may lend some support to the BENSON AND KING[19] hypothesis of anesthesia.

The fatty acids equilibrate extremely quickly across the erythrocyte membrane, the half-times of permeation for butyric and valeric acids being 379 and 218 msec, respectively[20]. Since the pre-incubation period was 5 min in these experiments, the fatty acids should have equilibrated across the membrane before the cells were tested for the anti-hemolytic effect.

The membrane concentrations indicate that there are at least two general categories of anesthetics which have grossly different intrinsic efficacies. It is interesting to note that these two categories are identical to those described by DEUTICKE[21], who found that neutral and positively charged drugs produced cup-shaped erythrocytes while negatively charged drugs induced crenation.

The present work does not indicate whether the anionic form or the neutral form of the fatty (or barbiturate) is active in the membrane phase itself. At pH 7 over 99 % of the fatty acid molecules are charged while only about 10 % of the barbiturate molecules are charged (see MATERIALS for pK_a values). In the membrane phase, however, the local pH and pK_a values for these compounds may be very different.

The membrane/buffer partition coefficients

The fact that the membrane/buffer partition coefficients are the same for erythrocyte membranes and excitable membranes further justifies the use of the erythrocyte as a simple and valid model for studying the action of anesthetics, and for testing various hypotheses of anesthesia. The octanol/water partition coefficients are useful primarily in predicting the membrane/buffer partition coefficients for neutral molecules; there was no correlation for positive molecules, since these do not truly partition between octanol and water[22].

Theories of anesthesia

There is no theory of anesthesia at present which adequately explains why the membrane anesthetizing concentrations of the negative anesthetics are 20-fold lower than the neutral or positive anesthetics. Although all anesthetics reduce the sodium conductance of the action potential[13], it may be that the negative anesthetics accomplish this by an entirely different mechanism[23].

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