Ionic Permeability of Thin Lipid Membranes

Effects of n-alkyl alcohols, polyvalent cations, and a secondary amine

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ABSTRACT Ultrathin (black) lipid membranes were made from sheep red cell lipids dissolved in *n*-decane. The presence of aliphatic alcohols in the aqueous solutions bathing these membranes produced reversible changes in the ionic permeability, but not the osomotic permeability. Heptanol (8 mM), for example, caused the membrane resistance (R_m) to decrease from $>10^8$ to about 10^5 ohm-cm^2 and caused a marked increase in the permeability to cations, especially potassium. In terms of ionic transference numbers, deduced from measurements of the membrane potential at zero current, T_{cat}/T_{Cl} increased from about 6 to 21 and T_K/T_{Na} increased from about 3 to 21. The addition of long-chain (C_8–C_{10}) alcohols to the lipid solutions from which membranes were made produced similar effects on the ionic permeability. A plot of log R_m vs. log alcohol concentration was linear over the range of maximum change in R_m, and the slope was -3 to -5 for C_2 through C_7 alcohols, suggesting that a complex of several alcohol molecules is responsible for the increase in ionic permeability. Membrane permselectivity changed from cationic to anionic when thorium or ferric iron (10^{-4} M) was present in the aqueous phase or when a secondary amine (Amberlite LA-2) was added to the lipid solutions from which membranes were made. When membranes containing the secondary amine were exposed to heptanol, R_m became very low (10^3–10^4 ohm-cm^2) and the membranes became perfectly anion-selective, developing chloride diffusion potentials up to 150 mv.

INTRODUCTION

Studies on bimolecular (black) lipid membranes are contributing to our understanding of the molecular structure and function of natural membranes. Although these synthetic lipid membranes are similar in several respects to biological membranes, their intrinsic permeability to ions is relatively low,

i.e., their electrical resistance is 10^7–10^9 ohm-cm^2. Various modifying agents, however, increase ionic permeability and induce varying degrees of ionic selectivity in synthetic lipid membranes (1–4).[1,2] This paper represents part of a continuing effort to identify substances which can increase ionic permeability and produce ionic selectivity in membranes made from sheep erythrocyte lipids. We will describe the effects of some alcohols, polyvalent cations, and a secondary amine on the ionic permeability of thin lipid membranes. We find that alcohols can greatly increase the cation, particularly potassium, permeability of black lipid films. However, alcohols can increase anion, rather than cation, conductance under certain conditions, e.g., when the membrane is positively charged either by adding polyvalent cations to the aqueous phase or by adding a secondary amine to the membrane-forming solution.

METHODS

Lipid Extraction and Analytical Methods

Blood from high potassium (HK) or low potassium (LK) sheep was drawn into a flask containing heparin. The plasma and buffy coat were removed by centrifugation, and the cells were washed three times in cold 0.16 M NaCl. The cells were then hemolyzed in cold 20 milliosmolar Tris-chloride, pH 7.65, and washed repeatedly with this solution until the membranes were white or cream-colored. The membranes were then washed three times with cold, distilled, deionized water and fractionated in a cold butanol-water system (5). About 30 ml of water-washed membranes (about 2 mg protein per ml) was shaken vigorously for 20 sec with 15 ml of cold *n*-butanol. After 20-25 min the white emulsion was centrifuged at 15,000 × *g* for 15 min. Centrifugation resolved the mixture into a lower butanol-saturated water phase and an upper water-saturated butanol phase, separated by a thin interfacial film. The butanol phase was then washed with an equal volume of cold water and centrifuged again. The butanol-lipid solution was collected in a cold syringe, transferred to an evaporating flask, and taken to dryness under vacuum. The lipids were then redissolved in chloroform to give a concentration of about 10 mg/ml. This colorless solution was stored at −16°C for up to 3 months without obvious signs of deterioration.

When this extraction procedure was used, we recovered about 88% of the original ghost phospholipids (estimated by phosphorous content [6, 7]) in the butanol phase and about 7% of the phospholipids in the aqueous phase. We found about 84% of the original ghost protein (measured by a modified Lowry method [8, 9]) in the aqueous phase and about 0.3% of the original protein in the butanol phase. These percentage recoveries of solubilized lipid and protein are similar to those reported

[1] Mueller, P., and D. O. Rudin. 1969. Translocators in bimolecular lipid membranes: their role in dissipative and conservative bioenergy transductions. *In* Current Topics in Bioenergetics. D. R. Sanadi, editor. Academic Press Inc., New York. 3: 157.

[2] Pressman, B. C., and D. H. Haynes. 1969. Ionophorous agents as mobile ion carriers. *In* Symposium on the Molecular Basis of Membrane Function. D. C. Tosteson, editor. Prentice Hall, Englewood Cliffs, N.J. 221.

by Maddy (5) and others (10, 11), using various mammalian (including sheep) erythrocytes.

We have not identified chemically the various lipids in our butanol extracts of erythrocyte ghosts. In various mammalian erythrocytes, butanol fractionation produces a roughly symmetrical distribution of lipids between the butanol and water phases, except for a high proportion of phosphatidylserine in the aqueous phase (10, 11). With this qualification we assume that the composition of our lipid extracts is similar to that reported for HK and LK sheep red cells (12), i.e., sphingomyelin 48%, phosphatidylethanolamine 29%, phosphatidylserine 14%, and phosphatidylinositol 4%. Cholesterol is also extracted by butanol (11), and in sheep erythrocytes the cholesterol:phospholipid molar ratio is 1:1 (14). The lipid composition of the black film may, of course, differ greatly from the lipid composition of the bulk solution from which membranes are made. We will not distinguish further between lipids from HK and LK erythrocytes, because these lipids are virtually identical in composition (14) and produce synthetic lipid membranes with similar properties (15).

In some experiments we used lipids extracted from a sheep red cell hemolysate with isopropanol-chloroform (15), and we also occasionally used human erythrocyte lipids extracted from ghosts with butanol. The results we obtained with these two lipid extracts, although incomplete, were similar to the results we obtained with butanol-extracted lipids of sheep red cell membranes.

Membrane Formation and Electrical Measurements

Membranes were formed from a solution of lipids in decane at a concentration of 20–30 mg/ml. Molar concentrations were estimated by assuming average molecular weights of 750 for phospholipids and 400 for cholesterol. The lipid solution was brushed across a circular hole (1.25 mm diameter) in a polyethylene partition separating two aqueous compartments in a Lucite chamber, described in detail by Andreoli et al. (15). The rear chamber was closed but was connected to a microsyringe by which volume adjustments could be made as necessary to keep the membrane planar. The solution in the front compartment was changed when necessary by means of a pump and aspirator (15). Most experiments were performed at 22–24°C, at which temperature the films became optically black in 2–10 min from the time of formation.

Membrane resistance (R_m), membrane voltage (V_m), and ionic transference numbers (T_i) were measured as described by Andreoli et al. (15). Briefly, the DC membrane resistance was calculated, using Ohm's law, from the V_m produced by applying a calibrated pulse (usually 40 mv) across the membrane plus a known resistance in series with the membrane. V_m was recorded as the potential difference between two calomel-KCl electrodes which made direct contact with the front and rear solution. Ionic transference numbers (T_i) were estimated from the steady V_m which developed in the presence of known activity gradients of Na, K, and Cl across the membrane. T_i was calculated from the relations $V_m = \sum_{i=1}^{n} T_i E_i$ and $\sum_{i=1}^{n} T_i = 1$. T_i is defined as G_i/G_m, where G_i is the conductance of the ith ion and G_m is the total membrane conductance. E_i is the equilibrium potential for the

ith ion, i.e., $\simeq 59 \log \frac{a_i^F}{a_i^R}$, where a_i^F and a_i^R are the ion activities in front and rear. The assumptions made in calculating transference numbers for Na, K, and Cl are that (*a*) electric charge is carried across the membrane only by these three ions and (*b*) concentration and electrical potential differences are the only significant driving forces for ion transport.

RESULTS

Effects of n-Alkyl Alcohols on Membrane Resistance

The effects of ethanol, pentanol, and heptanol on the electrical resistance of ultrathin lipid membranes are shown in Fig. 1. Similar curves were obtained

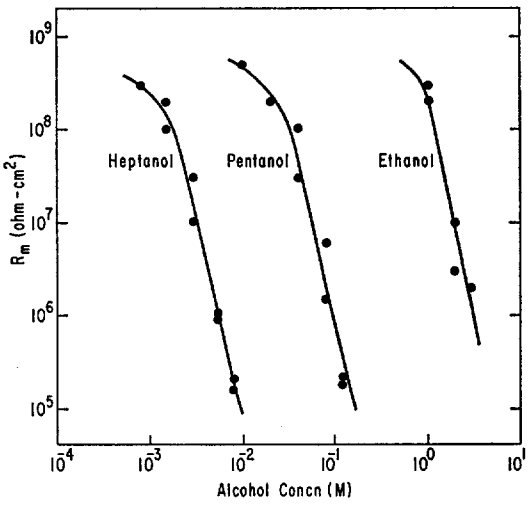

FIGURE 1. Effects of some *n*-alkyl alcohols on the DC resistance of ultrathin lipid membranes. Aqueous phase contained KCl (100 mM) plus alcohols at the concentrations indicated. Each point represents a single membrane.

with propanol, butanol, and hexanol. The membranes were formed with identical concentrations of salt (0.1 M KCl) and alcohol in front and rear compartments. The values shown in Fig. 1 represent stable resistances which varied by less than a factor of 2 over 30–60 min. The time required for R_m to reach this stable value varied from about 15 min with ethanol to about 30 min with heptanol. Alcohols at the concentrations shown in Fig. 1 produced no conspicuous effects on the stability, thinning rate, or surface properties of the films. However, higher concentrations of alcohols, e.g., concentrations sufficient to produce R_m's below 10^5 ohm-cm^2, usually caused the films to rupture. The alcohol effect was completely reversible, as demonstrated by forming a membrane in an alcohol-free solution, flowing alcohol into the front chamber, observing the characteristic decrease in R_m, then replacing the alcohol solution with an alcohol-free solution and observing a return of R_m to the control value within 10–20 min for short-chain alcohols or 30–40 min for long-chain

alcohols. Only optically black films showed a low R_m in the presence of alcohols, and even the addition of alcohols to the membrane-forming solutions did not produce low resistance membranes prior to the appearance of black areas. These observations argue against the possibility that the alcohol effect is due to electrical leakage through the torus at the perimeter of the membrane. Membrane resistances were constant over a range of ±90 mv, either in the absence (16) or presence of alcohols in the aqueous phase.

Table I shows the concentration of *n*-alkyl alcohols in 0.1 M KCl required to lower R_m from $>10^8$ to 10^6 ohm-cm². For a given aqueous concentration of alcohol, long-chain alcohols were much more effective than short-chain alcohols in reducing membrane resistance. However, when alcohol concentrations were expressed as thermodynamic activities, i.e., as fractions (C/C_o) of saturated aqueous solutions, the order of effectiveness was reversed and the differences between alcohols became much smaller. If equal thermodynamic activities result in equal numbers of molecules of the *n*-alkyl series penetrating the membrane (as observed in lipid monolayers by Bangham et al. (17)), then butanol is two to three times more effective per molecule than heptanol in reducing R_m (Table I).

TABLE I

EFFECTS OF *n*-ALKYL ALCOHOLS ON ELECTRICAL RESISTANCE OF THIN LIPID MEMBRANES

Alcohol	Concentration in aqueous phase required to give $R_m \simeq 10^6$ ohm-cm² (C)	Saturation concentration* (C_O)	Thermodynamic activity at $\simeq 10^6$ ohm-cm² (C/C_O)
	M	*M*	
Ethanol	3.0	Miscible	—
n-Propanol	1.4	Miscible	—
n-Butanol	0.30	1.07	0.28
n-Pentanol	0.10	0.306	0.33
n-Hexanol	0.028	0.058	0.48
n-Heptanol	0.0054	0.0078	0.69
n-Octanol	>0.00038	0.00038†	>1.0

The front and rear compartments contained KCl (0.1 M) plus alcohols at the concentrations indicated.

* From Handbook of Chemistry and Physics. 1960. Chemical Rubber Publishing Co., Cleveland, Ohio. 42nd edition.

† From Kelley, L. 1957. Organic Chemistry. McGraw-Hill Book Co., New York. 108.

The presence of cholesterol in the membrane was not necessary for the alcohol effect. Membranes formed from cholesterol-free (i.e., acetone-extracted [18]) lipids showed the same R_m in the presence of heptanol as did cholesterol-containing membranes.

The osmotic permeability of these membranes to water, measured as de-

scribed by Andreoli et al. (19), was not appreciably affected by alcohol. The osmotic permeability coefficient, either with or without heptanol (7.8 mM) in the aqueous phase, was 2–3 $\times$ 10^{-3} cm sec^{-1} (19).

Water-insoluble alcohols, octanol, nonanol, and decanol, were added to the lipid solutions from which membranes were formed. These alcohols produced low resistance membranes, but large amounts of alcohol relative to lipid were required (Fig. 2). For example, to produce a membrane resistance of about 10^6 ohm-cm^2, molar ratios of octanol:lipid of about 40 or decanol:lipid of about 65 were required. In membranes containing octanol or nonanol, but not decanol, R_m increased slowly with time, suggesting a slow diffusion of these alcohols from the membrane into the aqueous phase. Octanol could also be

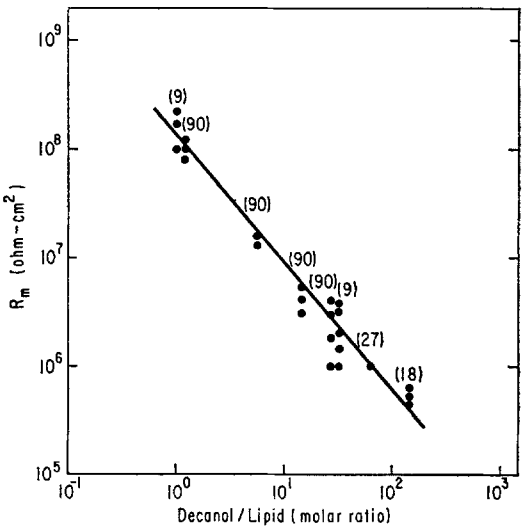

FIGURE 2. Electrical resistance of ultrathin lipid membranes as a function of the decanol: lipid molar ratio in the membrane-forming solution. Aqueous phase contained 100 mM KCl. Numbers in parentheses are the approximate concentrations (mM) of red cell lipids in the membrane-forming solution. Each point represents a single membrane.

added in small amounts to the aqueous phase (Table I), but the effect on membrane resistance was small.

The increase in membrane conductance with increasing decanol:lipid molar ratios (Fig. 2) suggests, but does not prove, a competition between alcohol and lipid molecules. Alternatively, decanol might compete with the solvent decane, i.e., the drop in R_m might reflect the replacement of decane by decanol in the nonpolar region of the membrane. This possibility was tested by the formation of membranes from lipid solutions in which the decanol:decane molar ratio varied while the lipid:decanol ratio was held constant. As shown in Fig. 3, membrane resistance was not sensitive to the decanol:decane ratio, which supports the hypothesis that the increase in membrane conductance is due primarily to a replacement of lipid molecules by decanol.

The slope of the log R_m vs. log alcohol concentration curves for C_2 through

C_7 alcohols was -3 to -5 (Fig. 1), suggesting that a complex of several alcohol molecules is responsible for the increase in membrane conductance. For octanol (not shown) the slope of the log R_m vs. log octanol:lipid molar ratio curve was about -2, and for decanol the slope was about -1 (Fig. 2), suggesting a somewhat different mode of action for these alcohols.

The effect of salt concentration on membrane resistance in the presence and absence of heptanol is shown in Fig. 4. In the absence of alcohols, membrane resistance was independent of salt concentration up to about 0.25 M (16). In the presence of 7.8 mM heptanol, R_m decreased markedly with increasing KCl concentration, but decreased only slightly with increasing NaCl concentration. The slope of the log R_m vs. log [KCl] curve was about -0.5, i.e., the membrane conductance was roughly proportional to the square root of the KCl

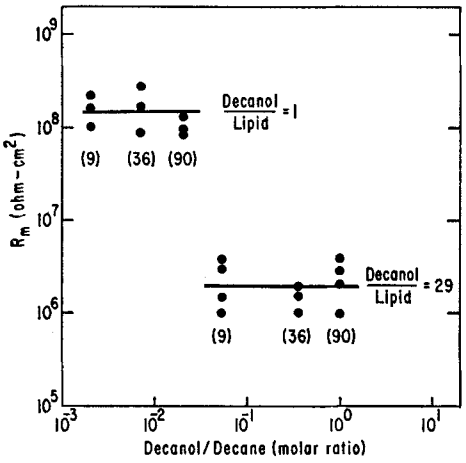

FIGURE 3. Electrical resistance of ultrathin lipid membranes as a function of the decanol: decane molar ratio in the membrane-forming solution. Aqueous phase contained 100 mM KCl. The numbers in parentheses are the approximate concentrations (mM) of red cell lipids in the membrane-forming solutions. Each point represents a single membrane.

concentration. Preliminary experiments suggest that the alcohol-induced increase in membrane conductance at very low salt concentrations (Fig. 4) is due to hydrogen ion conductance.

Effects of Alcohols on Ionic Selectivity

Ionic transference numbers for Na, K, and Cl were estimated by measuring concentration potentials (e.g., 0.1 M KCl, 0.01 M NaCl vs. 0.01 M KCl, 0.001 M NaCl) and biionic potentials (e.g., 0.1 M KCl, 0.01 M NaCl vs. 0.01 M KCl, 0.1 M NaCl), as described by Andreoli et al. (15). Table II shows that heptanol (7.8 mM) increased the permeability of black lipid membranes to potassium, relative to both sodium and chloride. In terms of ionic transference numbers, $T_{cat}:T_{Cl}$ increased from about 6 to 21 and $T_K:T_{Na}$ increased from about 3 to 21 in the presence of heptanol. Similar results were obtained for other *n*-alkyl alcohols. Ionic transference numbers were independent of pH over the range of pH 5–7 in either the presence or absence of alcohols.

An aromatic alcohol, guaiacol (2-methoxyphenol), also produced good K selectivity when present at an aqueous concentration of 30 mM (Table II). The ability of thick guaiacol membranes to discriminate between Na and K was first observed by Osterhout (44), who proposed the guaiacol membrane as a model for the K transport system in *Valonia*, a marine alga.

The transport number sequence for the alkali metal ion series was determined by measuring V_m when front and rear chambers contained equal concentrations of two different salts, e.g., 0.1 M KCl vs. 0.1 M NaCl. In the absence of alcohols the relative transport numbers were in the sequence Rb > K > Cs > Na > Li. Alcohols caused a reversal in the order of Rb and K in this series and increased the difference between the transport numbers for Rb and K compared to Na and Li.

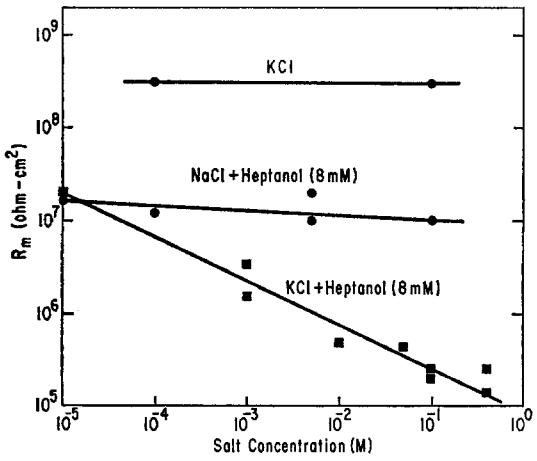

FIGURE 4. Electrical resistance of ultrathin lipid membranes as a function of salt concentration in the presence and absence of heptanol. Each point represents a single membrane.

The potassium selectivity induced by alcohol was observed only in thin (black) membranes rather than in thick (colored) films. For example, with lipid solutions containing octanol:lipid at a molar ratio of 26, $T_{cat}:T_{Cl}$ was 3 and $T_K:T_{Na}$ was 1.5 before any black areas appeared in the membrane. With the appearance of black areas, $T_{cat}:T_{Cl}$ increased rapidly to about 32 and $T_K:T_{Na}$ increased to about 26. This absence of potassium selectivity induced by alcohol in thick membranes was confirmed by making membranes 3 mm thick in a Lucite spacer where the lipid-water interfaces were formed with cellophane dialysis tubing (20). As shown in Table III, the addition of octanol to these thick lipid membranes produced no change in the transference numbers for Na, K, or Cl. Similar results were obtained when the lipid solution used for making the thick membranes was first equilibrated with a large volume of 0.1 M KCl and 8 mM heptanol. This absence of drug-induced ion selectivity in thick membranes differs from the behavior of membranes con-

taining valinomycin or monactin-dinactin in which K selectivity is produced in thick as well as in thin membranes (20, 21).

Effects of Alcohols on Membranes Containing Polyvalent Cations or Secondary Amines

The cationic permselectivity of membranes made from sheep red cell lipids (Table II) may be attributable to the net negative charge on some membrane components, i.e., phosphatidylinositol, phosphatidylserine, and possibly phosphatidylethanolamine (13, 15). If so, we would expect that conditions

TABLE II

EFFECT OF *n*-HEPTANOL AND GUAIACOL ON IONIC PERMEABILITY OF THIN LIPID MEMBRANES

Alcohol	Salt solutions				Transference Nos. (approximate)			
	Front	Rear	R_m	V_m	T_{cat}	T_{Cl}	T_{Na}	T_K
	mM	*mM*	*ohm-cm²*	*mv*				
None	KCl 10 NaCl 1	KCl 100 NaCl 10	10^8	−30 to −45	0.86	0.14	—	—
Heptanol (7.8 mM)	KCl 10 NaCl 1	KCl 100 NaCl 10	10^5	−47 to −53	0.96	0.046	—	—
Guaiacol (30 mM)	KCl 10 NaCl 1	KCl 100 NaCl 10	10^7	−48 to −49	0.94	0.055	—	—
None	KCl 10 NaCl 100	KCl 100 NaCl 10	10^8	−10 to −35	—	—	0.22	0.64
Heptanol (7.8 mM)	KCl 10 NaCl 100	KCl 100 NaCl 10	10^6	−49 to −52	—	—	0.044	0.91
Guaiacol (30 mM)	KCl 10 NaCl 100	KCl 100 NaCl 10	10^7	−50 to −53	—	—	0.030	0.91

Membranes were formed with identical solutions in front and rear compartments. After the membrane was optically black, the front solution was changed and the steady-state V_m was recorded. V_m is the potential of the rear, relative to the front (ground), chamber. Values of V_m represent the range observed in five to nine experiments in which at least two different lipid preparations were used. Transference numbers were estimated as described in the Methods section.

which alter the negative surface charge might alter the permselective properties of the film. Such conditions might be, for example, low pH, the presence of polyvalent cations in the aqueous phase, or the inclusion of positively charged components in the membrane itself. We have found, as expected, that all these conditions can produce anion permselectivity in ultrathin lipid membranes. Here we will describe the apparent interactions between alcohols and agents which induce anion selectivity.

In the absence of alcohols, the polyvalent cations Th^{4+} and Fe^{3+} (10^{-4} M) in the aqueous phase reversed the permselectivity of thin lipid membranes so that, for example, a 10-fold concentration gradient of NaCl or KCl generated an anionic rather than cationic V_m of 30–45 mv (Table IV). Membrane re-

TABLE III

EFFECT OF *n*-OCTANOL ON IONIC PERMEABILITY OF THICK LIPID MEMBRANES

Membrane-forming solution	Salt solutions		R_m	V_m	Transference Nos. (approximate)			
	Front	Rear			T_{cat}	T_{Cl}	T_{Na}	T_K
	mM	*mM*	*ohm-cm²*	*mv*				
Red cell lipids (4 mM) in decane	KCl 10 NaCl 1	KCl 100 NaCl 10	3×10^9	−30 to −34	0.80	0.20	—	—
Red cell lipids (4 mM) plus *n*-octanol (400 mM)	KCl 10 NaCl 1	KCl 100 NaCl 10	6×10^8	−30 to −33	0.80	0.20	—	—
Red cell lipids (4 mM) in decane	KCl 10 NaCl 100	KCl 100 NaCl 10	3×10^9	−6 to −10	—	—	0.33	0.47
Red cell lipids (4 mM) plus *n*-octanol (400 mM)	KCl 10 NaCl 100	KCl 100 NaCl 10	6×10^8	−7 to −8	—	—	0.33	0.47

Membranes were made in a Lucite spacer, 3 mm thick, with cellophane dialysis tubing separating the lipid and aqueous phases, as described by Tosteson et al. (20). The surface area of the membrane was 0.65 cm². The lipid concentration of 4 mM corresponds to a concentration by weight of about 2 mg/ml. The values of V_m represent the range observed in two experiments with a single lipid preparation.

TABLE IV

EFFECT OF *n*-HEPTANOL, THORIUM, AND A SECONDARY AMINE ON IONIC PERMSELECTIVITY OF THIN LIPID MEMBRANES

Membrane-forming solution	Modifying agent in aqueous phase	Salt solutions		R_m	V_m	Transference Nos. (approximate)	
		Front	Rear			T_{cat}	T_{Cl}
		mM	*mM*	*ohm-cm²*	*mv*		
Red cell lipids (20 mg/ml) in decane	Th^{4+} (0.1 mM)	KCl 10 NaCl 1	KCl 100 NaCl 10	10^8–10^9	30–45	0.16	0.84
Red cell lipids (20 mg/ml) in decane	Th^{4+} (0.1 mM) Heptanol (8 mM)	KCl 10 NaCl 1	KCl 100 NaCl 10	10^5–10^6	30–42	0.17	0.83
Red cell lipids (5 mg/ml), Amberlite LA-2 (400 mg/ml), decane (600 mg/ml)	None	KCl 10 NaCl 1	KCl 100 NaCl 10	10^5–10^6	50–53	0.04	0.96
Red cell lipids (5 mg/ml), Amberlite LA-2 (400 mg/ml), decane (600 mg/ml)	Heptanol (5–8 mM)	KCl 10 NaCl 1	KCl 100 NaCl 10	10^3–10^4	54–57	<0.01	>0.99

Membranes were formed with identical solutions in front and rear compartments. After the membrane was optically black, the front solution was changed and the steady-state V_m was recorded. V_m is the potential of the rear, relative to the front (ground), compartment. Values of V_m indicate the range of 4–13 measurements in which at least two different lipid preparations were used. Thorium was added as the nitrate salt.

sistance increased two- to fourfold in the presence of Th^{4+} or Fe^{3+}, suggesting that the permselectivity change was due chiefly to a reduction in cation, rather than an increase in anion, permeability. When both Th^{4+} (10^{-4} M) and heptanol (7.8 mM) were present in the aqueous phase, R_m decreased to 10^5–10^6 ohm-cm^2 but the anionic permselectivity remained (Table IV, Fig. 5). Thus heptanol, which increased chiefly cationic conductance in otherwise unmodified membranes, increased mainly anionic conductance in the presence of Th^{4+}. These heptanol- and thorium-containing membranes showed low Na-K selectivity; a biionic concentration gradient of the type described in Table II gave a V_m of 0 ± 1 mv, indicating a $T_K : T_{Na}$ of < 1.3. Similar results were obtained with Fe^{3+} and heptanol. The ionic selectivity changes

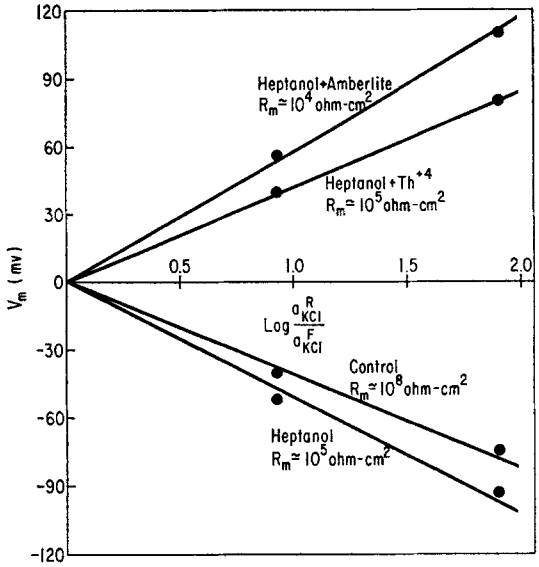

FIGURE 5. Effects of heptanol (8 mM), heptanol plus $Th(NO_3)_4$ (0.1 mM), and heptanol plus Amberlite LA-2 on the membrane voltage produced by activity gradients of KCl with 100 mM KCl in the rear compartment. Heptanol and Th^{4+} were present in the aqueous phase, whereas the Amberlite comprised 40% of the membrane-forming solution, which also contained decane (60%) and sheep red cell lipids (0.5%). Each curve represents a single membrane.

induced by Fe^{3+} and Th^{4+} were reversible; washing the membrane with the chelating agent EDTA (10^{-4} M) restored cation selectivity within 15 min. Some other polyvalent cations, La^{3+}, Ca^{2+}, and Ba^{2+}, at 10^{-4}–10^{-3} M did not alter the alcohol-induced increase in potassium conductance, although they did reduce cation transference numbers in the absence of alcohols.

The liquid anion exchanger, Amberlite LA-2 (Rohm & Haas, Philadelphia, Pa.), is a mixture of secondary amines with molecular weights ranging from 353 to 395, e.g., *n*-lauryl (trialkylmethyl) amine,

$$CH_3(CH_2)_{10}CH_2—\underset{\displaystyle H}{\overset{\displaystyle |}{N}}—\underset{\displaystyle R''}{\overset{\displaystyle R}{\overset{|}{\underset{|}{C}}}}—R',$$

where R + R′ + R″ = 11 to 14 carbons. Although we have been unable to

produce stable black films of pure Amberlite or Amberlite plus cholesterol, we were able to add large amounts of Amberlite to the sheep red cell lipid solutions. Stable membranes were formed from lipid solutions containing, for example, 60% decane, 40% Amberlite, and 0.5% red cell lipids (w/w). These films had DC resistances of 10^5–10^6 ohm-cm^2 and showed good, but not perfect, anion-selectivity (Table IV). When these Amberlite-containing membranes were exposed to heptanol (5–8 mM), R_m decreased to 10^3–10^4 ohm-cm^2, and the films became perfectly anion-selective (Table IV, Fig. 5). For example, a 10-fold KCl concentration gradient generated a steady V_m of 56.2 ± 0.8 mv (mean ± SE, n = 13 membranes), compared with a theoretical (Nernst) potential of 55 mv. Thus, in membranes containing a secondary amine, alcohol again produced a large increase in anion conductance. Anion selectivity in membranes containing the secondary amine was not affected by pH over the range 5–7, and a pH gradient of 2 units (pH 5 vs. pH 7) in 0.1 M KCl generated no potential difference across the membrane.

DISCUSSION

That alcohols can alter the permeability of natural or synthetic membranes has been known for at least 30 years (22–24), but an alcohol-induced increase in potassium selectivity as seen in our ultrathin lipid membranes (Table II, Fig. 4) has not been previously reported. In mammalian erythrocytes *n*-butanol produces a reversible cation leakage (25, p. 128), although the permeability to certain nonelectrolytes is concomitantly reduced (26, 27). In human red cells, 0.3 M butanol increases potassium permeability about 100-fold at 25°C (28), which is quantitatively similar to the effect of 0.3 M butanol on our synthetic lipid membranes (Tables I and II). In the squid axon alcohols at rather low concentrations decrease, rather than increase, cation conductance (29, 30), but the relation between the thermodynamic activity and the relative effectiveness of alcohols (29) is similar to that seen in our thin lipid membranes (Table I).

The concentrations of alcohols required to produce appreciable changes in the electrical resistance of our synthetic membranes are somewhat higher than the concentrations producing narcosis in living systems (17, 31). In liposomes, however, anesthetic concentrations of *n*-alkyl alcohols produce appreciable increases in cation leakage (17). The decane in our thin lipid membranes may reduce the sensitivity to alcohols by filling intermolecular spaces created in the core of the membrane when alcohol molecules become inserted into the lipid monolayers. This hypothesis that alcohols (or other short-chain, surface-active molecules) inserted into lipid monolayers disrupt the hydrophobic bonds between fatty acid chains of membrane lipids leads to the prediction that the relative effectiveness of alcohols in the membrane will decrease with increasing chain length (32), which is probably true in our membranes as well as in liposomes (17) and the squid axon (29).

In addition to disrupting hydrophobic bonds in lipid membranes, alcohols might increase ionic permeability by increasing the dielectric constant near charged groups and by partially replacing the water of hydration with polar hydroxyl groups. The alcohol-induced changes in ionic selectivity might arise from a shift in the balance of forces (*a*) between ions and water and (*b*) between ions and charged sites on the membrane (35, 36). It is apparent, at any rate, that the increase in potassium permeability caused by alcohols does not involve rigid steric restrictions as seen in complexes between ions and certain macrocyclic compounds (33, 34).[3] Additional information on these points may be gained by testing the ability of other organic compounds to induce ionic permeability changes as a function of their lipid solubility, dielectric constant, and molecular structure. The fact that alcohols increase ion selectivity only in bimolecular (black) lipid films and not in thick membranes (Tables II and III) will limit the utility of thick lipid membranes, which have proved useful for studying the mechanism by which certain macrocyclic antibiotics induce ionic selectivity (20).

Th^{4+}, Fe^{3+}, and secondary amines probably produce anion permselectivity by reversing the sign of the net charge on the membrane, thus making the membrane more accessible to aqueous anions than to aqueous cations. The greater effectiveness of Th^{4+} and Fe^{3+} compared to La^{3+}, Ca^{2+}, and Ba^{2+} has been observed in other systems, e.g., gall bladder (37), and may be due to the formation of hydrolysis products and complex ions of Th^{4+} and Fe^{3+}, which are probably more lipid-soluble than simple hydrated ions. Th^{4+} and Fe^{3+}, as well as the secondary amines, thus could conceivably act as fixed charges, mobile carriers, or both. Whether alcohols play a specific or nonspecific role in increasing the anion conductance of positively charged membranes remains to be determined.

A method for producing bimolecular lipid membranes with perfect anion selectivity and high conductance (Table IV, Fig. 5) has not been previously reported. The secondary amine used in our membranes is only one of several related compounds which might be expected to produce anion-selective bilayers, based on earlier studies with thick membrane systems (38). However, some substances which might be expected to produce anion selectivity in black lipid membranes evidently do not. For example, the cationic detergents, cetylpyridinium chloride and dodecylbenzene trimethyl ammonium chloride, lower membrane resistance without producing anion selectivity, in contrast to anionic detergents which, as expected, increase chiefly cationic conductance in thin lipid films (39). On the other hand, some compounds which do produce considerable anion selectivity in thin lipid membranes would not, a priori,

[3] Shemyakin, M. M., V. K. Antonov, L. D. Bergelson, V. T. Ivanov, G. G. Malenkov, Y. A. Ovchinnikov, and A. M. Shkrob. 1969. Chemistry of membrane-affecting peptides, depsipeptides, and depsides (structure-function relations). *In* Symposium on the Molecular Basis of Membrane Function. D. C. Tosteson, editor. Prentice-Hall Inc., Englewood Cliffs, N.J. 173.

be expected to, e.g., the polyene antibiotics, nystatin and amphotericin B (18, 40). Recently Pagano and Thompson (41) observed high chloride fluxes across black lipid membranes containing only lecithin and tetradecane (plus trace amounts of chloroform and methanol). The chloride movements were electrically silent, i.e., by exchange diffusion, a process which would seem more likely to occur in a bilayer containing liquid ion exchangers which are known to function as mobile carriers in thick membrane systems (38, 42). We have not yet measured chloride movements isotopically, and thus we cannot assess the possibility of exchange diffusion in our membranes.

Our interest in the production of anion-selective bilayers stems mainly from the well-known permselectivity of erythrocyte membranes and the fact that the mechanism of anion selectivity in red cell membranes is not completely understood (43). The extent to which anion-selective bilayers can provide useful models for the erythrocyte membrane will be a subject for future study.

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REFERENCES

1. ROTHFIELD, L., and A. FINKELSTEIN. 1968. Membrane biochemistry. *Annu. Rev. Biochem.* **37**:463.
2. BANGHAM, A. D. 1968. Membrane models with phospholipids. *Progr. Biophys. Mol. Biol.* **18**:29.
3. TIEN, H. T., and A. L. DIANA. 1968. Bimolecular lipid membranes: a review and a summary of some recent studies. *Chem. Phys. Lipids.* **2**:55.
4. MUELLER, P., and D. O. RUDIN. 1968. Resting and action potentials in experimental bimolecular lipid membranes. *J. Theor. Biol.* **18**:222.
5. MADDY, A. H. 1966. The properties of the protein of the plasma membrane of ox erythrocytes. *Biochim. Biophys. Acta.* **117**:193.
6. BARTLETT, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234**: 466.
7. MARINETTI, G. V. 1962. Chromatographic separation, identification, and analysis of phosphatides. *J. Lipid Res.* **3**:1.
8. JACOBS, E. E., M. JACOB, D. R. SANADI, and L. B. BRADLEY. 1956. Uncoupling of oxidative phosphorylation by cadmium ion. *J. Biol. Chem.* **223**:147.
9. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
10. REGA, A. F., R. I. WEED, C. F. REED, G. G. BERG, and A. ROTHSTEIN. 1967. Changes in the properties of human erythrocyte membrane protein after solubilization by butanol extraction. *Biochim. Biophys. Acta.* **147**:297.
11. ZWAAL, R. F. A., and L. L. M. VAN DEENEN. 1968. Protein patterns of red cell membranes from different mammalian species. *Biochim. Biophys. Acta.* **163**:44.

12. NELSON, G. J. 1968. Studies on the lipids of sheep red blood cells. II. The incorporation of phosphorus into phospholipids of HK and LK cells. *Lipids.* **3**:267.
13. PAPHADJOUPOULOS, D. 1968. Surface properties of acidic phospholipids: interaction of monolayers and hydrated liquid crystals with uni- and bi-valent metal ions. *Biochim. Biophys. Acta.* **163**:240.
14. NELSON, G. J. 1967. Studies on the lipids of sheep red blood cells. I. Lipid composition in low and high potassium red cells. *Lipids.* **2**:64.
15. ANDREOLI, T. E., J. A. BANGHAM, and D. C. TOSTESON. 1967. The formation and properties of thin lipid membranes from HK and LK sheep red cell lipids. *J. Gen. Physiol.* **50**:1729.
16. ANDREOLI, T. E., M. TIEFFENBERG, and D. C. TOSTESON. 1967. The effect of valinomycin on the ionic permeability of thin lipid membranes. *J. Gen. Physiol.* **50**:2527.
17. BANGHAM, A. D., M. M. STANDISH, and N. MILLER. 1965. Cation permeability of phospholipid model membranes: effect of narcotics. *Nature (London).* **208**:1295.
18. ANDREOLI, T. E., and M. MONAHAN. 1968. The interaction of polyene antibiotics with thin lipid membranes. *J. Gen. Physiol.* **52**:300.
19. ANDREOLI, T. E., V. W. DENNIS, and A. M. WEIGL. 1969. The effect of amphotericin B on the water and nonelectrolyte permeability of thin lipid membranes. *J. Gen. Physiol.* **53**: 133.
20. TOSTESON, D. C., T. E. ANDREOLI, M. TIEFFENBERG, and P. COOK. 1968. The effects of macrocyclic compounds on cation transport in sheep red cells and thin and thick membranes. *J. Gen. Physiol.* **51**(5, Pt. 2):373.
21. TOSTESON, D. C. 1968. Effect of macrocyclic compounds on the ionic permeability of artificial and natural membranes. *Fed. Proc.* **27**:1269.
22. NETSKY, M. G., and M. H. JACOBS. 1939. Some factors affecting the rate of hemolysis of the mammalian erythrocyte by n-butyl alcohol. *Biol. Bull. (Woods Hole).* **77**:319.
23. JACOBS, M. H., and M. WILLIS. 1947. Preparation and properties of cation permeable erythrocytes. *Biol. Bull. (Woods Hole).* **93**:223.
24. PONDER, E. 1947. K-Na exchange accompanying the prolytic loss of K from human red cells. *J. Gen. Physiol.* **32**:53.
25. WHITTAM, R. 1964. Transport and Diffusion in Red Blood Cells. Williams & Wilkins, Baltimore. 228 pp.
26. HUNTER, F. R. 1961. The effect of n-butyl alcohol on the permeability of erythrocytes to non-electrolytes. *J. Cell. Comp. Physiol.* **58**:203.
27. HUNTER, F. R., J. GEORGE, and B. OSPINA. 1965. Possible carriers in erythrocytes. *J. Cell. Comp. Physiol.* **65**:299.
28. TOSTESON, D. C. 1955. Sodium and potassium transport in red blood cells. *In* Electrolytes in Biological Systems. A. M. Shanes, editor: American Physiological Society, Washington, D. C. 123.
29. AMSTRONG, C. M., and L. BINSTOCK. 1964. The effects of several alcohols on the properties of the squid giant axon. *J. Gen. Physiol.* **48**:265.
30. MOORE, J. W., W. ULBRICHT, and M. TAKATA. 1964. Effect of ethanol on the sodium and potassium conductances of the squid axon membrane. *J. Gen. Physiol.* **48**:279.
31. SKOU, J. C. 1961. The effect of drugs on cell membranes with special reference to local anaesthetics. *J. Pharm. Pharmacol.* **13**:204.
32. HAYDON, D. A., and J. TAYLOR. 1963. The stability and properties of bimolecular lipid leaflets in aqueous solutions. *J. Theor. Biol.* **4**:281.
33. KILBOURN, B. T., J. C. DUNITZ, L. A. R. PIODA, and W. SIMON. 1967. Structure of the K^+ complex with nonactin, a macrotetrolide antibiotic possessing highly specific K^+ transport properties. *J. Mol. Biol.* **30**:559.
34. PEDERSEN, C. J. 1967. Cyclic polyethers and their complexes with metal salts. *J. Am. Chem. Soc.* **89**:7017.
35. EISENMAN, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2**(2, Pt. 2):259.
36. DIAMOND, J. M., and E. M. WRIGHT. 1969. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annu. Rev. Physiol.* **31**:581.

37. WRIGHT, E. M., and J. DIAMOND. 1968. Effects of pH and polyvalent cations on the selective permeability of gall-bladder epithelium to monovalent ions. *Biochim. Biophys. Acta.* **163**:57.
38. SHEAN, G. M., and K. SOLLNER. 1966. Carrier mechanisms in the movement of ions across porous and liquid ion exchanger membranes. *Ann. N. Y. Acad. Sci.* **137**:759.
39. SEUFERT, W. D. 1965. Induced permeability changes in reconstituted cell membrane structure. *Nature (London).* **207**:174.
40. FINKELSTEIN, A., and A. CASS. 1968. Permeability and electrical properties of thin lipid membranes. *J. Gen. Physiol.* **52**(1, Pt. 2):145.
41. PAGANO, R., and T. E. THOMPSON. 1968. Spherical lipid bilayer membranes: electrical and isotopic studies of ion permeability. *J. Mol. Biol.* **38**:41.
42. EISENMAN, G., J. P. SANDBLOM, and J. L. WALKER. 1967. Membrane structure and ion permeation. *Science (Washington).* **155**:965.
43. TOSTESON, D. C. 1967. Electrolyte composition and transport in red blood cells. *Fed. Proc.* **26**:1805.
44. OSTERHOUT, W. J. V. 1940. Some models of protoplasmic surfaces. *Cold Spring Harbor Symp. Quant. Biol.* **8**:204.