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THE HYDROPHOBIC EXPANSION OF ERYTHROCYTE MEMBRANES BY THE PHENOL ANESTHETICS

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SUMMARY

Nineteen phenol anesthetics protected human erythrocytes from hypotonic hemolysis. The concentration for 50 % anti-hemolysis (AH_{50} %) ranged from 7.8 mM for phenol to 0.113 mM for 3-CH_3-4-Cl phenol; the other substituents were 4-OCH_3; 4-F; 3-$N(CH_3)_2$; 4-$COOCH_3$; 3-NO_2; 3-CH_3; 4-OC_2H_5; 4-CH_3; 2-Cl; 2,6-$(CH_3)_2$; 3,5-$(CH_3)_2$; 4-Cl; 2-CF_3; 4-Br; 2,4-Cl_2; 3-I and 4-tert.-C_4H_9.

2. The coefficient of correlation between the AH_{50} % concentrations and the octanol/water partition coefficients was very high (0.963), indicating a hydrophobic interaction between the membrane and the phenol anesthetic. The anti-hemolytic effect is associated with membrane expansion. The correlation between AH_{50} % and pK_a or Hammett σ constants was very low, suggesting a negligible role of charge in the interaction.

3. The adsorption isotherms of 4-bromo-phenol, [^{14}C]phenol and 3-methyl, 4-chlorophenol to erythrocyte ghost membranes were obtained. The membrane/buffer partition coefficients of these phenols were independent of the free concentrations of the phenols.

4. The membrane/buffer partition coefficient for each of the 3 phenols was about one-fifth of the value for the octanol/water partition coefficient. Since this factor of 5 also holds for other alcohols, it seemed justified to compute all the membrane/buffer partition coefficients directly from the octanol/buffer partition coefficients. Separate experiments indicated that the octanol/buffer and the octanol/water partition coefficients were identical.

5. The membrane concentrations of the phenol anesthetics at 50 % anti-hemolysis ranged from 0.02 to 0.08 mole of anesthetic per kg dry membrane. The mean value for the membrane concentration was 0.04 mole/kg membrane, as predicted by the Meyer–Overton rule of anesthesia.

INTRODUCTION

Ever since the classical work of H. MEYER[1] and OVERTON[2], it has been known that the potency of a general anesthetic is directly proportional to its oil/water parti-

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tion coefficient[3]. In determining these partition coefficients, there is no agreement on which organic solvent best simulates the hydrophilic–hydrophobic nature of the biological membrane. Some researchers have preferred to study the correlation between anesthetic potency and anesthetic partitioning into a lipid[4,5] or lipoprotein monolayer[4] or into a nerve lipid/water system[6].

The most desirable way of studying the relation between the partition coefficient and the anesthetic potency would be to obtain this information on the same cell membrane. This is what has been done in the present study where both the membrane-expanding potencies and the membrane/water partition coefficients of a systematic series of phenol anesthetics have been obtained on the human erythrocyte membrane.

It is known that the erythrocyte membrane is a quantitative model for studying the effects of anesthetics on membranes since all lipid-soluble anesthetics tested so far have an erythrocyte-protecting effect[7,8] which is brought about by the membrane being expanded by the anesthetics[9].

METHODS

Determination of phenol concentration which inhibited osmotic hemolysis by 50 % (AH_{50} %)

The earlier procedure of SEEMAN AND WEINSTEIN[10] for determining the anti-hemolytic potency of an anesthetic compound was modified to obtain greater precision. The final procedure was as follows:

(1) Human blood was withdrawn by venepuncture and heparinized (50 I.U./ml blood).

(2) The erythrocytes were washed 3 times in 0.9% NaCl in 15 mM Tris-HCl buffer (pH 7), using at least 4 vol. of washing solution per volume of erythrocytes and discarding the buffy coat of leucocytes and thrombocytes on each occasion. The cells were finally resuspended at a hematocrit of between 5 and 7 %. Precise microhematocrit determinations were obtained by means of a Clay-Adams hematocrit centrifuge operating at 15000 × *g* for 3 min.[11].

(3) Using a 1-ml Eppendorf pipette (with plastic disposable tip; Brinkmann Instruments) which delivered to within 2.5 %, 1 ml of drug solution in isotonic medium was delivered into a 12 mm × 75 mm glass test-tube.

(4) An aliquot of 200 μl of the erythrocyte suspension was then added to the 12 × 75 mm tube by means of an Eppendorf pipette (2 % reproducibility). The contents of the tube were then rapidly mixed for 2 sec with a vortex mixer, and the tube was set aside for 5 min, thus allowing the drug to equilibrate across the cell membrane under isosmotic conditions.

(5) A third and final aliquot of 1.4 or 1.5 ml of drug solution (in 15 mM Tris–HCl, pH 7) was then added using a calibrated syringe (4 % reproducibility) and injecting rapidly into the tube while mixing constantly on the vortex mixer. The stainless steel needle attached to the syringe was filed and crimped at the tip such that 2 streams of solution were ejected at 90° to one another. The precise volume delivered was adjusted to give a final hemolysis (in the control samples) of 20–30 % hemoglobin release.

(6) After remaining at room temperature (23 ± 1°) for a further 10–15 min, the tubes were centrifuged at about 1500 × *g* for 90–120 sec in a Sorvall General Lab centrifuge (GLC-1). The supernatants were then directly decanted into Zeiss spectro-

photometer cuvettes, and the optical density of the hemoglobin measured at 540 nm. It was convenient to do 6 test tubes simultaneously, the first and last of these differing only by about 60 sec, which is small compared to the 10–15 min allowed for the entire time period for osmotic hemolysis. Two centrifuges were used. The absorbances for 100 % hemolysis and 0 % hemolysis were obtained by using solutions of 0.9 % NaCl and 15 mM Tris–HCl (pH 7), respectively, and treating the test-tubes in the same way.

(7) There was a small but progressive shift in the amount of absolute hemolysis in the control samples throughout the several hours of the experiment. The accuracy of the results was slightly improved, therefore, by noting the absolute time of the day when each set of 6 tubes was started, both for control tubes and for drug-containing tubes. In this way, it was possible to graph the percentage absolute hemolysis in the control tubes as a function of the absolute time of the day (control tubes were done every 45–60 min). The precise control percentage hemolysis for any particular time of the day could then be interpolated from this graph.

The adsorption of phenols to erythrocyte ghost membranes

Erythrocyte ghost membranes were prepared as previously described[12,13], The adsorption of [^{14}C]phenol (Mallinckrodt Nuclear; specific activity 25.9 mC/mmole) to these membranes was carried out by a method described previously[13]. The adsorption of the non-radioactive phenols to the membranes was done in the following way.

Using a 500-μl Eppendorf pipette (with plastic disposable tip), an aliquot of erythrocyte ghost membranes (having a dry weight of 1 %) was added to 2.0 ml of drug solution in a 10 mm $\times$ 75 mm testtube. The tube was covered with "Parafilm" and mixed for 2 sec on a Vortex mixer. After 15 min incubation at room temperature (23 $\pm$ 1°) the tube was centrifuged at 30000 $\times$ g for 10 min at 22° in a temperature-controlled centrifuge (Sorvall RC2B). Using a 1000-μl Eppendorf pipette adapted to accept a glass Pasteur pipette, a 1.0-ml aliquot of supernatant was removed and delivered into a 16 mm $\times$ 75 mm screw-capped culture tube containing 0.1 ml of colorimetric reagent. The colorimetric reagent was adapted from FEIGL[14] and was prepared by dissolving 53.0 g of mercury (specially distilled, Johnson Matthey and Mallory, Ltd.) in 45 ml of concentrated nitric acid, and diluting with 20 ml of distilled water. The tube was capped, buzzed on a Vortex mixer and heated for 2 min at 90° in a water bath. The tube was cooled in ice water for 5 min and the contents were transferred into a glass cuvette (10 mm path length) and the absorbance measured at 500 nm in a Zeiss spectrophotometer against a blank prepared as above, except that buffer replaced the drug. The concentrations in the supernatant were determined from a calibration curve prepared with known drug concentrations.

The octanol/buffer partition coefficients and the pK_a values for the phenols

The pK_a values and the octanol/buffer (pH 7) partition coefficients were determined by Dr. A. Reuter of Dr. Karl Thomae Gmbh (Biberach, West Germany), using methods previously described[15]. The phenol substituent, π, is defined as log P – log P_{phenol}, where P is the partition coefficient of the phenol derivative. The partition coefficients and the pK_a values were determined on the same phenol material which was used for testing the anti-hemolytic potency.

MATERIALS

Phenol was obtained from Mallinckrodt Chemical Works, Montreal. Dr. Karl Thomae, Gmbh, Biberach, West Germany, generously donated 4-OCH_3-phenol, 4-F-phenol, 3-$N(CH_3)_2$-phenol, 4-$COOCH_3$-phenol, 3-NO_2-phenol, 3-CH_3-phenol, 4-OC_2H_5-phenol, 4-CH_3-phenol, 2-Cl-phenol, 2,6-$(CH_3)_2$-phenol, 3,5-$(CH_3)_2$-phenol, 4-Cl-phenol, 2-CF_3-phenol, 4-Br-phenol, 2,4-Cl_2-phenol, 3-I-phenol, 4-*tert.*-C_4H_9-phenol and 3-CH_3-4-Cl-phenol All the compounds were dissolved and recrystallized in the cold from petroleum ether, heptane or benzene, and dried *in vacuo* to remove solvents.

RESULTS

The anti-hemolytic potencies of the phenol anesthetics (AH_{50} % values)

All the phenols protected human erythrocytes from osmotic hemolysis. Fig. 1 shows typical experiments for phenol, 3,5-$(CH_3)_2$-phenol, 4-Br-phenol, 3-I-phenol and 4-*tert.*-C_4H_9-phenol; the ordinate represents relative hemolysis where the value of 1.0 stands for the amount of hemolysis which occured in the absence of any phenol (relative hemolysis of 1.0 = absolute hemolysis of 25 % hemoglobin release). The

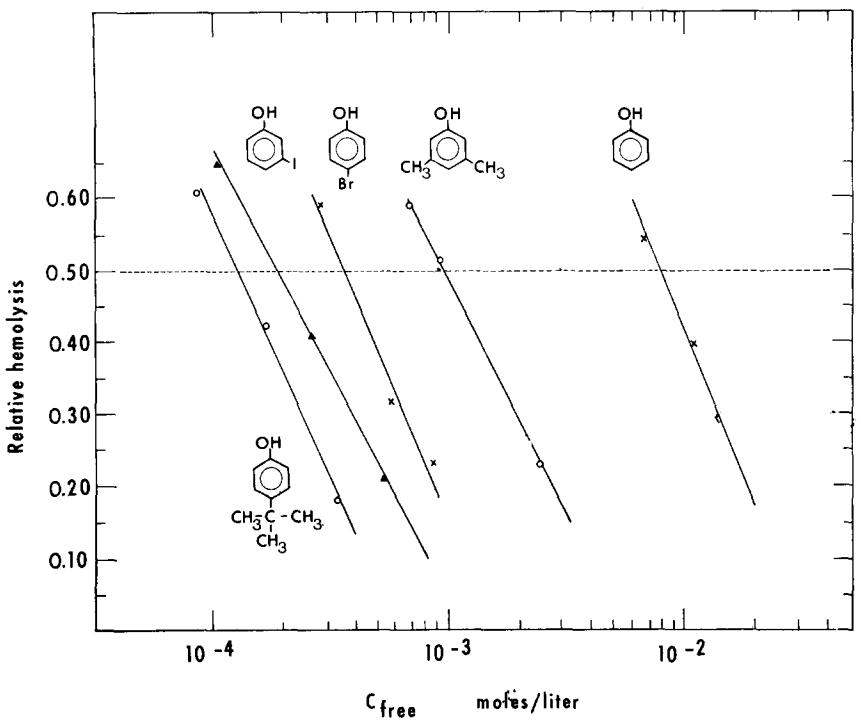

Fig. 1. Typical examples of the anti-hemolytic effects of the phenol anesthetics on human erythrocytes. The drugs are, from left to right, 4-*tert.*-C_4H_9-phenol, 3-I-phenol, 4-Br-phenol, 3,5-$(CH_3)_2$-phenol and phenol. A relative hemolysis of 1.0 indicated 25% hemoglobin release in hypotonic solution without drug. The anti-hemolytic concentration for a relative hemolysis of 0.5 (*i.e.* the AH_{50}%) was obtained by interpolation. The anti-hemolytic effect is accounted for by an expansion of the membrane area.

TABLE I

For the substituted phenols, a summary of the anti-hemolytic potencies (AH_{50} % values in column 2), the pK_a values (column 3), the partition coefficients in octanol/buffer (column 4) and the calculated membrane/buffer partition coefficients (based on the relation in Fig. 6 that $P_{m/b}$ is one-fifth that of $P_{o/b}$). The membrane concentrations of the phenols at 50% anti-hemolysis (last column) average out to 0.04 mole of drug per kg dry membrane, in excellent agreement with the Meyer–Overton prediction of 1899.

Phenol substituent	*Experimental concn. of phenol for 50% anti-hemolysis*	*Experimental Ionization*	*Experimental Octanol / Buffer partition coefficient*	*Calculated Membrane / Buffer partition coefficient*	*Calculated Membrane concn. of phenol at 50% anti-hemolysis*
	AH_{50} %	pK_a	$P_{o/b}$	$P_{m/b} = (P_{o/b})/5$	$c^{50\%}_{membrane} = (AH_{50}\%) \cdot P_{m/b}$
	moles/l		*moles/l octanol / moles/l water*	*moles/kg membrane / moles/l water*	*mole/kg membrane*
H	$7.8 \cdot 10^{-3}$	10.05	30.2	6.04	0.047
4-OCH_3	$6.0 \cdot 10^{-3}$	10.29	23.4	4.7	0.028
4-F	$3.0 \cdot 10^{-3}$	9.96	64.6	12.9	0.039
3-$N(CH_3)_2$	$2.8 \cdot 10^{-3}$	10.04	37.2	7.4	0.028
4-$COOCH_3$	$2.4 \cdot 10^{-3}$	8.37	91.2	18.2	0.044
3-NO_2	$2.2 \cdot 10^{-3}$	8.39	102.3	20.5	0.045
3-CH_3	$1.7 \cdot 10^{-3}$	10.15	89.1	17.8	0.030
4-OC_2H_5	$1.6 \cdot 10^{-3}$	10.27	64.6	12.9	0.021
4-CH_3	$1.4 \cdot 10^{-3}$	10.32	89.1	17.8	0.025
2-Cl	$1.15 \cdot 10^{-3}$	8.52	147.9	29.6	0.034
2,6-$(CH_3)_2$	$1.05 \cdot 10^{-3}$	10.69	229.1	45.8	0.048
3,5-$(CH_3)_2$	$9.5 \cdot 10^{-4}$	10.24	223.9	44.8	0.043
4-Cl	$7.4 \cdot 10^{-4}$	9.38	223.9	44.8	0.033
2-CF_3	$5.5 \cdot 10^{-4}$	8.42	631.0	126.2	0.069
4-Br	$3.6 \cdot 10^{-4}$	9.37	446.7	89.3	0.032
2,4-Cl_2	$2.0 \cdot 10^{-4}$	7.88	1995	399.0	0.080
3-I	$1.9 \cdot 10^{-4}$	9.03	1000	200.0	0.038
4-*tert.*-C_4H_9	$1.27 \cdot 10^{-4}$	10.27	871	174.2	0.022
3-CH_3-4-Cl	$1.13 \cdot 10^{-4}$	9.59	1259	251.8	0.028
					Mean ± S.D. 0.039 ± 0.15

AH_{50} %, or the phenol concentration at which the relative hemolysis was 0.5, was thus obtained by interpolation, as shown in Fig. 1. The complete list of the AH_{50} % values for the 19 phenols are presented in Table I.

Although the control amount of absolute hemolysis (in the absence of any drug) varied slightly from experiment to experiment (e.g. between 20 % and 30 % hemoglobin release), the AH_{50} % was independent of the control absolute hemolysis. Despite this independence, however, the majority of the AH_{50} % determinations were made near the control value of 25 % absolute hemolysis by appropriate adjustments of the amount of hypotonic solution injected by the syringe (step 5 in METHODS).

The correlation between the AH_{50} % and the octanol/buffer partition coefficient

The octanol/buffer partition coefficients of the phenols are tabulated in Table I. These values are virtually identical to the octanol/water partition coefficients, as de-

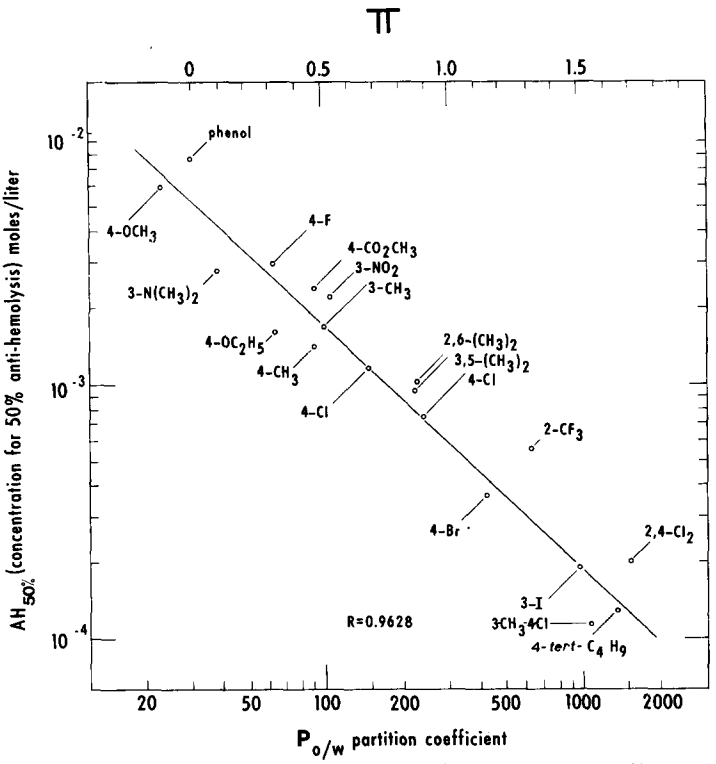

Fig. 2. The correlation between the AH_{50}% values and the octanol/water partition coefficients has a correlation coefficient of 0.963 for the substituted phenols. The high correlation indicates a hydrophobic interaction between the membrane and the phenol anesthetics.

TABLE II

SIMILARITY BETWEEN PHENOL SUBSTITUENTS IN OCTANOL/BUFFER AND OCTANOL/WATER SYSTEMS

For the substituted phenols, there is good agreement between the octanol/water partition coefficients and the octanol/buffer (pH 7) partition coefficients. π is defined as log $P_{\text{substituted phenol}}$ — — log P_{phenol}, where P is the partition coefficient. Values for $\pi_{\text{octanol/water}}$ in the last column were obtained from the literature[15].

Phenol substituent	*Octanol/buffer*		*Octanol/water*		*From ref. 15*
	Log $P_{o/b}$	*$\pi_{o/b}$*	*Log $P_{o/w}$*	*$\pi_{o/w}$*	*$\pi_{o/w}$*
H	1.50	0	1.48	0	0
4-OCH_3	1.37	−0.13	1.37	−0.11	−0.12
4-F	1.79	0.29	1.81	0.33	0.31
3-$N(CH_3)_2$	1.59	0.09	1.57	0.09	0.10
4-$COOCH_3$	1.95	0.45	1.96	0.48	
3-NO_2	1.84	0.34	2.01	0.53	0.54
3-CH_3	1.97	0.47	1.95	0.47	0.56
4-OC_2H_5	1.77	0.27	1.81	0.33	
4-CH_3	1.92	0.42	1.95	0.47	0.48
2-Cl	2.19	0.69	2.17	0.69	0.69
2,6-$(CH_3)_2$	2.10	0.60	2.36	0.88	
3,5-$(CH_3)_2$	2.4	0.9	2.35	0.87	
4-Cl	—	—	2.35	0.87	0.93
2-CF_3	2.5	1.0	2.8	1.32	
4-Br	2.35	0.85	2.65	1.17	1.13
2,4-Cl_2	2.13	0.63	3.3	1.8	1.62
3-I	3.0	1.5	3.0	1.52	1.47
4-*tert.*-C_4H_4	3.0	1.5	2.94	1.46	1.85
3-CH_3-4-Cl	2.7	1.2	3.1	1.6	1.49

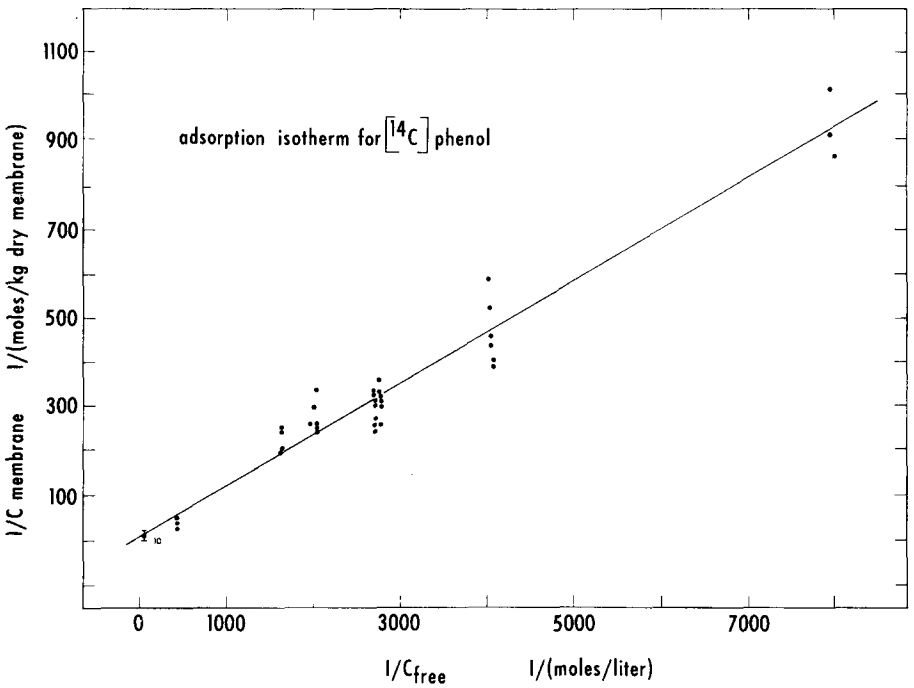

Fig. 3. Double reciprocal plot of the membrane concentration of [^{14}C] phenol versus the free concentration of phenol. The intercept on the ordinate indicates the number of binding sites. The point for the highest concentration (*i.e.* lowest $1/c_{free}$) was repeated 10 times, the vertical bar indicating the standard deviation. The straight line indicates 1 set of binding sites with affinity constant of 63.7 l/mole phenol.

monstrated in Table II. The phenol substituents have identical values for $\pi_{o/b}$ and $\pi_{o/w}$, where π = log P (of the substituted phenol) − log P (of phenol).

There is a high correlation between the AH_{50} % and the octanol/buffer partition coefficient. This is shown in Fig. 2, where the correlation coefficient is 0.963.

A low correlation was obtained between the AH_{50} % values and the pK_a values (correlation coefficient of 0.247) or between the AH_{50} % values and the Hammett σ constants[15].

The adsorption of phenols to erythrocyte ghost membranes

The adsorption of 4-Br-phenol, [^{14}C]phenol, and 3-CH_3-4-Cl-phenol to erythrocyte ghost membranes was measured. The results for two of these phenols are presented in Figs. 3 and 4, where the reciprocal of the membrane concentration is plotted versus the reciprocal of the phenol free concentration.

The ordinate intercepts (of the adsorption isotherms of Figs 3 and 4) represent the number of phenol binding sites at saturation[13]. The mean number of binding sites for phenol, 4-Br-phenol and 3-CH_3-4-Cl-phenol was 160 mmoles/kg dry membrane. This compares with a value of 66 mmoles/kg dry membrane found for the alcohols[13].

The membrane/buffer partition coefficients for the phenols.

The membrane/buffer partition coefficients were calculated directly from the ad-

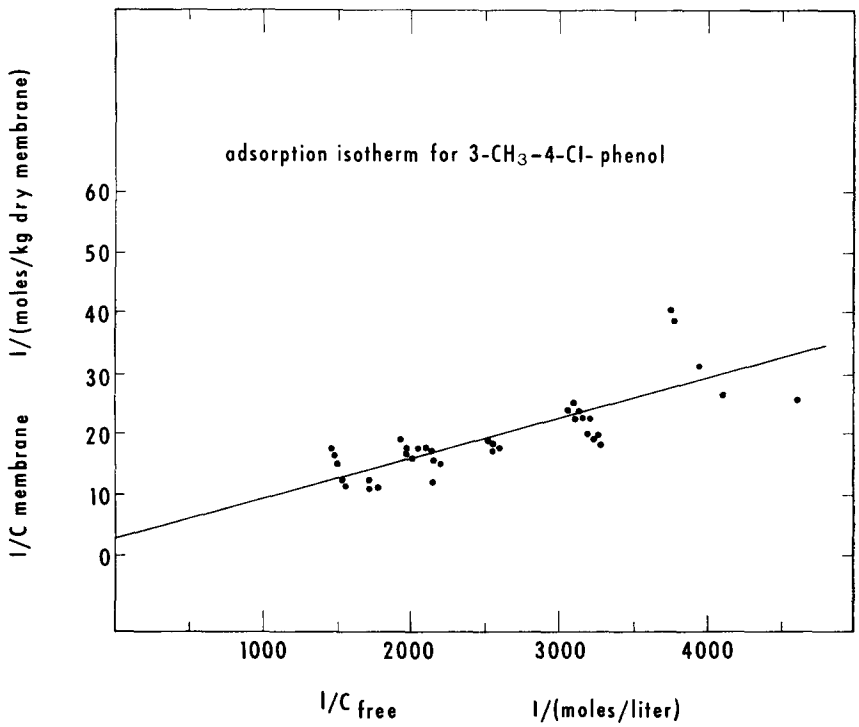

Fig. 4. The adsorption of 3-CH_3-4-Cl-phenol to erythrocyte membranes is plotted as a double reciprocal plot, as in Fig. 3 for phenol. The affinity constant is 409 l/mole of 3-CH_3-4-Cl-phenol.

sorption isotherms, and these are shown in Fig. 5 for the 3 phenols. The membrane/buffer partition coefficients of the phenols were essentially independent of the free phenol concentrations (Fig. 5), as found previously with the alcohols[13].

The correlation between $P_{m/b}$ and $P_{o/b}$

The correlation between $P_{m/b}$ and $P_{o/b}$ is shown in Fig. 6 for the three phenols. It appears that the membrane/buffer partition coefficients are approximately one-fifth the octanol/buffer partition coefficients; this is identical to the relation for the alcohols[13,15]. The correlation between the AH_{50} % and the $P_{m/b}$ is shown in Fig. 7.

The anesthetizing membrane concentration

The Meyer–Overton rule of anesthesia states that the concentration in the "lipid phase" (or hydrophobic phase) should be around 0.02 to 0.08 moles/l when anesthesia occurs[16,12,13]. It is possible to calculate the phenol concentration in the membrane phase, using the data already presented, and using Eq. 1.

$$c^{50\%/o}_{membrane} = (AH_{50\%/o}) \cdot \frac{P_{o/b}}{5} \quad (1)$$

where $(P_{o/b})/5$ is the calculated membrane/buffer partition coefficient, using the relation presented in Fig. 6.

The calculated values for $c^{50\,\%}_{membrane}$ are tabulated in the last column of Table I.

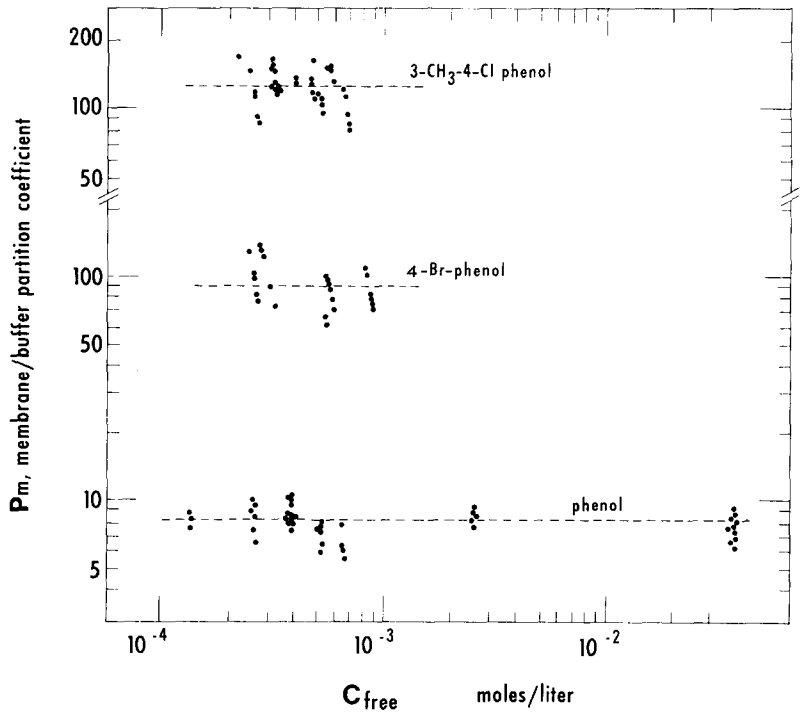

Fig. 5. Showing that the membrane/buffer partition coefficients are independent of the free concentrations of the phenol anesthetics.

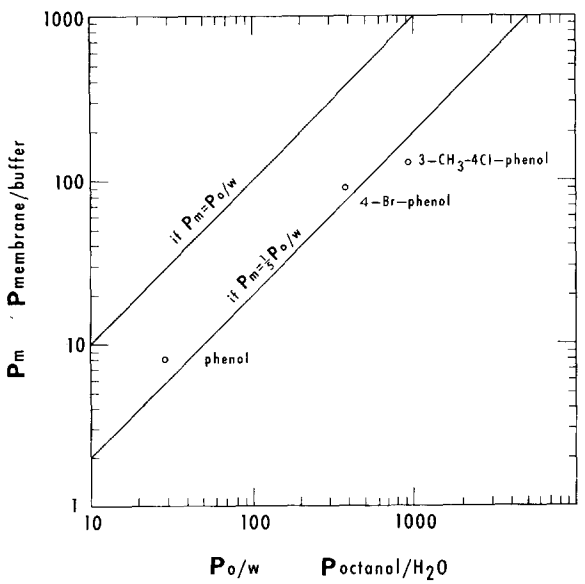

Fig. 6. Demonstrating that the membrane/buffer partition coefficients are of the order of one-fifth the octanol/water partition coefficients of the substituted phenols. The top line shows the relation expected if the two sets of coefficients were identical, the lower line if the two sets differed by a factor of 5; the values for phenol, 4-Br-phenol and 3-CH_3-4-Cl-phenol fall near the latter line.

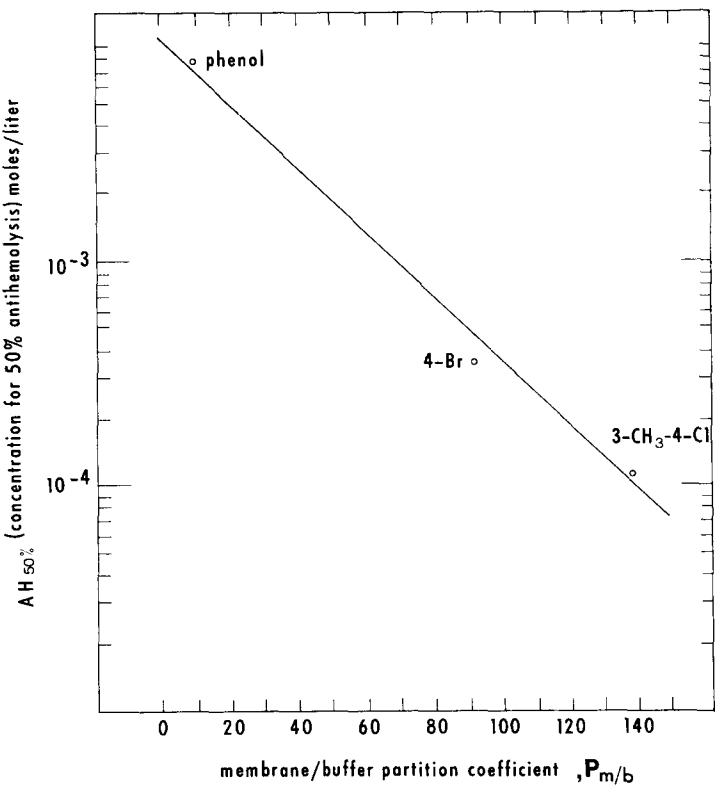

Fig. 7. The correlation between the anti-hemolytic potencies (AH_{50} %) and the membrane/buffer partition coefficients for 3 phenols. Compare with Fig. 2.

The values for $c^{50\,\%}_{\text{membrane}}$ range from 0.02 to 0.07 mole/kg dry membrane with the mean value at about 0.04 mole/kg dry membrane.

DISCUSSION

The three main findings are: (1) That the anti-hemolytic potencies of the phenols are directly related to the membrane/buffer or octanol/buffer partition coefficients, the correlation coefficient with $P_{o/b}$ being 0.963. (2) That the membrane/buffer partition coefficients for the phenols are about one-fith the octanol/buffer partition coefficients. And (3), most important of all, that the anesthetizing membrane concentrations of the phenols are between 0.02 and 0.08 mole/kg dry membrane, in approximate agreement with the rule of anesthesia predicted by MEYER[1] and OVERTON[2] in 1899.

The correlation between AH_{50} % and the partition coefficient

It has been demonstrated repeatedly that the narcotic activity of miscellaneous anesthetics correlates much better with the nonaqueous/aqueous partition coefficient than with any other parameter of the anesthetic molecule, such as polarizability, molecular weight, or molar attraction constant[15,17]. The new feature about

the present results is that this general correlation between narcotic potency and partition coefficient now extends to the membrane/buffer partition coefficient as well. This correlation provides further support to the classical idea that the membrane-anesthetic interaction is hydrophobic in nature (see refs. 13, 18 and 19 for further discussion).

The membrane/buffer partition coefficients for the phenols

The adsorption isotherms of 4-Br-phenol, [^{14}C]phenol and 3-CH_3-4-Cl-phenol (Figs. 3 and 4) indicate: (1) that in the membrane-stabilizing region there is one set of binding sites: (2) that the affinity constants of these binding sites are 63.72 l/mole for phenol, 409.4 l/mole for 3-CH_3-4-Cl-phenol and 1140.4 l/mole for4-Br-phenol, where the affinity constant is calculated according to Refs. 41 and 42; (3) that the mean number of binding sites for the phenols is 161 mmoles/kg dry membrane; (4) that the membrane/buffer partition coefficient for each phenol is independent of the free phenol concentration, a situation similar to that found with the alcohols[13]; this differs from the anesthetic amines, such as chlorpromazine, which have partition coefficients which fall markedly as the C_{free} increases[12]; and (5) that the membrane/buffer partition coefficient for each phenol is approximately one-fifth the octanol/buffer partition coefficient. This finding indicates that the membrane is considerably more hydrophilic than octanol. This is not surprising since it is known that the membrane is 30 % hydrated[20]. The correlation between $P_{m/b}$ and $P_{o/b}$ is the same for both phenols and alcohols[13], but the situation with amines or acids is much more complicated[22].

The anesthetic potency of the phenols

There is a paucity of information concerning the anesthetic effect of phenols on nerve fibers[21]. It is known, however, that phenol concentrations of 10 mM or higher block action potentials of A and C fibers[21] and that the minimum concentration of phenol required for blocking the action potential of single sartorius muscle fibers (frog) is 10 mM (ref. 22). These values compare with a value of 7.8 mM for the AH_{50} % for phenol.

The active form of the phenol anesthetic

In solution the phenol compound (designated by HP) dissociates into H^+ and P^-. Since the majority of the pK_a values for the phenols range between 9 and 10, this means that the majority (over 99 %) of the phenol molecules are in the undissociated form (HP) with 1 % or less in the ionized form (P^-). For 2,4-dichlorophenol, however, only about 85 % is in the undissociated form. The pH in the membrane phase is not known, and it may be that the percentage of ionized molecules may be very different in the membrane region. These experiments, therefore, do not indicate which form of the phenol is active in anesthetizing or expanding the membrane. The fact that the AH_{50} % in relation to the pK_a has a low correlation coefficient (0.247) suggests that the ionized form of the phenol is not important under the conditions of the present experiments.

The Meyer-Overton rule of anesthesia

The values of $C^{50\,\%}_{membrane}$ for the phenols all ranged between 0.02 and 0.08 mole/kg dry membrane, almost exactly as predicted by the Meyer-Overton rule of anesthesia.

This is a small range of variation (4-fold) compared to the 1000-fold variation in the range of the AH_{50} %. Together with the results on chlorpromazine[12], such data provide strong and direct support for the validity of the Meyer–Overton rule for neutral and positively charged anesthetics.

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