Ionic Permeability of Thin Lipid Membranes

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

JOHN GUTKNECHT and D. C. TOSTESON

From the Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27706

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 osmotic permeability. Heptanol (8 mM), for example, caused the membrane resistance ($R_m$) to decrease from $>10^8$ to about $10^4$ 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, $T_{\text{cat}}/T_{\text{Cl}}$ increased from about 6 to 21 and $T_{\text{K}}/T_{\text{Na}}$ increased from about 3 to 21. The addition of long-chain (C$_6$-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). 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 X 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 $-16^\circ$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

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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 $V_m = \sum T_i E_i$ and $\sum T_i = 1$. $T_i$ is defined as $G_i / G_m$, where $G_i$ is the conductance of the $i$th ion and $G_m$ is the total membrane conductance. $E_i$ is the equilibrium potential for the
ith ion, i.e., \(-59 \log \frac{a_i^r}{a_i^f}\), where \(a_i^r\) and \(a_i^f\) 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 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 10⁴ ohm-cm², 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-cm\(^2\). For a given aqueous concentration of alcohol, long-chain alcohols were much more effective than short-chain alco-

| Alcohol       | Concentration in aqueous phase required to give \( R_m \geq 10^8 \) ohm-cm\(^2\) | Saturation concentration* \( (C) \) \( (C_0) \) | Thermodynamic activity at \( R_m = 10^6 \) ohm-cm\(^2\) \( C/C_0 \) |
|---------------|---------------------------------|---------------------------------|--------------------------|
| Ethanol       | 3.0 Miscible                    | Miscible                        | —                        |
| n-Propanol    | 1.4 Miscible                    | Miscible                        | —                        |
| n-Butanol     | 0.30 1.07 0.28                  | 0.36 0.33                       | 0.28                     |
| n-Pentanol    | 0.10 0.306 0.33                 | 0.058 0.48                      | 0.69                     |
| n-Hexanol     | 0.028 0.058 0.48                | 0.0078 0.69                     | 1.0                      |
| n-Heptanol    | 0.0054 0.0078 0.69              | 0.00038 1.0                     |
| n-Octanol     | >0.00038 1.0                    | >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 \(2-3 \times 10^{-4} \, \text{cm sec}^{-1}\) (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 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...
C₇ 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 Rₘ 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, Rₘ decreased markedly with increasing KCl concentration, but decreased only slightly with increasing NaCl concentration. The slope of the log Rₘ vs. log [KCl] curve was about -0.5, i.e., the membrane conductance was roughly proportional to the square root of the KCl 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, Tₓ: Tₒ 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](image-url)

**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, $T_{oct}:T_{cl}$ was 3 and $T_{K}:T_{Na}$ was 1.5 before any black areas appeared in the membrane. With the appearance of black areas, $T_{oct}:T_{cl}$ 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-
Containing 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 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 Th4+ and Fe3+ (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 II

| Alcohol         | Salt solutions | Transference Nos. (approximate) |
|-----------------|----------------|---------------------------------|
| Alcohol         | Front          | Rear                           |
| Heptanol (7.8 mm) | KCl 10        | KCl 1000                       |
| Heptanol (30 mm)  | KCl 10        | KCl 1000                       |
| Guaiacol (7.8 mm) | KCl 10        | KCl 1000                       |
| Guaiacol (30 mm)  | KCl 10        | KCl 1000                       |
| 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.

Membranes containing valinomycin or monactin-dinactin in which K selectivity is produced in thick as well as in thin membranes (20, 21).
TABLE III
EFFECT OF n-OCTANOL ON IONIC PERMEABILITY OF THICK LIPID MEMBRANES

| Membrane-forming solution | Salt solutions | Transference Nos. (approximate) |
|----------------------------|----------------|---------------------------------|
|                            | Front          | Rear                           | $R_m$ | $V_m$ | $T_{sat}$ | $T_{Cl}$ | $T_{Na}$ | $T_K$ |
|                            | $mM$           | $mM$                           | $ohm$m$^2$m | $mv$  |        |        |        |       |
| Red cell lipids (4 mM) in decane | KCl 10 | KCl 100 | $3 \times 10^9$ | -30 to -34 | 0.80 | 0.20 | --- | --- |
| Red cell lipids (4 mM) plus n-octanol (400 mm) | NaCl 1 | NaCl 10 | --- | --- | --- | --- | --- | --- |
| Red cell lipids (4 mM) in decane | KCl 10 | KCl 100 | $6 \times 10^8$ | -30 to -33 | 0.80 | 0.20 | --- | --- |
| Red cell lipids (4 mM) plus n-octanol (400 mm) | NaCl 1 | NaCl 10 | --- | --- | --- | --- | --- | --- |

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 cm$^2$. 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 | Transference Nos. (approximate) |
|---------------------------|---------------------------------|----------------|---------------------------------|
|                            |                                 | Front          | Rear                           | $R_m$ | $V_m$ | $T_{sat}$ | $T_{Cl}$ |
|                            |                                 | $mM$           | $mM$                           | $ohm$m$^2$m | $mv$  |        |        |
| Red cell lipids (20 mg/ml) in decane | Th$^{4+}$ (0.1 mM) | KCl 10 | KCl 100 | $10^9$-$10^9$ | 30-45 | 0.16 | 0.84 |
| Red cell lipids (20 mg/ml) in decane | Heptanol (8 mM) | NaCl 1 | NaCl 10 | $10^6$-$10^6$ | 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 | KCl 100 | $10^6$-$10^6$ | 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) | NaCl 1 | NaCl 10 | $10^9$-$10^9$ | 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.
istance increased two- to fourfold in the presence of Th⁴⁺ or Fe³⁺, suggesting that the permselectivity change was due chiefly to a reduction in cation, rather than an increase in anion, permeability. When both Th⁴⁺ (10⁻⁴ M) and heptanol (7.8 mM) were present in the aqueous phase, \( R_m \) decreased to 10⁶–10⁸ ohm-cm² 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 Th⁴⁺. 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 Fe³⁺ and heptanol. The ionic selectivity changes induced by Fe³⁺ and Th⁴⁺ were reversible; washing the membrane with the chelating agent EDTA (10⁻⁴ M) restored cation selectivity within 15 min. Some other polyvalent cations, La⁺⁺, Ca²⁺, and Ba²⁺, 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, \( \text{CH}_3(\text{CH}_2)_m\text{CH}_3—\text{N—C—R'} \),

\[ \text{R}'' \]

where \( \text{R} + \text{R}' + \text{R}'' = 11 \) 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 $10^3$-$10^4$ 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). 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).

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

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 dodecyl benzene 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,
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.

We thank Dr. V. Dennis for measuring the osmotic permeability coefficients, Drs. V. Dennis, M. Tieffenberg, C. M. Armstrong, G. Kepner, and P. K. Lauf for helpful suggestions, and Paul Cook for technical assistance.

This work was supported in part by United States Public Health Service Grant 5PO1-HE12157 and by National Science Foundation Grant GB-6714.

Dr. Gutknecht was supported in part by a postdoctoral fellowship (GM-13,804) from the National Institutes of Health.

Received for publication 24 September 1969.

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