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The effect of anesthetics (chlorpromazine, ethanol) on erythrocyte permeability to water

The results presented in this study indicate that anesthetics increase the hydraulic permeability coefficient of the human erythrocyte membrane. The experiments were carried out for the following two reasons.

(1) SHANES[1,2] and SKOU[3] predicted that local anesthetics would reduce the passive permeability of the membrane to cations and other molecules. The passive permeability of erythrocytes to K^+ is increased by phenothiazines[4] (W. KWANT AND P. SEEMAN, unpublished) and by the alcohol anesthetics[5,6]. The effect of anesthetics on the passive hydraulic permeability coefficient (L_p) of human erythrocytes[7-9] was studied, therefore.

(2) All lipid-soluble anesthetics have an anti-hemolytic effect at concentrations which are virtually identical to those which anesthetize nerve fibers[4,10,11]. This protective effect may be explained by the fact that the compounds expand the membrane area of the erythrocyte by 1–6 % (refs. 12, 13). The anesthetics also "fluidize" the membrane, disordering the membrane components[15,16]. Since expansion and fluidization of the membrane might increase or decrease the membrane permeability, it is necessary to measure directly the equivalent pore radius[17] or the L_p.

The hydraulic conductivity, L_p, was measured by the stop-flow method described by SHA'AFI *et al.*[7]. For each separate measurement, L_p was determined by computer averaging and least-square fitting of data from at least three runs and three controls. The anesthetics were added to both the isosmolar medium (containing the erythrocytes) and the hyperosmolar medium in identical concentration. Stearic acid was dissolved in ethanol and then added to the media; the final ethanol concentration in the stearic acid series was always 0.013 M. Chlorpromazine hydrochloride was kindly donated by Poulenc Laboratories, Montreal. All other compounds were from Fisher Chemical Co., Toronto.

The results, shown in Fig. 1 and Table I, indicate that chlorpromazine and stearic acid increased the hydraulic permeability of the erythrocyte membrane. The optical method used precluded obtaining L_p values at high concentrations of stearic acid because of turbidity. Concentrations of chlorpromazine above $2 \cdot 10^{-5}$ M caused hemolysis, presumably because of the surface activity of this compound[18].

It is evident from Table I and Fig. 1 that pentanol, hexanol and octanol increase L_p with increasing concentration. At first observation ethanol appears to decrease L_p. It has been shown, however, that increasing the osmolality of the medium decreases hydraulic conductivity of red cells[9]. After correcting the observed L_p values of the ethanol-treated cells for this osmolality effect it can be seen that ethanol increased L_p to a value greater than expected for an inactive solute. At 0.35 M ethanol the osmolality effect should reduce L_p from the control value of $0.87 \cdot 10^{-11}$ $cm^3 \cdot dyne^{-1} \cdot sec^{-1}$ (Table I) to $0.55 \cdot 10^{-11}$ $cm^3 \cdot dyne^{-1} \cdot sec^{-1}$. Since the observed value at 0.35 M ethanol was $0.74 \cdot 10^{-11}$ $cm^3 \cdot dyne^{-1} \cdot sec^{-1}$ ethanol increased the permeability of the membrane to water by 34 % (see Fig. 1). The results for hexanol are erratic, presumably because of impurities (Fisher "practical" grade was used as supplied); the work will have to be repeated with purer hexanol. Variations between experiments may arise from slightly different hematocrits and from different time intervals elapsed before the L_p was measured.

The concentration range chosen for both chlorpromazine and the alkanols is the same as that for local anesthesia (see refs. 3–5). Stearic acid, $1 \cdot 10^{-5}$ M, is estimated to be anesthetic[19, 20].

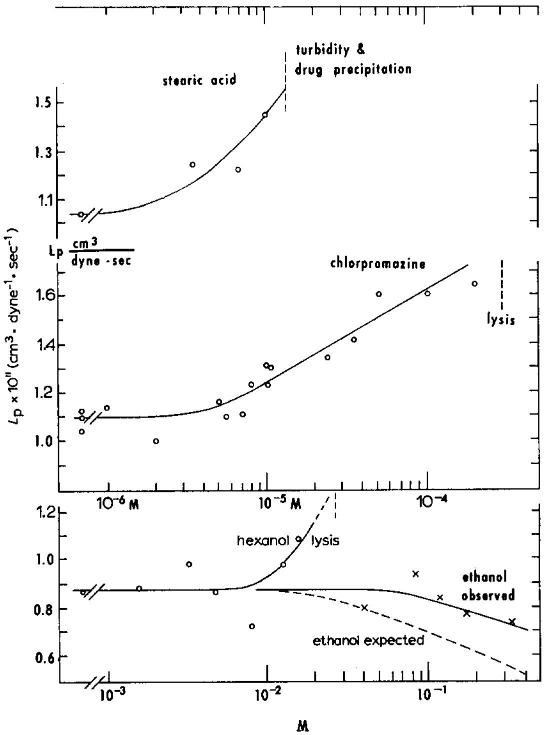

Fig. 1. Anesthetics increase the hydraulic permeability of the erythrocyte membrane. The hydraulic coefficient, Lp, has dimensions of 10^{-11} $cm^3 \cdot dyne^{-1} \cdot sec^{-1}$. The open circles in the bottom graph represent hexanol. Hexanol lysis occurs at 28 mM.

The drug-induced increase in L_p may reflect an increase in the hydrated state of the membrane, normally estimated[21–23] to be 20–40 % water.

The effect of anesthetics on L_p does not appear to be related to the concentration of Ca^{2+} in the membrane since chlorpromazine and stearic acid displace Ca^{2+} from the membrane, while ethanol and other low alkanols markedly increase the membrane concentration of Ca^{2+} (refs. 24, 25).

The present results do not support PAULING's[26] clathrate theory of anesthesia, but rather suggest that the membrane water is in a more mobile state in the presence of an anesthetic, in agreement with other work[15].

In summary, this work shows that neutral, positive and negatively charged anesthetics all increase the water permeability of the membrane. This finding is in agreement with the increase in passive permeability that occurs with alcohols[6] and with chlorpromazine[4].

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TABLE I

EFFECT OF ANESTHETICS ON THE HYDRAULIC PERMEABILITY OF THE ERYTHROCYTE MEMBRANE

Drug	*Drug concn.* (*M*)	$L_p \times 10^{11}$ ($cm^3 \cdot dyne^{-1} \cdot sec^{-1}$)*	*% Increase of* L_p
Ethanol	0	0.87	0
5 Sept. '69	0.04	0.80	1**
	0.084	0.94	29**
	0.120	0.84	22**
	0.174	0.77	20**
	0.350	0.74	34**
Pentanol	0	0.96	0
6 Aug. '69	$1.85 \cdot 10^{-2}$	1.34	40
Hexanol	0	0.84	0
19 Nov. '69	$1.58 \cdot 10^{-3}$	0.84	0
	$3.17 \cdot 10^{-3}$	0.96	14
	$4.77 \cdot 10^{-3}$	0.87	4
	$7.95 \cdot 10^{-3}$	0.72	−14
	$1.27 \cdot 10^{-2}$	0.98	17
	$1.58 \cdot 10^{-2}$	1.03	23
Octanol	0	0.84	0
19 Nov. '69	$1.24 \cdot 10^{-4}$	0.75	−11
	$3.75 \cdot 10^{-4}$	0.86	3
6 Aug. '69	$2.5 \cdot 10^{-4}$	1.49	55
Chlorpromazine	0	1.04	0
6 Aug. '69	$1 \cdot 10^{-6}$	1.21	16
	$2 \cdot 10^{-6}$	0.99	− 5
	$5 \cdot 10^{-6}$	1.16	12
	$8 \cdot 10^{-6}$	1.23	18
	$1 \cdot 10^{-5}$	1.31	25
	$2.4 \cdot 10^{-5}$	1.34	30
	$5 \cdot 10^{-5}$	1.60	54
7 Aug. '69	0	1.10	0
	$1 \cdot 10^{-5}$	1.30	18
	$3.5 \cdot 10^{-5}$	1.41	28
	$1 \cdot 10^{-4}$	1.60	45
	$2 \cdot 10^{-4}$	1.64	49
5 Aug. '69	0	1.13	0
	$1 \cdot 10^{-6}$	1.14	1
	$5.5 \cdot 10^{-6}$	1.10	− 3
	$7 \cdot 10^{-6}$	1.11	− 2
	$1 \cdot 10^{-5}$	1.23	9
Stearic acid	0	1.14	0
4 Oct. '69	$3.5 \cdot 10^{-6}$	1.34	18
	$7 \cdot 10^{-6}$	1.13	15
	$1 \cdot 10^{-5}$	1.55	36

* Each value is an average of at least three separate determinations.

** Correction has been made for the osmolality of the final medium containing the drug (see text and ref. 9).

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