Permeability and Electrical Properties of Thin Lipid Membranes

ALAN FINKELSTEIN and ALBERT CASS

From the Departments of Physiology and Neurology, Albert Einstein College of Medicine, New York 10461, and The Rockefeller University, New York 10021

ABSTRACT We present and discuss the permeability and electrical properties of thin lipid membranes, and the changes induced in these properties by several agents added to the aqueous phases after the membranes have formed. The unmodified membrane is virtually impermeable to ions and small "hydrophilic" solutes, but relatively permeable to water and "lipophilic" molecules. These properties are consistent with those predicted for a thin film of hydrocarbon through which matter is transported by dissolving in the membrane phase and then diffusing through it. The effect of cholesterol in reducing the water and "lipophilic" solute permeability is attributed to an increase of the "viscosity" of the hydrocarbon region, thus reducing the diffusion coefficient of molecules within this phase. The selective permeability of the membrane to iodide (I^-) in the presence of iodine (I_2) is attributed to the formation of polyiodides (perhaps I_5^-), which are presumed to be relatively soluble in the membrane because of their large size, and hence lower surface charge density. Thus, I_2 acts as a carrier for I^-. The effects of "excitability-inducing material" and the depsipeptides (particularly valinomycin) on ion permeability are reviewed. The effects of the polyene antibiotics (nystatin and amphotericin B) on ion permeability, discussed in greater detail, are the following: (*a*) membrane conductance increases with the 10th power of nystatin concentration; (*b*) the membrane is anion-selective but does not discriminate completely between anions and cations; (*c*) the membrane discriminates among anions on the basis of size; (*d*) membrane conductance decreases extraordinarily with increasing temperatures. Valinomycin and nystatin form independent conductance pathways in the same membrane, and, in the presence of both, the membrane can be reversibly shifted between a cation and anion permeable state by changes in temperature. It is suggested that nystatin produces pores in the membrane and valinomycin acts as a carrier.

Workers investigating the physical-chemical mechanisms underlying the permeability properties of biological membranes are faced with two formidable questions. First, can the permeability to a given species be ascribed to certain of the membrane constituents, such as lipid, protein, and carbohydrate, or is it the organization of these constituents that is of prime importance in determining permeability? Second, does the very thinness of the membrane

(less than 100 A), irrespective of its actual organization, necessitate the consideration of physical phenomena that can generally be ignored in the discussion of "macroscopic" membranes (such as ion exchange membranes or dialysis tubing)? For example, such thermodynamic concepts as the "concentration" of a species within the membrane might be completely inadmissible, and the treatment of transport, instead of beginning with the familiar flux equations, might have to start de novo from considerations of fluctuation theory and Brownian motion. Or, again, the intense electrical fields created by a few millivolts potential difference across a 100 A structure may introduce such formidable subjects as the Wien effect and dielectric breakdown.

It was therefore of great interest when Mueller et al. (1–3) reported a technique for forming thin (less than 100 A) membranes from phospholipids plus hydrocarbon additives. A system was now available for the study of a spontaneously formed lipid membrane devoid of protein, but to which protein could be added, either during or after formation. Furthermore, it was possible for the first time to measure electrical potential differences and transport of matter across an experimentally created thin film separating two aqueous phases.

It is our purpose to present and discuss the results we and others have obtained on the permeability of these thin lipid membranes to water, neutral solutes, and ions. A presentation of the permeability characteristics of these membranes naturally divides itself into two general topics. One is the properties of the unmodified membrane. The other is the alteration of these properties by certain materials added to the aqueous phase either before or after the membrane has formed. In the first half of the paper, we shall consider the unmodified membrane. Except with respect to water, the data available at present are rather meager; it seems possible, nevertheless, to explain the existing data using very simple assumptions, which can be tested further as additional experimental findings become available. We shall show that the presently known permeability properties of the unmodified membrane can be understood from the *bulk* characteristics of hydrocarbons, without invoking more sophisticated concepts, such as those alluded to above. In the second half of the paper we shall describe the modifications of ion permeability produced in these membranes by several agents, with particular reference to the effect of the polyene antibiotics.

UNMODIFIED MEMBRANE

Water Permeability

RELATIONSHIP BETWEEN P_f AND P_d There are two kinds of measurements which reflect the permeability of a membrane to water The first is made in an osmotic experiment, in which a difference in concentration (Δc_s) of an impermeant solute is established across the membrane and the rate of

water movement observed. From this rate, the filtration (or osmotic) permeability coefficient, P_f, is determined by the equation

$$\Phi_w = P_f A \phi \, \Delta c_s \tag{1}$$

where Φ_w = the flux of water (in moles per unit time)
A = the area of the membrane
ϕ = the osmotic coefficient
Δc_s = the difference in concentration of the impermeant species. (For a uni-univalent salt, $2 \times \Delta c_s$ instead of Δc_s appears in equation 1.)

The second type of measurement is made in a tagged water experiment, in which a concentration difference (Δc_w^*) of isotopically labeled water is established across the membrane in the absence of an osmotic gradient, and the flux, Φ_w^*, of the labeled water measured. From this flux, the diffusion permeability coefficient, P_d, is determined by the equation

$$\Phi_w^* = -P_d A \, \Delta c_w^* \tag{2}$$

[For the derivations of equations 1 and 2, see Cass and Finkelstein (4).]

There has been a great deal of interest in the biological literature on the relative magnitudes of P_f and P_d. This arises from the fact that in membranes that water traverses through aqueous pores, $P_f > P_d$ (5), whereas in "liquid" membranes, which water traverses by solution, diffusion, and dissolution, it is anticipated that $P_f \approx P_d$. The finding that P_f is greater than P_d for most plasma membranes has been taken as evidence for the existence of aqueous pores and, in fact, their "equivalent pore radii" have been calculated from these data (6). It is therefore of considerable interest to determine the relationship between P_f and P_d in these experimental thin lipid membranes.

We have found (4) that for membranes formed from solutions of ox brain lipids plus *dl*-α-tocopherol plus cholesterol, dissolved in chloroform-methanol,

$$P_f = P_d \tag{3}$$

and that for the particular membranes we dealt with, $P_f \approx 1 \times 10^{-3}$ cm/sec.

The equality of P_f and P_d was established only after considerable effort in the tagged water experiment in reducing unstirred layers, which, if present, lead to erroneously low values for P_d. Because of the presence of unstirred layers, the values of P_d reported by other investigators (7, 8) are too low [a fact recognized by Hanai et al. (9)]; consequently, earlier reports that P_f exceeds P_d in membranes formed from egg lecithin plus tetradecane dissolved in chloroform-methanol (7) or in membranes formed from egg lecithin plus cholesterol and decane (8) are probably not valid. There is, in fact, no evidence to indicate that P_f is significantly greater than P_d in any of these thin lipid membranes. We wish to point out, however, that it will be particularly

difficult to establish the equality or inequality of P_f and P_d for the membranes of Huang and Thompson (7), which have large values of P_f (6–10 $\times$ 10^{-3} cm/sec); with such permeable membranes, the unstirred layer problem in the P_d determinations is formidable.

The unstirred layer problem in the determination of P_d for plasma membranes has been emphasized by Dainty (10), who seriously questioned the general finding that P_f exceeds P_d for cells. Our own difficulties in reducing unstirred layers in our relatively simple experimental situation cause us to place credence in Dainty's critique.

THE EFFECT OF COMPOSITION OF MEMBRANE-FORMING SOLUTION, ESPECIALLY WITH RESPECT TO CHOLESTEROL, ON WATER PERMEABILITY The values of P_f obtained by several investigators using various kinds and mixtures of phospholipids, cholesterol, and additives (decane, tetradecane, *dl*-α-tocopherol) range from 0.5 $\times$ 10^{-3} to 10 $\times$ 10^{-3} cm/sec (4, 7, 8). (It may be noted in passing that these values are comparable with those of plasma membranes, and, in fact, the highest of these is equal to that of the highly permeable red blood cell.) We have found that P_f is markedly dependent on the molar ratio of cholesterol to phospholipid in the membrane-forming solution (11). Fig. 1 shows a plot of P_f vs. molar ratio of cholesterol to phospholipid; in the upper curve the phospholipid is egg lecithin, and in the lower curve it is the mixture obtained from ox brain white matter. In the former case P_f falls from 4.2 $\times$ 10^{-3} to 0.75 $\times$ 10^{-3} cm/sec as the cholesterol:phospholipid molar ratio increases from 0:1 to 8:1, and in the latter case P_f falls from 1.8 $\times$ 10^{-3} to 0.49 $\times$ 10^{-3} cm/sec as this ratio increases from 0:1 to 2:1. Ergosterol gives comparable results. We found no significant dependence of P_f on the additive (tetradecane or *dl*-α-tocopherol) used, or on the chloroform-methanol content (if any) of the membrane-forming solutions. In addition, membranes formed from egg lecithin completely saturated by hydrogenation had a P_f of 1.7 $\times$ 10^{-3}, compared with 4.2 $\times$ 10^{-3} cm/sec for the partially unsaturated natural egg lecithin.

THE MECHANISM OF WATER TRANSPORT The fact that $P_f = P_d$ implies that the osmotic transfer of water occurs via independent movement of water molecules (as in diffusion) rather than through cooperative phenomena (as in laminar flow). There are many possible mechanisms consistent with this type of movement, particularly for a thin membrane where fluctuating defects in the membrane or "hopping" by energetic water molecules must be considered. It has been noted, however, that the magnitude of P_f (or P_d) is predictable by simply treating the membrane as a thin hydrocarbon film (8, 12).

The picture is as follows (see Fig. 2): water dissolves in the membrane at the two membrane-water interfaces in proportion to its mole fractions in the

aqueous phases, and then diffuses through the membrane subject to the boundary conditions thus established. It is here assumed that the boundary processes are very rapid, so that the rate-limiting step is the diffusion of water within the membrane. The majority of the fatty acid chains of egg lecithin are 16 or 18 carbon atoms in length. For hexadecane at 35°C, Schatzberg (13, 14) gives a water solubility of 64 × 10^{-6} g/ml and a diffusion coefficient for

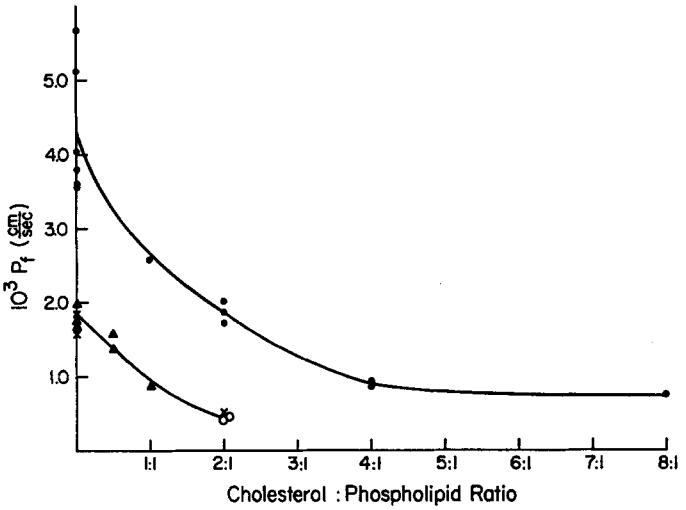


FIGURE 1. P_f as a function of cholesterol:phospholipid molar ratio. All films were formed at 36° ± 0.01°C, by the technique of Mueller et al. (3), in solutions either of unbuffered 100 mM NaCl or of 100 mM NaCl + 0.2 mM $MgSO_4$ + 5 mM histidine (pH 7). After the membrane had become completely black, the concentration of NaCl was increased on one side, and the rate of water movement determined (4). The "mixed lipid" was extracted from ox brain white matter by the method of Mueller et al. (3), twice split with water to remove protein, and passed through a Unisil column to remove cholesterol. ●, lecithin membranes formed with tetradecane as the additive; ×, "mixed lipid" membranes formed with tetradecane as the additive; ▲, "mixed lipid" membranes formed with *dl*-α-tocopherol as the additive; ○, "mixed lipid" membranes formed with ergosterol instead of cholesterol. (Both tetradecane and *dl*-α-tocopherol were present as additives.)

water of 5 × 10^{-5} cm^2/sec. Taking the thickness of the hydrocarbon region of the membrane as 50 A (15, 16) and using these values, we obtain P_f = 6.4 × 10^{-3} cm/sec, in remarkable agreement with our value of 4.2 × 10^{-3} cm/sec for lecithin membranes at 36°C.

Pursuing this model further, we predict that P_f will be affected by any modification of the membrane that alters the water solubility in it or the diffusion coefficient of water within it. Can the effect of cholesterol be explained on this basis? Assuming that cholesterol is anchored at or near the water interface via its hydroxyl group, we have taken cholestane as a model

for the contribution of cholesterol to the hydrocarbon region of the membrane. Fig. 3 is a plot of our determinations of the viscosity (η) of a solution of cholestane in 1-hexadecene as a function of the molar ratio of cholestane to 1-hexadecene. For a 1:3 ratio, which corresponds to a cholesterol:phospholipid ratio of 2:3, the viscosity has doubled, which should result in a signifi-

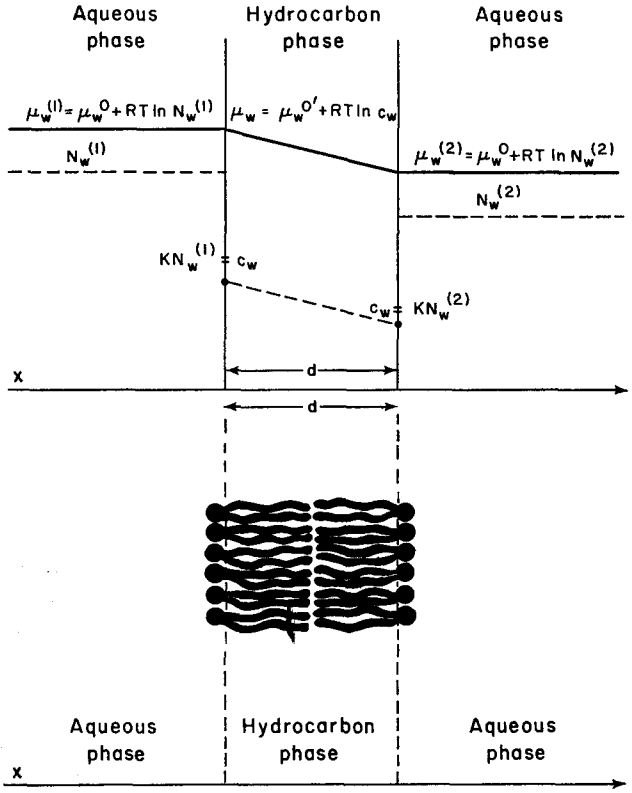


FIGURE 2. Postulated mechanism of water transport across a thin lipid membrane. The upper part of the figure gives the profiles of the chemical potential (μ_w) of water, the mole fractions (N_w), and the concentrations (c_w) of water in the various phases. (Note that with this mechanism the pressure is assumed constant throughout all phases.) In the lower part, we indicate schematically a bimolecular leaflet; the hydrocarbon phase is made up of the fatty acid chains, drawn as wavy lines. This phase may also contain some of the additive used to form the membranes, such as tetradecane. The filled circles represent the polar ends of the phospholipids.

cant reduction of the diffusion coefficient of water. Thus, the reduction of P_f by cholesterol can be attributed to an increase in the viscosity of the hydrocarbon region of the membrane, resulting in a decrease of the diffusion coefficient of water within this phase.

The fact that the permeability of the saturated lecithin membrane is low

compared with that of the partially unsaturated, natural lecithin membrane is also explicable in the same terms. Thus, the viscosity of 1-hexadecene is 12% less than that of hexadecane (17), and the water solubility in 1-hexadecene is 15% greater than in hexadecane (11). Both properties would make the P_f value of the saturated lecithin membrane lower than that of the unsaturated one, although the quantitative agreement might be better. (Saturated lecithin forms rather solid films, so that the "viscosity" of the hydrocarbon region might be expected to be greater than that of bulk hexadecane.)

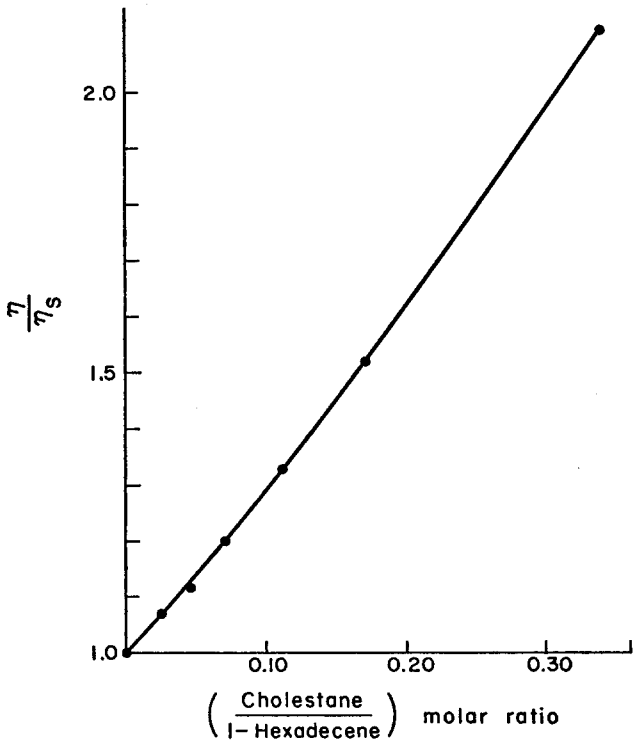


FIGURE 3. Relative viscosity of solutions of cholestane in 1-hexadecene. The measurements were performed in an Übbelohde viscosimeter at 35.0°C. (η is the viscosity of a solution of cholestane in 1-hexadecene; η_s is the viscosity of 1-hexadecene alone.)

The picture presented above relegates the polar ends of the phospholipid to the role of making it possible to establish a thin hydrocarbon film between two aqueous phases; it is assumed that they themselves introduce no additional energy barrier for water transport. The differences between the P_f's of membranes formed from egg lecithin and those of membranes formed from the mixture of phospholipids in ox brain (see Fig. 1) may argue against this, but any conclusion must await the comparison of P_f's of membranes formed from lipids with different polar heads but identical hydrocarbon tails. We may

summarize by saying that *at present the water permeability of thin lipid membranes is adequately explained by the properties of the hydrocarbon portion of the membranes, and these properties are consistent with the bulk properties of the corresponding hydrocarbons.* We shall see below that this picture is also adequate for explaining the permeability of these membranes to ions and other neutral molecules.

Ion Permeability

GENERAL The outstanding characteristic of the ion permeability of these membranes is their extreme impermeability; their high electrical resistance (10^6–10^9 Ω-cm^2) (1, 15, 18) and the failure of ionic tracers to exchange across them (12) make this evident. [From measurements of potential difference across the membranes in the presence of salt gradients, it has been concluded that they are somewhat more permeable to cations than to anions (19).] The high degree of impermeability is compatible with the membrane being a thin hydrocarbon film. It is sufficient to note that there are no data available for the partition coefficients of simple salts between water and hydrocarbon, since the amount of salt entering a bulk hydrocarbon phase is immeasurably small. Thus, the ion impermeability is a simple consequence of the inability of ions to partition into the membrane phase. We shall discuss below the quantitative aspects of this phenomenon.

PERMEABILITY TO THE IODIDE ION In contrast to these general results Läuger et al. (20) have reported that the electrical resistance of thin lipid membranes formed from lecithin dissolved in decane is lowered approximately 1000-fold if iodide (I^-) is present in concentrations greater than 1 mM. In addition, if a 10:1 iodide concentration ratio is established across the membrane, there appears a 60 mv potential difference, with the concentrated iodide solution positive. This potential difference appears even in the presence of chloride concentrations 100 times greater than that of iodide, indicating that the effect is highly specific for iodide. As Läuger et al. pointed out, their results might be due either to the selective permeability of the lipid membrane to iodide, or to electronic conduction through the membrane, with oxidation ($2\ I^- \rightarrow I_2 + 2e^-$) occurring at one interface and reduction (perhaps involving oxygen) at the other.

In further investigating this phenomenon, we have found that the lipid membranes we have studied indeed become selectively permeable to iodide (I^-), but only if molecular iodine (I_2) is present.[1] We wish to summarize and discuss these results, as they illustrate the nature of the energy barrier of the membrane to ions and demonstrate one way in which this barrier can be lowered. A more detailed description of the methods, results, and theory will be presented elsewhere.[2])

[1] Unless a reducing atmosphere is present, there is always some I_2 present in I^- solutions, and it may be that this was the case in the experiments of Läuger et al.

[2] A. Cass and A. Finkelstein. Manuscript in preparation.

The membranes we worked with were formed from solutions of ox brain lipid plus *dl*-α-tocopherol and cholesterol dissolved in chloroform-methanol; such membranes in 0.1 M KCl had electrical resistances of approximately 2.5×10^7 Ω-cm². If in addition 10^{-2} M KI was present on both sides, the resistance fell only 10-fold. However, if in the presence of 10^{-3} or 10^{-2} M KI the I_2 concentration on one or both sides was raised to approximately 10^{-5} M, the resistance invariably fell within seconds to stable values of less than 10^4 Ω-cm².

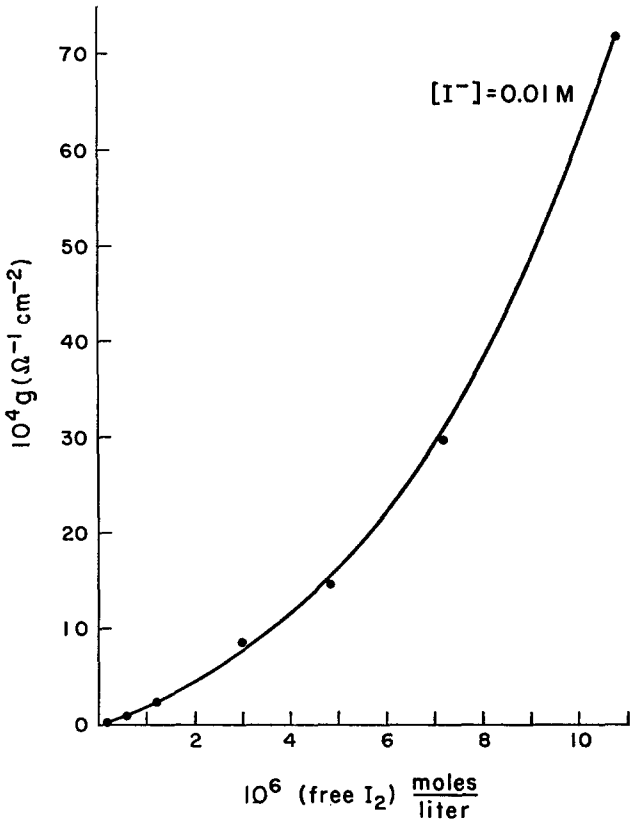


FIGURE 4. Conductance of individual thin lipid membranes as a function of the concentration of free iodine in the solutions in which they were formed. The aqueous phases contained 10 mM KI, in addition to 100 mM KCl, 5 mM phosphate buffer (pH 7), and 0.2 mM $MgSO_4$, plus the amount of free iodine indicated. This amount is computed from the dissociation constant for the reaction $I_2 + I^- \rightleftarrows I_3^-$. The conductance was obtained from the steady-state potential difference developed across the membrane during a small step of current. Temperature = 33° ± 1°C.

Similarly, membranes formed in the presence of 10^{-5} M I_2 but without added KI had resistances of approximately 10^6 Ω-cm², but upon subsequent addition of KI the resistance fell to 10^4 Ω-cm² or lower. It is therefore apparent

that both I^- and I_2 are required to lower the resistance drastically. The small effects with I^- alone can be attributed to trace amounts of I_2.[3] With a given membrane, the high resistance is completely restored by reducing the I_2 to I^- with an excess of thiosulfate ($S_2O_3^=$), and is again lowered upon addition of excess I_2. Thus, successive additions of I_2 and reductions of I_2 by $S_2O_3^=$ produce immediate 10^4-fold changes in resistance. Fig. 4 is a plot of membrane conductance in 10^{-2} M I^- as a function of "free" I_2.[4]

With I^- and I_2 present on both sides of the membrane, the membrane potential varies linearly as the logarithm of the ratio of I^- concentrations, with a slope of 60 mv (Fig. 5). On the other hand, with equal concentrations of I^- on both sides, gradients of I_2 concentration give rise to potential differences of a sign opposite to that anticipated if the conduction process were linked to oxidation-reduction reactions. This establishes that these effects are not due to electronic conduction, but rather to selective permeability of the membrane to I^-.

The high permeability of these membranes to I^- in the presence of I_2 can be understood in terms of the well-known ability of I^- and I_2 to combine to form polyiodides. For example, the reactions

$$I^- + I_2 \rightleftarrows I_3^-$$

$$2\,I^- + 6\,I_2 \rightleftarrows I_{14}^=$$

occur in aqueous solution, producing the polyiodides I_3^- and $I_{14}^=$. The former reaction dominates in aqueous solution (21) and has a dissociation constant at 40°C of

$$K = \frac{[I^-][I_2]}{[I_3^-]} = 1.7 \times 10^{-3}$$

The polyiodides I_5^-, I_9^-, etc. are also known (22).

We suggest that I_2 dissolves in the hydrocarbon region of the membrane (the partition coefficient of I_2 between hexadecane and water is 50:1) (23) and at each interface reacts with I^- to form I_3^-, I_5^-, or some higher complex. Such polyiodides, particularly the higher complexes, would be expected to be more soluble in the membrane than is I^-, for two reasons. First, the lipophilic

[3] The different lipid preparation used by Läuger et al. gave higher initial resistances than did the preparations we used; this fact, and the uncertain concentration of trace I_2, may explain the larger effect that they obtained with I^- alone. Similarly, the lowering of resistance we have observed by I_2 alone might be partially due to trace amounts of I^-. We have found, however, that the rate of membrane formation is considerably reduced by I_2 and that membranes tend to break when exposed to large I_2 concentrations. It is possible, therefore, that the lowering of resistance by I_2 alone may be due to alteration in membrane structure produced by the reaction of I_2 with the membrane lipids (perhaps the double bonds in the hydrocarbon chains).

[4] By "free" I_2 we mean the total iodine present less the amount complexed as I_3^- (see below).

nature of iodine should compensate to some extent for the electrostatic forces tending to exclude charged species; second, and more important, the electrostatic forces should be considerably reduced by the spreading of charge over the increased surface area of the large polyiodide ions.

In order to appreciate this latter point, let us follow Born (24) and assume that the energy necessary to transfer an ion from water to hydrocarbon is

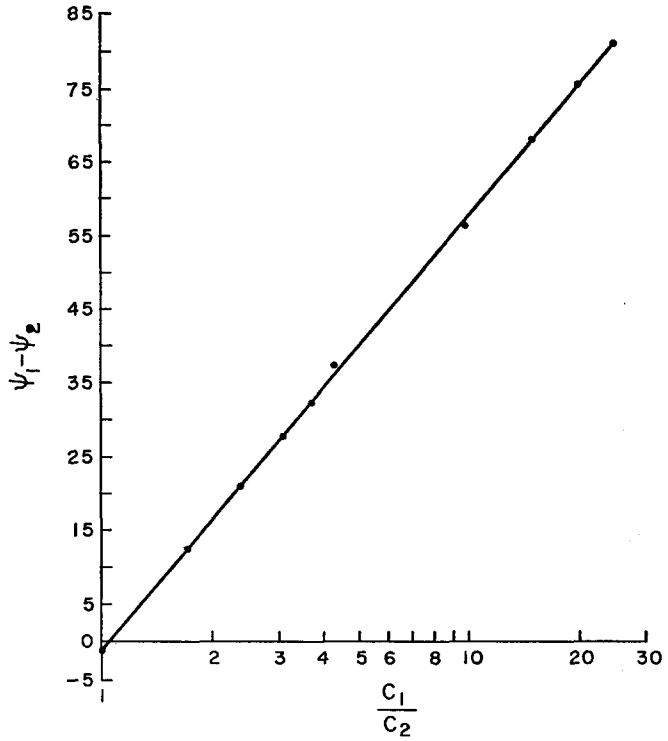


FIGURE 5. Potential difference (in millivolts) across a single thin lipid membrane as a function of the ratio of iodide concentrations in the two aqueous phases. The film was formed in a solution containing 100 mM KCl, 5 mM phosphate buffer (pH 7), 0.2 mM $MgSO_4$, 10 mM KI, and 10^{-5} M I_2. After the film had formed, concentration differences of I^- were established by adding small amounts of concentrated KI solution to one side. The potential difference across the membrane was measured with saturated calomel electrodes coupled to the solutions through saturated KCl bridges. Temperature = 33° ± 1°C.

simply the electrostatic energy, U, necessary to transfer a charge, q, from a medium of dielectric constant 80 to one of dielectric constant 2. Assuming the ion is a sphere of radius r, then from electrostatic theory we obtain

$$U(r) = \frac{q^2}{2r}\left(\frac{1}{2} - \frac{1}{80}\right) \qquad (4)$$

The radius of I^- is approximately 2 A, and, substituting this into equation 4, we obtain

$$U_{I^-} \approx 1.5 \text{ ev} \tag{5}$$

The concentration, c_o, of an ion in the organic phase is related to its concentration, c_w, in the aqueous phase through the Boltzmann distribution,

$$c_o = c_w e^{-U(r)/kT} \tag{6}$$

At room temperature ($T \approx 300°K$) we obtain, upon substituting equation 5 into equation 6,

$$\frac{c_o}{c_w} \equiv P_{I^-} = e^{-60} \approx 10^{-26} \tag{7}$$

where P_{I^-} is the partition coefficient of I^- between hydrocarbon and water. We see from this simple electrostatic calculation why the partition coefficient of ions between water and hydrocarbon is so low, and consequently why a thin layer of hydrocarbon will be virtually impermeable to simple ions. On the other hand, we note from equation 4 that a change in r produces a reciprocal change in the *exponent* of equation 6, so that, if r were to triple from 2 A to 6 A, the partition coefficient would increase by a factor of 10^{17}. It is probably this effect that operates when I^- combines with I_2 to form polyiodides. In addition, the polyiodides are not spherical but linear, L-shaped, or T-shaped. This spreads the charge out over a greater area than for a sphere and hence lowers the electrostatic energy even more.

As an example, a rough calculation indicates that

$$U_{I_5^-} \approx 0.3 \text{ ev}$$

giving from equation 6 a partition coefficient of $\approx 10^{-5}$, other things being equal. When this is multiplied by a factor of 50 (resulting from the lipophilic nature of iodine, which originates from van der Waals' forces), we obtain

$$P_{I_5^-} \approx 5 \times 10^{-4} \tag{8}$$

Thus, if we assume that a reasonable concentration of I_5^- exists at the membrane interfaces, sufficient I_5^- will enter the membrane to account for the observed lowering of resistance. The membrane potential follows the concentration ratio of I^- (Fig. 5) and not of I_5^-, because the high membrane permeability to I_2 keeps I_2 concentrations at the interfaces nearly equal. Thus, the ratio of *interfacial* I_5^- concentrations equals the ratio of bulk solution I^- concentrations.

If we are correct in this analysis, we have here a simple example of carrier transport of an ion across a lipid membrane. The carrier in this case is neutral and the complex charged. Within the membrane, electroneutrality cannot hold, there being no comparable mechanism to permit the entry of counterions. It is interesting in this respect that the low resistance (high permeability)

is very much dependent on the fact that the membrane is thin enough to permit the space-charge region to extend throughout the membrane. Thus, a thick hydrocarbon membrane should in principle display the same behavior as these membranes, but the resistance would be enormous, as the I_n^- concentration in the membrane would fall to very small values a short distance from the membrane interfaces.[5] The present system of fairly well understood reactions offers the opportunity to study in detail how a carrier mechanism can operate to transport ions across lipid membranes.

Neutral Solute Permeability

The few measurements of the membranes' permeability to neutral solutes are easily summarized. To "hydrophilic" solutes such as urea, acetamide, erythritol, glycerol, and sugars the membrane manifests very low, or immeasurably low, permeability. This has been established both from tracer permeability measurements (12) and from reflection coefficient determinations in osmotic studies (4, 8) (Fig 6). In contrast, "lipophilic" molecules such as indole and its derivatives are highly permeant (25). These findings are obviously compatible with a solubility diffusion mechanism of transport of matter across a hydrocarbon film. It is interesting in this connection that cholesterol reduces the membrane permeability for the "lipophilic" molecules,[6] which is consistent with the "viscosity" mechanism we have suggested to explain the cholesterol effect on P_f (see page 150).

Summary and Conclusions

We have attempted to show that the water, neutral solute, and ion permeability of unmodified thin lipid membranes can be at least semiquantitatively derived assuming that the bulk properties of hydrocarbon apply to regions only 50 A thick. A corollary of this is that the major barrier to transport is the hydrophobic region of the membrane phase. In the case of water, the rate-limiting step is the diffusion of water within the hydrocarbon region, the solution and dissolution of water into this phase occurring so rapidly that the water concentration just within the membrane can be calculated from equilibrium considerations. On the other hand, the rate of ion movement is immeasurably small because of the extreme insolubility of ions in the hydrophobic region. It is somewhat surprising that the polar region of the membrane does not appear to play a significant role in controlling permeability, but merely serves to anchor the hydrocarbon region between two aqueous phases. We may anticipate, however, that when the membrane has been

[5] When membranes are formed in solutions containing I^- and I_2, the onset of low resistance parallels the appearance of "black" regions in the film, as one would predict from the above considerations.
[6] R. C. Bean. Personal communication.

"opened up" to ions by such substances as discussed in the next sections, the polar groups may be found to play a more critical part.[7]

The iodide-iodine system presents a new aspect of the relationship between the properties of these membranes and those of bulk hydrocarbon. Here, the *thinness* of the hydrocarbon region is crucial for significant transport, although

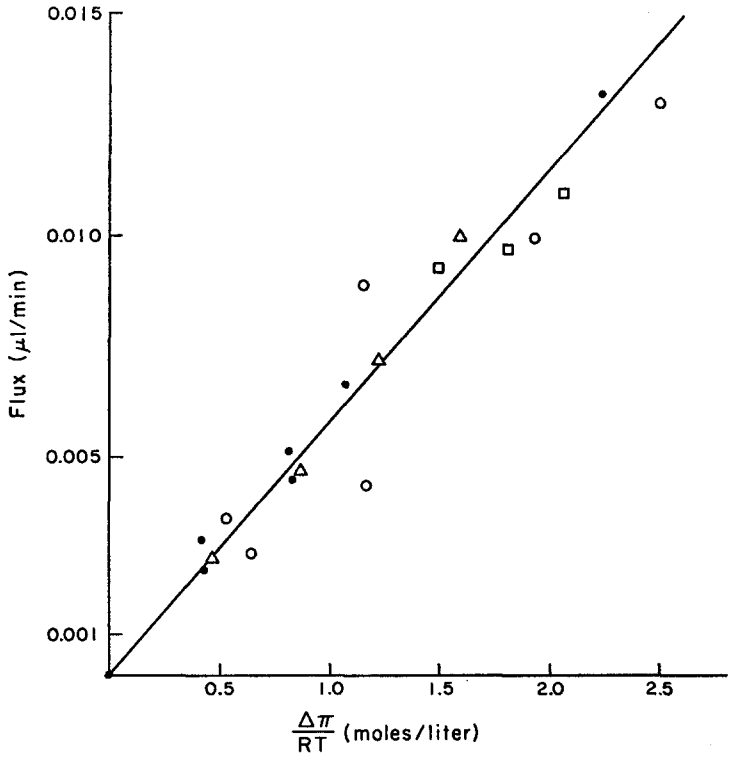


FIGURE 6. Flux of water across thin lipid membranes as a function of osmotic pressure differences for several solutes. All membranes were formed at 36° ± 0.01°C in 100 mM NaCl from a single membrane-forming solution of ox brain lipid + *dl*-α-tocopherol + cholesterol, dissolved in 2:1 chloroform-methanol. After the membrane had become completely black, the concentration of the solute of interest was increased on one side, and the rate of water movement determined (4). The solutes were: ●, NaCl; □, urea; △, acetamide; ○, glycerol. Note that all of the solutes can be fitted reasonably well to the straight line for NaCl, thus demonstrating that their reflection coefficients are approximately the same as that for NaCl, which is 1.

the calculations are still based on consideration of macroscopic phases. We see how new phenomena can arise when the membrane thickness becomes

[7] This already appears to be the case in the iodine-iodide system. The resistance lowering produced by iodide in membranes formed from phosphatidylinositol is dependent on neutral salt concentration; apparently the negative charge on the membrane created by the phosphate groups can reduce the iodide concentration at the membrane/solution interfaces (26).

comparable with the dimensions of the space-charge region, and the constraint of electroneutrality no longer applies.

MODIFIED MEMBRANE

Review of Some Modifiers

In the previous sections we considered the permeability of unmodified thin lipid membranes. We wish now to discuss the ways in which the ion permeability can be altered by certain substances added to the aqueous phase after the membrane has formed. Most common laboratory reagents do not significantly alter the ion permeability. An exception is detergents (27), which disrupt the membrane structure and break the film. Several types of agents, however, have been observed to increase ion permeability (increase membrane conductance) in a controlled manner. The three which we shall discuss and compare are, first, excitability-inducing material (EIM); second, the depsipeptides—in particular valinomycin; and, third, the polyene antibiotics—in particular nystatin and amphotericin B. Since the first two have been described previously, we shall review them only briefly before turning to a discussion of polyene antibiotics.

EXCITABILITY-INDUCING MATERIAL (1, 2, 28–30) This is a protein, or protein-containing material, as yet incompletely purified and characterized, obtained from the culture fluid of certain bacteria and a wide variety of other sources. When added in microgram per milliliter amounts to one or both of the chambers separated by a membrane, EIM reduces membrane resistance from 10^9–10^7 Ω-cm^2 to values of 10^4 Ω-cm^2 or less. This fall in resistance is a consequence of increased cation permeability; the membrane is selectively permeable to cations, but does not significantly discriminate among different cations. In this low-resistance state, the membrane resistance is voltage-dependent, rising regeneratively, at a threshold potential, by a factor of 5–10. With an applied constant current, this resistance increase produces a voltage response whose rising phase is not unlike that of an action potential (1, 2, 28). If, in addition to EIM, protamine is added to the solution, full-fledged action potentials can be observed; with membranes of appropriate lipid composition in the presence of salt gradients, repetitive firing and self-sustained oscillations can occur (29, 30). These phenomena are due to "flipping" of the membrane from a state of cation to anion permeability and back again. For a detailed discussion of these fascinating results and their interpretation, see Mueller and Rudin (30).

DEPSIPEPTIDES (VALINOMYCIN) (31–33) When added in micromolar amounts to one or both of the aqueous phases, the cyclic depsipeptide valinomycin lowers the membrane resistance by several orders of magnitude,

again because of an increase in cation permeability. In this case, however, the membrane not only is selectively permeable to cations, but also discriminates significantly among different cations (31–33). The membrane is approximately 300 times more permeable to K^+ than to Na^+ as measured either by the relative membrane resistances in NaCl and KCl solutions or by the bi-ionic potentials developed when the membrane separates equal concentrations of NaCl and KCl. The selectivity for alkali cations is in the order $Rb^+ > K^+ > Cs^+ > Na^+ \approx Li^+$. The cyclic depsipeptides of the enniatin series have effects on these membranes similar to the effects of valinomycin (31); the discrimination among cations is dependent on ring size. Membrane resistance is not voltage-dependent in the presence of any of these molecules. Thus, to summarize: Both EIM and the cyclical depsipeptides make the membrane cation selective. With EIM there is no selectivity among cations, but the resistance (and, therefore, permeability) is voltage-dependent; with the depsipeptides there is selectivity among cations, but the resistance is not voltage-dependent.

The Polyene Antibiotics (Nystatin, Amphotericin B)

The polyenes are a group of antibiotics whose members each contain a large lactone ring with conjugated double bonds, and whose biological activity seems to result from their ability to make cell membranes "leaky" to small solutes. A large amount of circumstantial evidence suggests that the compounds are active exclusively on sterol-containing membranes [see Lampen (34)]. For example, they are active against fungi, whose membranes contain sterols, but not active against bacteria, whose membranes are sterol-free. They attack only those cultures of *Mycoplasma* which have incorporated cholesterol into their membranes, and they hemolyze red blood cells. When injected beneath monolayers formed at an air/water interface, the polyenes interact with mixed monolayers of cholesterol and phospholipid, but not with those formed from phospholipid alone (35). Finally, van Zutphen et al. (36) have shown that this sterol requirement extends to the thin lipid membranes; i.e. filipin and nystatin will not break films formed from lecithin alone, but will break those formed from mixtures of lecithin and cholesterol.

We now describe some of our studies of the action of polyenes on thin lipid membranes. These membranes were formed from mixtures of ox brain lipid plus *dl*-α-tocopherol and cholesterol dissolved in 2:1 chloroform-methanol. The only action of filipin that we have observed is the destruction of the membranes shortly after the electrical resistance begins to fall. No ion selectivity has been observed in the evanescent period between onset of action and membrane breakdown. Amphotericin B and nystatin at appropriate concentrations, however, lowered the membrane resistance in a controlled and

reproducible manner without causing breakage; it is the results obtained with these two antibiotics that we shall describe. A more detailed report will be presented elsewhere.[2]

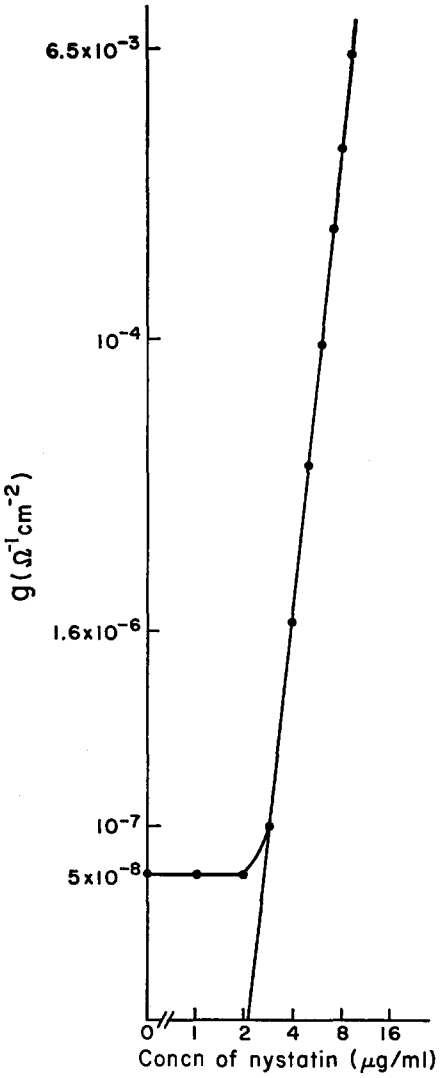


FIGURE 7. Conductance of a single thin lipid membrane as a function of nystatin concentration on both sides of the membrane. The membrane was formed in a solution containing 100 mM NaCl, 0.2 mM $MgSO_4$, and 5 mM sodium phosphate (pH 7). After the membrane had been formed, the concentration of nystatin was raised stepwise on both sides. The conductance reached a steady value within 5 min after each addition of nystatin. The conductance was obtained from the steady-state potential difference developed across the membrane during a small step of current. Temperature = 32° ± 0.5°C.

RESISTANCE When added only to the solution on one side of the membrane, amphotericin B and nystatin manifest variable effectiveness in lowering the membrane resistance; e.g. with a given membrane a certain concentration might lower the resistance by a factor of 10^4, whereas 5 times this concentration acting on another film formed from the same membrane-

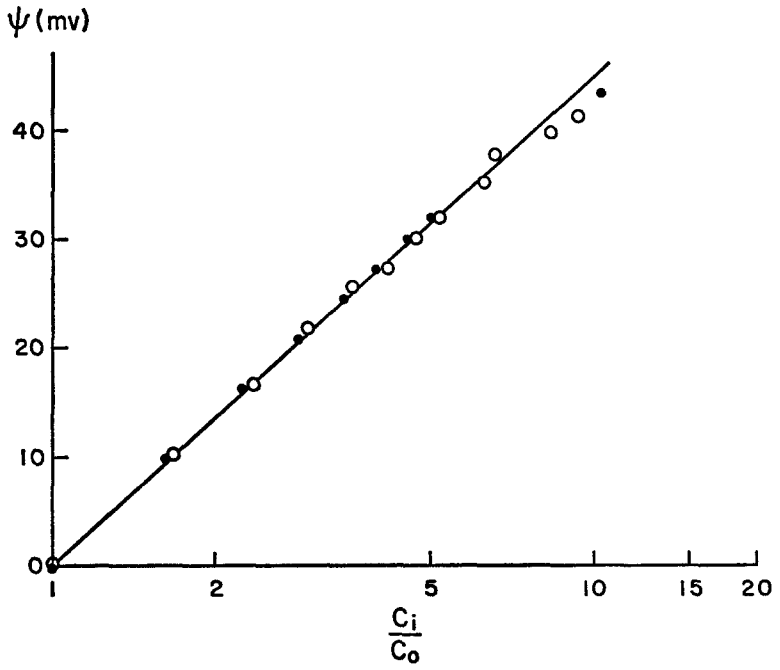


FIGURE 8. Potential difference (in millivolts) across a thin lipid membrane treated with amphotericin B, as a function of the ratio of LiCl concentrations in the two aqueous phases. Membranes were formed in 10 mM LiCl. After the membrane had formed, amphotericin B was added to one side to give a concentration of 20 μg/ml. After 3 min, resistance had fallen to 1000 Ω-cm². At this time, LiCl concentration differences were established by additions of small amounts of concentrated LiCl to one side, and the potential difference across the membrane was measured as described in Fig. 5. (● and ○ refer to two different membranes.) Temperature = 31° ± 1°C. Note the bending from a straight line at the higher concentration ratios. For similar experiments begun in 100 mM LiCl, the slope was the same as here (≈45 mv), but the bending occurred at smaller concentration ratios. The same slope was obtained in single salt experiments with NaCl and with KCl, but bending was more pronounced and occurred at smaller concentration ratios, especially with KCl. Essentially the same data were obtained for membranes treated with nystatin. (Corrections for activity coefficients have been incorporated into the concentration values, and therefore the abscissa is actually the ratio of activities.)

forming solution might have no effect.[8] On the other hand, when nystatin, for example, was added to both sides in micromolar amounts, reproducible resistance changes over a range of five orders of magnitude were observed. Comparable results were obtained when the membranes were formed in

[8] Amphotericin B was added dropwise from a concentrated aqueous solution of Fungizone (Squibb), which contains equal amounts of amphotericin B and deoxycholate. Nystatin was added dropwise from a concentrated methanol solution of Mycostatin (Squibb). Controls with deoxycholate and methanol showed no effect on the membranes.

solutions already containing the antibiotic. In this case the onset of low resistance paralleled the appearance of "black" regions in the film, thus demonstrating that the significant action of these antibiotics is on the thin membrane. Fig. 7 is a log-log plot of conductance as a function of nystatin concentration on *both sides*; the conductance is seen to vary as the 10th power of the nystatin concentration. Nystatin on one side alone produced a negligible change in the conductance of membranes formed from this particular lipid solution. If, however, the polyene was present in excess on one side, the conductance of the film varied as approximately the 6th power of the concentration on the other side. If nystatin was flushed out from one of the chambers, the membrane resistance returned to its previously high value within a few minutes. Reintroduction of nystatin to the chamber again lowered the resistance. We shall comment further below on the large power dependences of conductance on concentration.

TABLE I

BI-IONIC POTENTIALS ACROSS THIN LIPID MEMBRANES TREATED WITH NYSTATIN

Side 1	Side 2	Potential difference*
		mv
0.01 M NaCl	0.01 M $NaSO_3CH_2CH_2OH$	55–60
0.1 M NaCl	0.1 M $NaSO_3CH_2CH_2OH$	55–60
0.1 M NaCl	0.1 M $NaSO_3CH_3$	40
0.03 M NaCl	0.03 M NaF	22
0.1 M NaCl	0.1 M NaI	3–8
0.1 M $NaSO_3CH_3$	0.1 M $NaSO_3CH_2CH_2OH$	20–25
0.1 M NaCl	0.1 M KCl	1–2

* In all cases, side 1 is positive with respect to side 2.

ION SELECTIVITY In the low-resistance state produced by nystatin or amphotericin B, the membrane is *anion*-selective.[9] Fig. 8 is a plot of membrane potential as a function of the log of the ratio of LiCl concentration on one side to that on the other. Note that the membrane does not discriminate completely between Li^+ and Cl^-, the slope being 45 mv instead of the theoretical 60 mv. Similar slopes are obtained with concentration gradients of sodium chloride and somewhat smaller slopes, of approximately 35 mv, with sodium isethionate, which might suggest little membrane selectivity between

[9] With excessive amounts of antibiotic, the membrane resistance drops to extremely low values, ion selectivity is lost, and the membrane generally breaks after a short time. It would appear that at higher concentrations these antibiotics completely disrupt the membrane structure and produce general "leakiness."

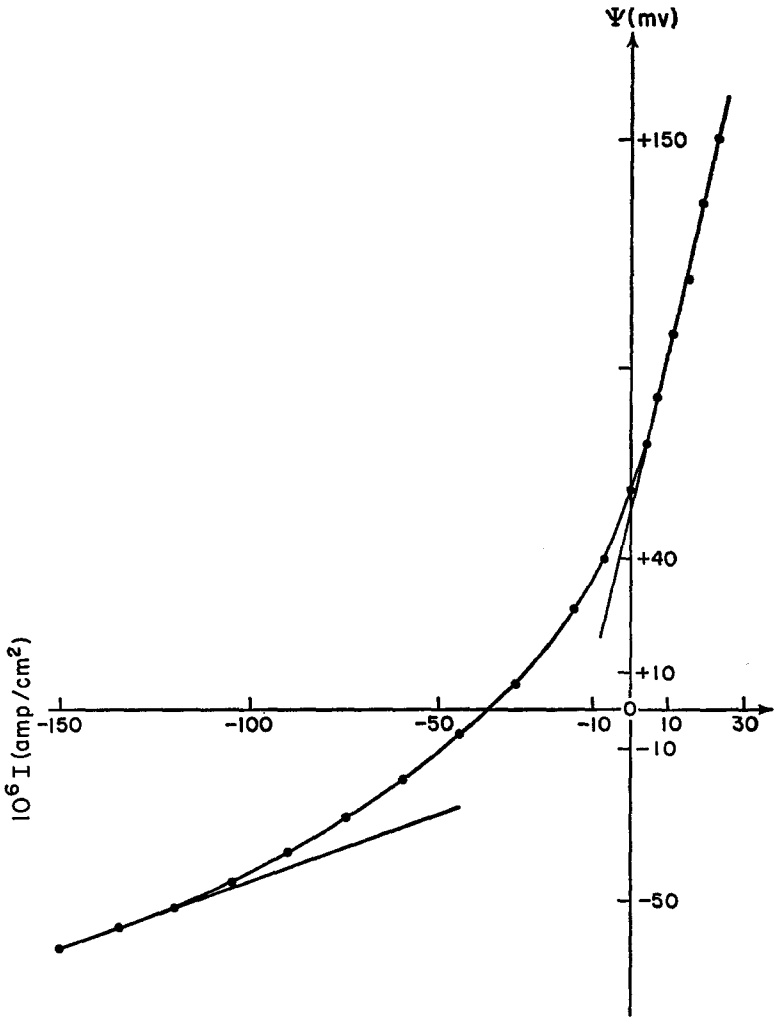


FIGURE 9. Current-voltage relationship of a nystatin-treated thin lipid membrane separating solutions containing equal concentrations of NaCl and $NaSO_3CH_2CH_2OH$. The membrane was formed in distilled water. After the membrane had formed, NaCl was added to one side to give a concentration of 10 mM, and $NaSO_3CH_2CH_2OH$ was added to the other side to give a concentration of 10 mM. Nystatin was then added to both sides to give a concentration of 5 μg/ml. Within 2 min, the resistance began falling and a potential difference developed across the membrane. After 20 min, the potential had reached a constant value of 60 mv (NaCl side positive), and the resistance (as measured by small steps of current) was also constant. The current-voltage curve was then obtained. Each point is the steady-state potential difference developed across the membrane during a given step of current. (The potential of the $NaSO_3CH_2CH_2OH$ solution is taken as 0, and positive current is defined as flowing from the NaCl solution to the $NaSO_3CH_2CH_2OH$ solution.) From the slopes of the two tangents, a slope-resistance ratio of 15 is obtained. (The slope-resistance ratio varies considerably from one membrane to another, even though the bi-ionic potentials are quite reproducible. This may be due to difficulties in defining boundary conditions for a bi-ionic situation, as is suggested by the consistent failure of the tangent for positive currents to pass through the origin.) Temperature = 32° ± 0.25°C.

chloride and isethionate ion. That this is not the case, however, is seen from bi-ionic potentials of approximately 60 mv, which develop across the membrane between equimolar concentrations of sodium chloride and sodium isethionate. Table I summarizes the bi-ionic potentials obtained between

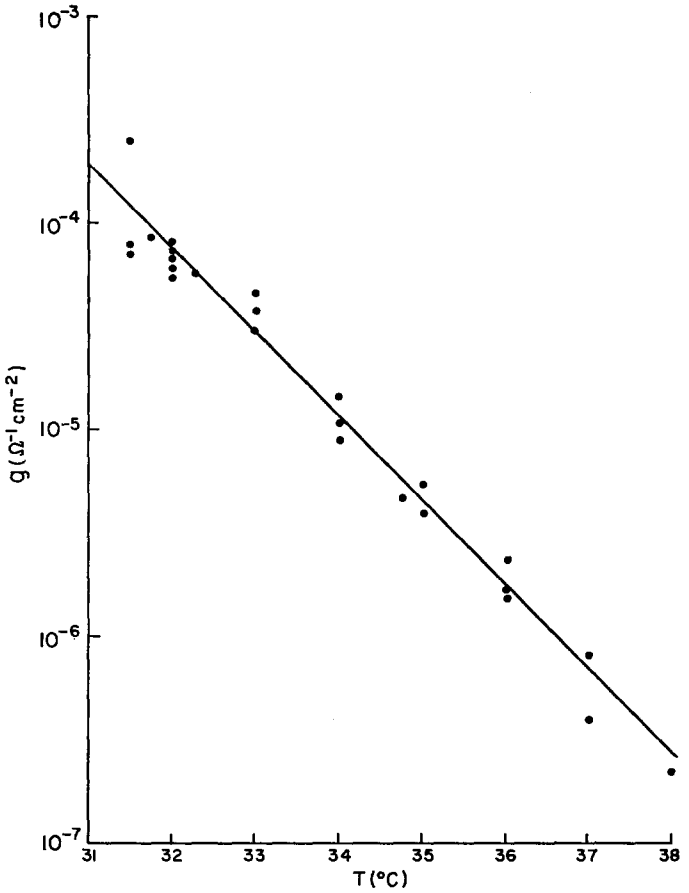


FIGURE 10. Temperature dependence of the conductance of a nystatin-treated thin lipid membrane. The membrane was formed at 31.5°C in a solution containing 100 mM NaCl, 0.2 mM $MgSO_4$, and 5 mM sodium phosphate (pH 7). After the membrane had formed, nystatin was added to both sides to give a concentration of 8 μg/ml. After 5 min, the temperature was continuously cycled between 31.5° and 38°C, and the conductance was measured during this time. Conductance was measured as in the experiment in Fig. 7.

several pairs of sodium salts The observed selectivity among anions appears to be dependent on ionic size. Fig. 9 shows the current-voltage relationship with 0.01 M sodium chloride vs. 0.01 M sodium isethionate; the rectification is qualitatively consistent with the selectivity predicted from the bi-ionic potential.

TEMPERATURE DEPENDENCE In the presence of nystatin, membrane conductance decreases extraordinarily with increasing temperature. Fig. 10 is a semilog plot of the membrane conductance of a single membrane as the temperature is repeatedly cycled between 31.5° and 38°C; a Q_{10} of approximately 10^4 is obtained! This appears to imply a ridiculously high desorption energy for nystatin. If we recall, however, the 10th power dependence of conductance on nystatin concentration, and assume from this that 10 nystatin molecules must be adsorbed to create a conductance channel, then, assuming

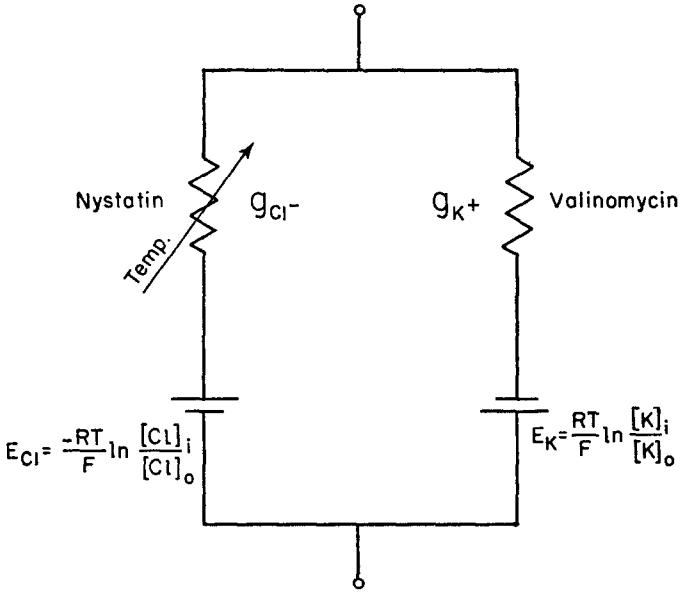


FIGURE 11. Equivalent circuit for a thin lipid membrane treated with both nystatin and valinomycin. The circuit shown is a specific example of the type commonly drawn to represent plasma membranes (38). (Since nystatin does not produce ideal chloride selectivity, the emf in the nystatin limb should be somewhat less than the theoretical "Nernst emf" that is drawn.) The arrow drawn through the chloride conductance indicates that this conductance is temperature-dependent.

that the energy of desorption for a given molecule is independent of the number of molecules already adsorbed, the Q_{10} of desorption of a single molecule becomes $\sqrt[10]{10^4} \approx 2.5$, which is quite reasonable.

MEMBRANE IN THE PRESENCE OF BOTH VALINOMYCIN AND NYSTATIN A prevalent idea in membrane physiology is that in a membrane simultaneously permeable to more than one species of ion there exist separate, parallel pathways, each permeable to one specific ion. This idea is generally depicted by the familiar equivalent circuit of the type shown in Fig. 11 (37, 38). These thin lipid membranes in the presence of both valinomycin and nystatin

appear to realize this description (Fig. 12). In the presence of valinomycin alone, the membrane is essentially K^+-selective, and, if there is a KCl concentration difference, a cationic potential difference is established. If now sufficient nystatin is added to make the Cl^- conductance larger than the K^+

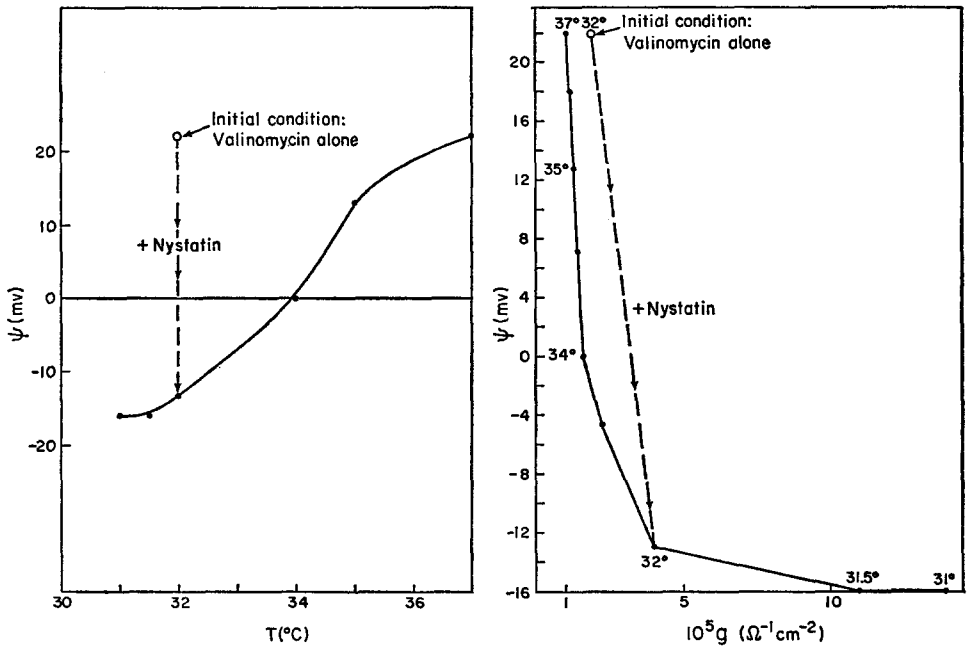


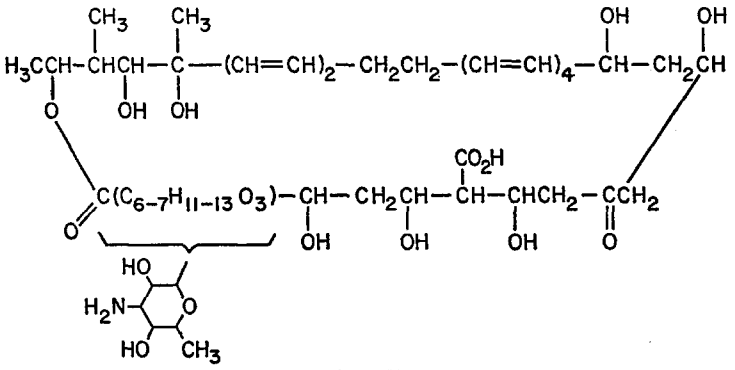
FIGURE 12. Effect of temperature on a thin lipid membrane treated with both nystatin and valinomycin. Left: potential vs. temperature: the membrane was formed in a solution containing 100 mM KCl, 0.2 mM $MgSO_4$ and 5 mM potassium phosphate (pH 7). After the membrane had formed, valinomycin was added to one side to give a concentration of 1 μg/ml. The KCl concentration was then increased on one side to 340 mM, and a potential difference of +22 mv appeared across the membrane. (All potentials are measured with respect to the 340 mM KCl solution.) This is taken as the "initial condition" in the figure. Nystatin was then added to both sides (dashed line) to a concentration of 4 μg/ml, and the potential then changed to −13 mv. The temperature was then cycled between 31° and 37°C, and the potential and conductance were continually recorded. Right: potential vs. conductance: this is the same experiment as in the left half of the figure. We are here plotting the potential difference as a function of the membrane conductance, which is changing with temperature. Indicated along the curve are the temperatures (all in degrees centigrade) corresponding to particular points.

conductance, the potential difference "flips" to that of an anion-selective membrane. Independence of these two conductance pathways can be demonstrated by changing the temperature. Increasing the temperature lowers the chloride conductance (see preceding section) and thus drives the membrane into the cation permeable state; decreasing the temperature increases the

chloride conductance and returns the membrane to the state of anion permeability.[10] (In terms of the equivalent circuit of Fig. 11, this transition is realized through the temperature dependence of the chloride conductance.) This system may be viewed as a model for a thermal receptor.

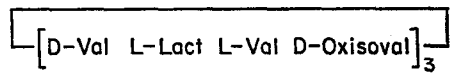
Summary and Conclusions

We have considered three substances which drastically alter the ion permeability of thin lipid membranes: EIM, valinomycin, and nystatin (and amphotericin B). The first is of unknown molecular structure; the last two are



NYSTATIN

$C_{46-47}H_{73-75}O_{18}N$



VALINOMYCIN

FIGURE 13. Formulae of nystatin and valinomycin.

cyclical structures (Fig. 13).[11] EIM produces cation selectivity and electrical excitability, but no selectivity among cations. Valinomycin and nystatin produce selectivity among cations and anions, respectively, but neither confers electrical excitability. We have seen that the nystatin-induced conductance varies as the 10th power of the nystatin concentration on the

[10] The effect of temperature on the valinomycin action is small compared with that on the nystatin action and is actually of opposite sign; i.e. increasing the temperature increases potassium conductance (31).

[11] Mueller and Rudin (30) have recently reported that the large cyclic peptide alamethicin reproduces much of the effects of EIM.

two sides, and approximately as the 6th power of the concentration on one side, if excess antibiotic is present on the other side. These data suggest that nystatin may produce pores in the membrane (through the proper arrangement of 10 molecules to form a channel). On the other hand, it has been reported, and we have confirmed, that the valinomycin-induced conductance varies linearly with the valinomycin concentration (33), which suggests that this molecule acts as a carrier, rather than as a channel former. [Pressman et al. (39) have reached this conclusion from other considerations.] Speculation about the mechanism by which valinomycin induces selectivity has centered around the ability of the carbonyl groups in the ring to substitute for the hydration shells around the cations (31, 33). Similarly, one may speculate that the hydroxyl groups in the lactone ring of nystatin can substitute for the everted hydration shells around anions and thus account for the anion selectivity.[12] (The inability of nystatin- and amphotericin B–treated membranes to discriminate completely between cations and anions may be related to the large size of the ring.)

We have seen that valinomycin and nystatin can interact independently with the same film to create a membrane with separate cation and anion permeable sites. This is rather appealing, as it is in keeping with that spirit among membrane physiologists that views plasma membranes as mosaic structures containing separate ion (or neutral molecule)–selective pathways. The molecular bases for these selective pathways in cell membranes is at present unknown. Whether structures formally analogous to the ones we have discussed (e.g. loops or rings, perhaps as parts of larger molecules) play a functional role in cell membranes remains to be seen.

This work was supported by a grant from the Office of Saline Water, U.S. Department of the Interior, and by U.S. Public Health Service grants 5T1 MH-6418 and NB-03356.

Addendum We have recently found that nystatin greatly increases the value of the osmotic permeability coefficient, P_f. The membranes were formed at 32°C in a solution of 100 mM NaCl + 5 mM sodium phosphate (pH 7) + 0.2 mM $MgSO_4$, containing nystatin at a concentration of 7.5 μg/ml. The electrical resistance of these membranes was approximately 100 Ω-cm^2, as compared to 3×10^7 Ω-cm^2 for membranes formed with the same lipid solution in a nystatin-free medium. The value of P_f for these membranes was 23×10^{-3} cm/sec, as compared to 0.4×10^{-3} cm/sec for membranes formed in the nystatin-free medium; that is, nystatin increased the water permeability approximately 50-fold. Furthermore, in contrast to unmodified membranes, which are virtually impermeable to small hydrophilic solutes (see text), nystatin-treated membranes show graded permeability to solutes on the basis of size.

[12] Since we have observed no changes in selectivity between pH 2.7 and pH 9.5, we consider it unlikely that the charge of the single carboxyl or single amino group in nystatin plays a role.

This was established by measurements of the reflection coefficients, σ, for several solutes. The results were:

$$\sigma_{\text{urea}} \approx 0$$

$$\sigma_{\text{ethylene glycol}} \approx 0.15$$

$$\sigma_{\text{glycerol}} \approx 0.33$$

$$\sigma_{\text{propionamide}} \approx 0.36$$

$$\sigma_{\text{erythritol}} \approx 0.79$$

$$\sigma_{\text{glucose}} \approx \sigma_{\text{sucrose}} \approx \sigma_{\text{NaCl}} \approx 1$$

We feel that these results (that is, the large increase in water permeability and the graded permeability to solutes) are strong evidence that nystatin (and by implication, amphotericin B) produces aqueous pores in thin lipid membranes. From the fact that the membrane is impermeable to glucose but slightly permeable to erythritol, we estimate the pore radius to be approximately 4 A.

REFERENCES

1. MUELLER, P., D. O. RUDIN, H. T. TIEN, and W. C. WESCOTT. 1962. Reconstitution of cell membrane structure *in vitro* and its transformation into an excitable system. *Nature.* **194**:979.
2. MUELLER, P., D. O. RUDIN, H. T. TIEN, and W. C. WESCOTT. 1962. Reconstitution of excitable cell membrane structure *in vitro*. *Circulation.* **26**:1167.
3. MUELLER, P., D. O. RUDIN, H. T. TIEN, and W. C. WESCOTT. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solutions. *J. Phys. Chem.* **67**:534.
4. CASS, A., and A. FINKELSTEIN. 1967. Water permeability of thin lipid membranes. *J. Gen. Physiol.* **50**:1765.
5. ROBBINS, E., and A. MAURO. 1960. Experimental study of the independence of diffusion and hydrodynamic permeability coefficients in collodion membranes. *J. Gen. Physiol.* **43**:523.
6. PAGANELLI, C. V., and A. K. SOLOMON. 1957. The rate of exchange of tritiated water across the human red cell membrane. *J. Gen. Physiol.* **41**:259.
7. HUANG, C., and T. E. THOMPSON. 1966. Properties of lipid bilayer membranes separating two aqueous phases: water permeability. *J. Mol. Biol.* **15**:539.
8. HANAI, T., and D. A. HAYDON. 1966. The permeability to water of bimolecular lipid membranes. *J. Theoret. Biol.* **11**:370.
9. HANAI, T., D. A. HAYDON, and W. R. REDWOOD. 1966. The water permeability of artificial bimolecular leaflets: a comparison of radiotracer and osmotic methods. *Ann. N.Y. Acad. Sci.* **137**:731.
10. DAINTY, J. 1963. Water relations of plant cells. *Advan. Botan. Res.* **1**:279.
11. FINKELSTEIN, A., and A. CASS. 1967. Water permeability of thin lipid membranes: the effect of cholesterol. *Nature.* **216**:717.
12. VREEMAN, H. J. 1966. Permeability of thin phospholipid films. *Koninkl. Ned. Akad. Wetenschap. Proc. Ser. B.* **69**:542.
13. SCHATZBERG, P. 1963. Solubilities of water in several normal alkanes from C_7 to C_{16}. *J. Phys. Chem.* **67**:776.
14. SCHATZBERG, P. 1965. Diffusion of water through hydrocarbon liquids. *J. Polymer Sci.* (No. 10, Pt. C):87.
15. HANAI, T., D. A. HAYDON, and J. TAYLOR. 1964. An investigation by electrical methods of lecithin-in-hydrocarbon films in aqueous solutions. *Proc Roy. Soc.* (*London*), *Ser., A.* **281**: 377.

16. TIEN, H. T. 1967. Black lipid membranes: thickness determination and molecular organization by optical methods. *J. Theoret. Biol.* **16**:97.
17. A.P.I. RESEARCH PROJECT 44. 1953. Selected Values of Physical Constants and Thermodynamic Properties of Hydrocarbons and Related Compounds. Carnegie Institute of Technology Press, Pittsburgh. 227.
18. HUANG, C., L. WHEELDON, and T. E. THOMPSON. 1964. The properties of lipid bilayer membranes separating two aqueous phases: formation of a membrane of simple composition. *J. Mol. Biol.* **8**:148.
19. 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.
20. LÄUGER, P., W. LESSLAUER, E. MARTI, and J. RICHTER. 1967. Electrical properties of bimolecular phospholipid membranes. *Biochim. Biophys. Acta.* **135**:20.
21. JONES, G. 1930. On the existence and behavior of complex polyiodides. *J. Phys. Chem.* **34**: 673.
22. HAVINGA, E., and E. WIEBENGA. 1959. A review of the interhalogen compounds and polyhalides. *Rec. Trav. Chim.* **78**:724.
23. HILDEBRAND, J. H., and C. JENKS. 1920. Solubility. IV. Solubility relations of naphthalene and iodine in the various solvents, including a method for evaluating solubility data. *J. Am. Chem. Soc.* **42**:2180.
24. GURNEY, R. W. 1962. Ions in Solution. Dover Publications, Inc., New York. 3.
25. BEAN, R. C., and W. C. SHEPHERD. 1967. Permeability of lipid, bimolecular leaflet membranes to organic solutes. *Federation Proc.* **26**:862.
26. LESSLAUER, W., J. RICHTER, and P. LÄUGER, 1967. Some electrical properties of bimolecular phosphatidyl inositol membranes. *Nature.* **213**:1224.
27. SEUFERT, W. D. 1965. Induced permeability changes in reconstituted cell membrane structure. *Nature.* **207**:174.
28. MUELLER, P., and D. O. RUDIN. 1967. Action potential phenomena in experimental bimolecular lipid membranes. *Nature.* **213**:603.
29. MUELLER, P., and D. O. RUDIN. 1963. Induced excitability in reconstituted cell membrane structure. *J. Theoret. Biol.* **4**:268.
30. MUELLER, P., and D. O. RUDIN. 1968. Resting and action potentials in experimental bimolecular lipid membranes. *J. Theoret. Biol.* **18**:222.
31. MUELLER, P., and D. O. RUDIN. 1967. Development of K^+-Na^+ discrimination in experimental bimolecular lipid membranes by macrocyclic antibiotics. *Biochem. Biophys. Res. Commun.* **26**: 398.
32. LEV, A. A., and E. P. BUZHINSKI. 1967. Cation specificity of model bimolecular phospholipid membranes with exposure to valinomycin. *Cytology* (Russian), **9**:102.
33. 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.
34. LAMPEN, J. O. 1966. Interference of polyenic antifungal antibiotics (especially nystatin and filipin) with specific membrane functions. *In* Biochemical Studies of Antimicrobial Drugs. B. A. Newton and P. E. Reynolds, editors. The Society for General Microbiology, Cambridge. 111.
35. DEMEL, R. A., and L. L. M. VAN DEENEN. 1965. Penetration of lipid monolayers by polyene antibiotics. Correlation with selective toxicity and mode of action. *J. Biol. Chem.* **240**: 2749.
36. VAN ZUTPHEN, H., L. L. M. VAN DEENEN, and S. C. KINSKY. 1966. The action of polyene antibiotics on bilayer lipid membranes. *Biochem. Biophys. Res. Commun.* **22**:393.
37. HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.*, (*London*). **117**:500.
38. FINKELSTEIN, A., and A. MAURO. 1963. Equivalent circuits as related to ionic systems. *Biophys. J.* **3**:215.
39. PRESSMAN, B. C., E. J. HARRIS, W. S. JAGGER, and J. H. JOHNSON. 1967. Antibiotic-mediated transport of alkali ions across lipid barriers. *Proc. Natl. Acad. Sci. U.S.* **58**:1949.

Note Added in Proof Two recent papers (Läuger, P., J. Richter, and W. Lesslauer. 1967. *Ber. Bunsenges. Physik. Chem.* **71**: 906; Rosenberg, B. and G. L. Jendrasiak, 1968. *Chem. Phys. Lipids* **2**: 47) recognize that I_2 is necessary for obtaining conductance increases with I^-. Both papers suggest that I_2 forms a charge transfer complex with lecithin, producing either electronic or ionic conduction. We consider that our membrane potential measurements with equal I^- concentrations on both sides of the membrane, but different I_2 concentrations, preclude electronic conduction with redox reactions at the interfaces, because the potentials (10–50 mv) were of the wrong sign: i.e., the solution with the low I_2 concentration was negative. Because of high permeability of the membrane to I_2, it is not possible to assure a significant ratio of interfacial I_2 concentrations unless the concentration on one side is driven almost to zero by a reducing agent, such as $S_2O_3^=$. The rise of membrane resistance to values comparable with those obtained in the absence of I^- and I_2 demonstrated that $S_2O_3^=$ significantly reduced the interfacial I_2 concentration. The accompanying potential difference showed that an asymmetry was established between the interfaces. (No potentials were obtained with $S_2O_3^=$ in the absence of I_2 and I^-.) These results are consistent with our suggestion of a polyiodide being the charge-carrying species, but seem incompatible with an electron conduction mechanism. As for a charge transfer mechanism giving rise to I^- conduction, we wish to note that we obtained similar conductance increases with films of saturated lecithin and films of unsaturated lipids. Since unsaturation greatly promotes formation of charge transfer complexes, this argues against such a mechanism.

Discussion

Question from the Floor: In regard to valinomycin, Lardy has pointed out that not only do you get a hole in the molecule as your model shows, but there is some interaction of the carbonyl group, and these antibiotics chelate with metal ions. You have to consider this in the explanation of ion selectivity.

Dr. Finkelstein: Yes. As Mueller and Rudin have pointed out, the carbonyl groups, which are facing into the ring, can substitute for hydration shells of the ion. I didn't mean to imply that this was just fitting a piece in a hole. Obviously there are various forces interacting. In the case of valinomycin you would invoke the carbonyl groups, and perhaps, in the case of nystatin, where you have OH groups, you would invoke these as substituting for the everted hydration shells.

Question from the Floor: All right. These functional groups do play a role.

Dr. Finkelstein: Oh, absolutely! I mean, I'm not even sure the ring plays a part.

Question from the Floor: Now, in this regard, if one uses these extremely small amounts of valinomycin, then one would be hard pressed to explain the permeation of these ions on the basis of, say, the ion interaction with valinomycin on one side and this penetrating through. You would almost have to take into account that the valinomycin gets incorporated into the bilayer, and then opens up and forms a semipermeable pore through the membrane, rather than going through and carrying ions *stoichiometrically*. Would you agree to that?

Dr. Finkelstein: I don't know that you could eliminate either one. I agree with you that the valinomycin is in the membrane. For instance, if you wash it out from the aqueous solution, the effect will still be there for some time. But I don't see that that

eliminates or establishes one or the other type of mechanism. I think that to date, the data, at least on these membranes, are equally consistent with a pore or with a so-called carrier, although, as we mentioned, the dependence of membrane conductance on the first power of the valinomycin concentration may indicate a carrier mechanism. The carrier, of course, will just shuttle back and forth within the lipid region.

Question from the Floor: You said that the polar groups of phospholipids don't play a role in ion permeation. Do you really believe that? What about these phospholipids, such as phosphatidylserine, which have been shown to bind ions to membranes? What are you going to say about those?

Dr. Finkelstein: Well, I was talking about the unmodified film. I have no vested interest one way or the other, but nevertheless (and I find it surprising) the results we and the others have obtained do not seem to depend on the nature of the polar ends of the phospholipids. Now, it is true, Dr. Katzman and his collaborators have done many studies on the binding of cations, monovalent and divalent, to phospholipids, but still the permeability properties of the unmodified lipid film do not seem to be significantly dependent on the polar groups.

I must admit that no detailed study has been done of films with identical hydrocarbon tails but different polar groups. Hopefully such studies will be done. I just tried to point out that the permeability data available at present are adequately explained simply by the properties of the hydrocarbon region.