J. Membrane Biol. 16, 237–256 (1974)

The Effect of Prymnesin on the Electric Conductivity of Thin Lipid Membranes

Arie Moran* and Asher Ilani

Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received 17 September 1973

Summary. A proteolipidic toxin, prymnesin, when added to the aqueous solutions around thin lipid membranes causes a marked increase in membrane conductance. The toxin-treated membrane is cation-permselective. The extent of cation permselectivity is dependent upon ionic strength of the aqueous solutions in a fashion similar to the dependence of cation permselectivity of a cation exchanger containing about 100 mM of fixed negative sites. Dose-response relationship studies reveal a linear relation between log prymnesin concentration and log membrane conductance. The slope of the curve is around 3 if the toxin is applied to one side of the membrane and is around 7 if the toxin is applied to both sides of the membrane. The membrane treated with toxin on one side only is clearly asymmetric in its properties. These characteristics are expressed by an asymmetric current-voltage relationship, and by asymmetric sensitivity of membrane conductance to pH and to salt concentration. The conductance of the toxin-treated membrane is inversely proportional to temperature. It is suggested that aggregates of toxin moieties assemble in the membrane to form negatively charged aqueous pores. There is roughly a good correlation between the increase in membrane conductance and the increase in membrane permeability to urea if both were attributed to the formation of aqueous channels in the membrane.

Artificial bileaflet membranes ("black" membranes) generally have a low electric conductivity compared with that of cellular membranes (Mueller, Rudin, Tien & Wescott, 1963). Many of the studies of the artificial membranes revolve around the problem of finding and characterizing "modifiers", namely, substances which will increase the membrane conductance by rendering it more permeable to ions (Finkelstein & Cass, 1968). The list of such modifiers is increasing. We report here the action of a new modifier, prymnesin, on black membranes. Preliminary reports of this study have appeared earlier (Moran & Ilani, 1971, 1972).

Prymnesin is a toxin extracted from the phytophlagellate, *Prymnesium parvum*. It is notorious for its biological activity in nature as causing death

* *Present address:* Department of Physiology and Biophysics, Haifa Medical School, Haifa, Israel.

to fish populations, i.e., it is ichtiotoxic (Shilo, 1971). Other activities of the toxin studied in the laboratory are hemolysis and cytolysis (Dafni & Shilo, 1966).

The exact chemical structure of the toxin is not known. The active purest preparations contain amino acids, phosphatides and sugars (Ulitzur & Shilo, 1970). The solubility characteristic of the toxin is similar to that of proteolipids. The nature and activities of the toxin have been reviewed lately by Shilo (1971).

The earliest studies of the action of prymnesin on black membranes were conducted on membranes formed from lecithin and cholesterol solution in methyl oleate (Moran & Ilani, 1971; for procedure of membrane formation *see* Moran & Ilani, 1970). The studies reported in this paper were carried out on oxidized cholesterol membranes (Tien, Carbone & Davidowicz, 1966). The latter had the advantage of having much lower conductivities (i.e., of the untreated membranes), a fact which enabled study of the prymnesin effect at lower concentrations. Qualitatively, the effects of prymnesin on oxidized cholesterol and on lecithin-cholesterol membranes were similar.

Materials and Methods

Black lipid membranes were formed in the usual fashion by applying a small drop of 4% oxidized cholesterol solution in decane to a hole punched in a Teflon partition interposed between aqueous solutions (Mueller *et al.*, 1963; Tien *et al.*, 1966). The diameter of the hole in the Teflon partition was 1.3 mm. Electrodes on the two sides of the membranes were connected either to a homemade Wheatstone Bridge for measurement of membrane capacitance or to a Keithly electrometer (Model 615). The output of the electrometer was connected to a recorder for continuous measurement of membrane potential. Current was applied to the membrane through another pair of electrodes or through the same electrodes used for measurement of membrane potential. (We did not find any significant difference between the two methods, and because of simplicity the latter method was generally used.)

In many experiments a constant voltage was applied to the membrane and the current was monitored by measuring the voltage across a 10-MΩ resistance placed in series with the membrane. This technique was used to minimize the time constant of the recorded response to changes in membrane conductance at high (>500 MΩ) membrane resistances.

Current-voltage curves were recorded directly on an X-Y recorder (Hewlett-Packard). In this case two pairs of electrodes were used. A 100-kΩ resistance was connected in series to the current electrodes and the voltage across the resistance was monitored on the Y-axis of the recorder. The output of the electrometer which measured the voltage across the membrane was connected to the X-input of the recorder. The current across the membrane was varied at a very low frequency ($\sim$0.03 cps); i.e., the measured current-voltage relationship represented the steady-state characteristics of the system.

Prymnesin was added to one or both compartments in small volumes of ethanol solutions. Control additions of similar volumes of pure ethanol did not show any effects on membrane conductance. Stirring of the solution was affected by magnetic stirrers.

Some of the membranes were formed at the edge of a polyethylene tube connected to a syringe. A silver-silver chloride wire introduced into the tube served as an electrode for the inner compartment of the membrane. A major problem in this set-up was the sealing of the tube at the point of penetration by the silver wire. If successfully done, it was possible to change the solution in the outer compartment without membrane breakdown. This set-up was used for study of the reversibility of the prymnesin effect on the membrane. To remove the toxin from the membrane, the outside solution was rinsed continuously by a large volume of prymnesin-free solution.

Oxidized cholesterol was prepared by the method of Tien *et al.* (1966). Prymnesin was purchased from Makor Inc. (POB 6570, Jerusalem). It was available at a concentration of about 50,000 Hu (hemolytic units)/cc in ethanol solution. In this preparation 2,000 Hu corresponds to 1 μg. (S. Ulizur, *personal communication*). All salts used were of reagent grade. Water was glass bi-distilled. Monactin was a gift obtained from Dr. Hans Bickel of Ciba, Basle, Switzerland.

The silver-silver chloride electrodes were generally connected to the aqueous solution through agar potassium chloride bridges. In experiments where the membranes were formed at the edge of the polyethylene tube the Ag-AgCl electrodes were connected directly to the solutions on both sides of the membrane.

The capacitance of membranes of this study was about 0.6 $\mu F/cm^2$. The resistance of the untreated membranes varied between 2×10^7 and 2×10^8 ohms cm^2.

Results

Time Course of Prymnesin Effects on Membranes

Fig. 1 shows the typical time-course of the change in membrane conductance after the addition of prymnesin to one side of the membrane.

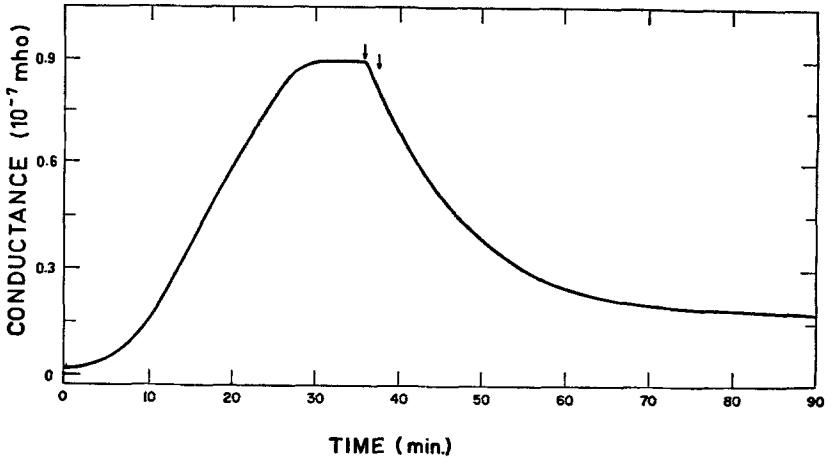

Fig. 1. Change in membrane conductance following addition at time zero of prymnesin to the solution on one side of the membrane. The membrane was formed at the edge of a polyethylene tube as described in Materials and Methods. The prymnesin-containing solution was replaced by prymnesin-free solution at the time interval indicated by the two arrows. 0.1 M KCl solutions on both sides of the membrane. Prymnesin concentration 5 Hu/cm^3. Membrane area about 0.01 cm^2

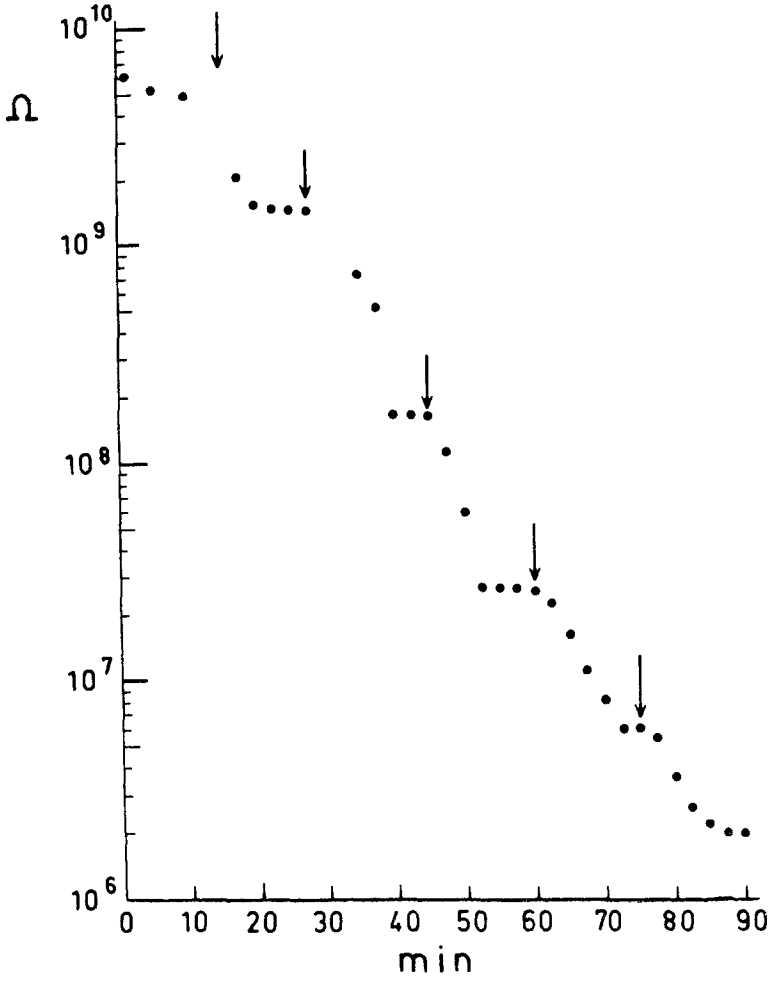

Fig. 2. Change in membrane resistance caused by consecutive additions (arrows) of prymnesin to the solution on one side of the membrane. After each addition the prymnesin concentration rose by 1 Hu/cc. Within 5 to 15 min after each addition the membrane attained a new steady level of resistance. 0.1 M KCl solutions on both sides of the membrane. Membrane area about 0.01 cm^2

Within about 10 min after the addition of the toxin the membrane conductance reached a new steady level. Subsequent addition of more toxin resulted in further increase of the conductance to a new steady level (Fig. 2). The highest conductance that could be obtained with prymnesin was around 10^{-2} mho/cm^2. Above this conductance level the membranes were not stable; i.e., a high enough prymnesin concentration led to rupture of the membrane. At least part of the prymnesin effect was reversible as can be deduced from Fig. 1, which shows that rinsing of the membrane with prymnesin-free solution led to a decrease in membrane conductance.

Dose-Response Relationship

From the result of experiments shown in Fig. 2 it was possible to study the dose-response relationship of the prymnesin-membrane interaction. The result of such analysis is shown in Fig. 3. The relation between log mem-

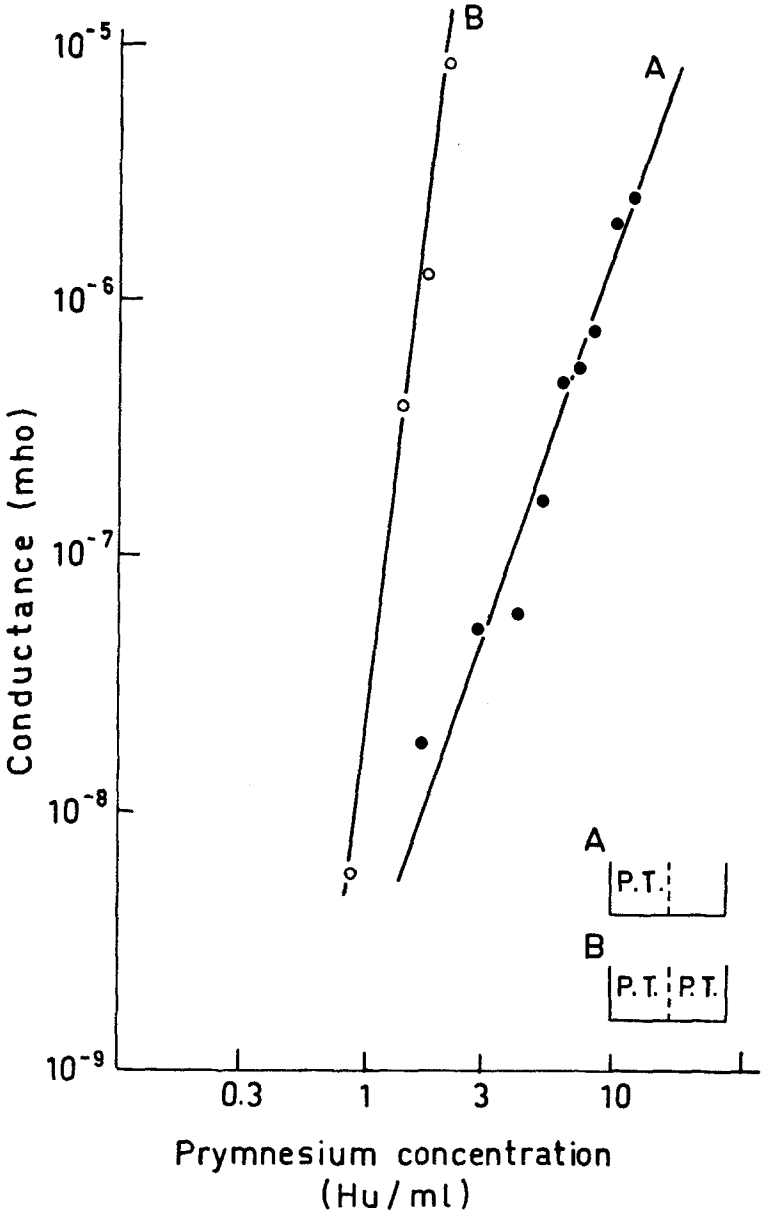

Fig. 3. Log membrane conductance as a function of log prymnesin (P.T.) concentration. Curve *A*: prymnesin added to one side of the membrane. Curve *B*: prymnesin added to both sides of the membrane. The points were determined from results of experiments such as that represented in Fig. 2. 0.1 M KCl solutions. Membrane area about 0.01 cm^2

brane conductance and log prymnesin concentration is linear and shows a slope of about 3 when the toxin is applied to one side of the membrane and a slope greater than 6 when applied to both sides of the membrane. These observations suggest that a complex of several molecules forms the units which contribute to the conductance of the membrane. Further analysis of these findings as well as comparison with dose-response relationships for other types of "modifiers" will be mentioned in the Discussion.

Temperature-Dependence of the Conductance of Prymnesin-Treated Membranes

Fig. 4 shows changes in conductance of prymnesin-treated membranes brought about by varying the temperature of the system. For comparison, the temperature effect on the conductance of a monactin-treated membrane is also shown. It is obvious that temperature-dependence of membrane conductance is completely different for two types of modifiers. In monactin-treated membrane the conductance increased with increase in temperature while for the prymnesin-treated membranes the relationship between temperature and membrane conductance was just the reverse. In the case of monactin, the temperature effect can be easily attributed to changes in the mobility of the charge carriers due to variations in viscosity of the membrane. The fact that in the prymnesin-treated membrane the conductance increased with decrease in temperature indicates that the number of "pores" or "ion carriers" in the prymnesin-treated membrane is inversely proportional to temperature. In this respect, prymnesin resembles the polyenes which are assumed to assemble into "pores" within the membrane (Cass, Finkelstein & Krespi, 1970).

Ion Discrimination by Prymnesin-Treated Membranes

The ability of the membrane to discriminate between various ions was studied by measuring the potential difference across membranes interposed between solutions of different composition.

Fig. 5 shows the potential difference across a potassium chloride concentration cell. At low concentrations the potential across the membrane was close to that of a theoretical cation electrode potential. The cation permselectivity declined with increase of salt concentration. This is a typical behavior of a cation-exchange membrane. The curves in Fig. 5 correspond to a theoretical potential across a membrane containing 100 mM of fixed monovalent negative sites. The calculations are based on the as-

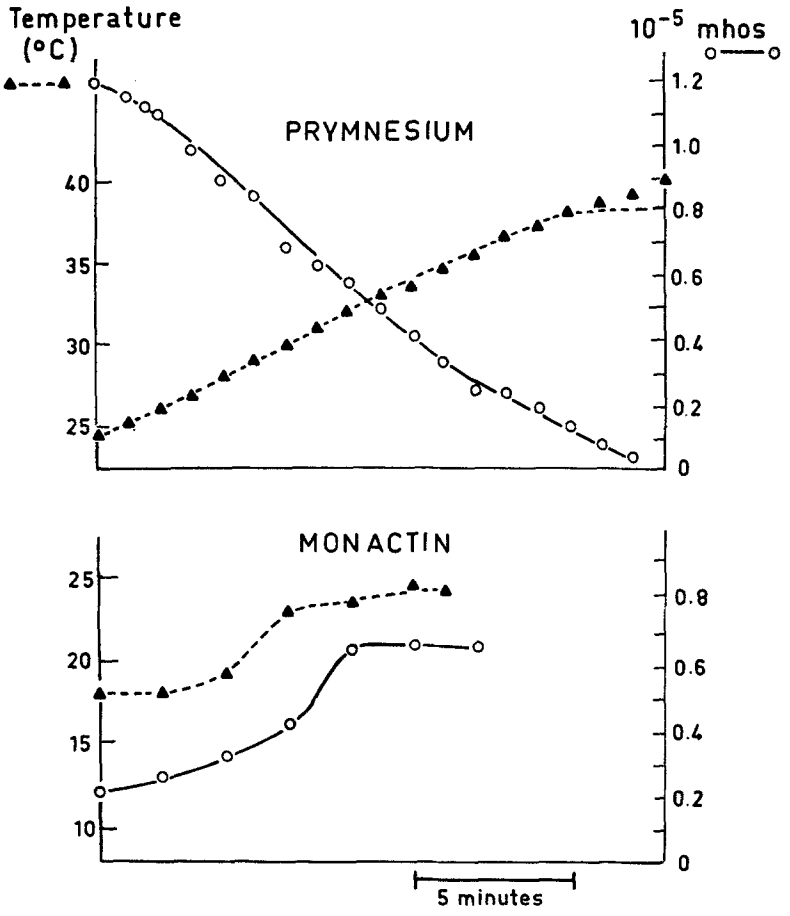

Fig. 4. Changes of temperature and of membrane conductance in a prymnesin- and in a monactin-treated membrane. 0.01 M KCl solutions. Prymnesin was present at a concentration of 7 Hu/cm^3. Monactin was present at a concentration of 10^{-6} M. Both modifiers were present only on one side of the membrane. It should be noted that the change in conductance of the prymnesin-treated membrane covers a range of two orders of magnitude

sumption that there is no diffusion potential inside the membrane (i.e., potassium and chloride have the same mobility) and that the potentials at the membrane-water interfaces are determined by the classic Donnan equations (Teorell, 1953).

The details of the way of obtaining the theoretical curves in Fig. 5 are as follows: It is assumed that the water and membrane phases behave ideally; namely, activity equals concentration. The potential at the interface is given by:

$$\psi^1 = \frac{RT}{F} \ln \frac{C_K^{1m}}{C_K^{1w}} = -\frac{RT}{F} \ln \frac{C_{Cl}^{1m}}{C_{Cl}^{1w}} \tag{1}$$

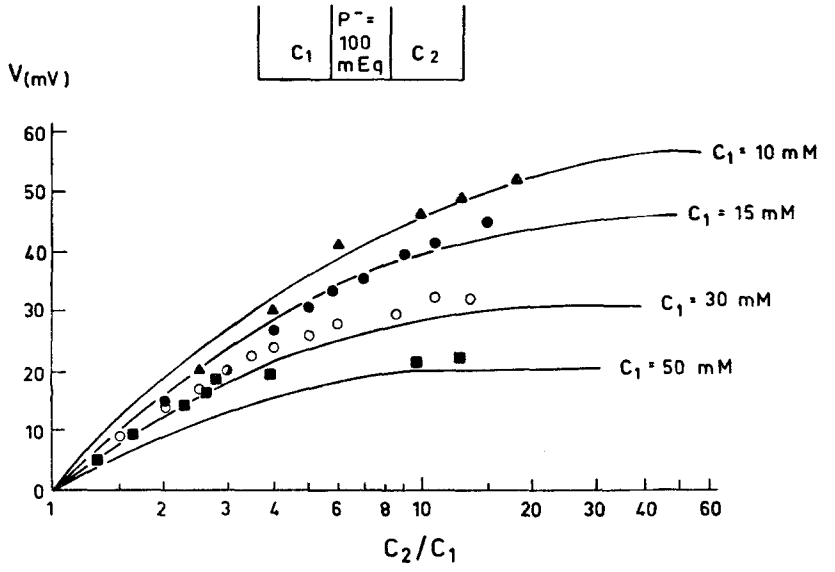

Fig. 5. The potential difference across prymnesin-treated membranes interposed between KCl solutions of concentration C_1 and C_2 as a function of the concentration ratio. The concentrated side was negative with respect to the dilute side. The concentration in the more dilute side (C_1) was kept constant while the concentration on the more concentrated side (C_2) was increased stepwise. Prymnesin was present in either side; i.e., there was no difference in membrane potential whether prymnesin was on the more dilute or more concentrated side of the membrane. The curves represent a theoretical potential difference across an ion exchange membrane containing 100 mM of monovalent fixed negative sites (inset). Details of calculations of the theoretical curves are given in the text

where C_K^1 and C_{Cl}^1 are the concentrations of K and Cl at membrane-water interface 1, respectively. Superscripts m and w refer to the membrane and water phases, respectively. ψ^1 is the potential difference at interface 1. A similar equation will apply to interface 2. That is,

$$\psi^2 = \frac{RT}{F} \ln \frac{C_K^{2m}}{C_K^{2w}}. \tag{2}$$

The total potential difference ψ_m will be given by

$$\psi_m = \psi^2 - \psi^1. \tag{3}$$

Donnan equilibrium requires that

$$C_K^{1m} * C_{Cl}^{1m} = C_K^{1w} * C_{Cl}^{1w} \tag{4}$$

and

$$C_K^{2m} * C_{Cl}^{2m} = C_K^{2w} * C_{Cl}^{2w}. \tag{5}$$

Table 1. The potential difference (p.d.) across prymnesin-treated membranes interposed between 20 mM solutions of chlorides of the indicated alkali metals ions [a]

Bi-ionic cell	p.d. (MV)	P_i/P_K	U_i/U_K
K-Li	−21	0.44	0.52
K-Na	−10	0.67	0.68
K-Cs	+1.0	1.05	1.05

[a] Polarity refers to K-side. The relative permeability P of the membrane to the ions was calculated by the "constant field equation" assuming that the permeability to chloride is negligible. The relative mobilities U_i of the ions in aqueous solutions are also listed.

Due to electroneutrality

$$C_K^w = C_{Cl}^w \tag{6}$$

and

$$C_K^m = C_{Cl}^m + X \tag{7}$$

where X is the concentration of fixed monovalent negative sites in the membrane. Eqs. (4), (5), (6) and (7) enable one to calculate C_K^m and C_{Cl}^m in terms of the concentration of KCl in the aqueous solutions and of X. Thus, ψ^1, ψ^2 and ψ_m can be calculated. The curves shown in Fig. 5 represent the results of such calculations. The membrane potential ψ_m, is shown as a function of KCl concentration on both sides of the membrane when X equals 100 mM.

It should be noted that there is a fairly good correspondence between the behavior of the prymnesin-treated membrane and an ion-exchange membrane of about 100 mM of fixed charge sites. It is therefore assumed that fixed negative charges present on the prymnesin molecule are responsible for the cation permselectivity of the treated membrane.

It is worthwhile to emphasize that the potential difference in a KCl concentration cell is the same whether the prymnesin is present in the more concentrated, the more dilute or both sides of the membrane. This indicates that the extent of cation permselectivity and its dependence on salt concentration is a symmetric property of the membrane. This fact stands out in comparison to other asymmetric properties of the membrane treated by prymnesin on one side only (*see* next sections).

Discrimination between various cations was studied by measuring the bi-ionic cell potential. The results are shown in Table 1. These studies indicate that the relative permeabilities of the prymnesin-treated membrane to the alkali metal ions correspond to their relative mobilities in aqueous solutions (Table 1).

Permeability to Water and Urea

The permeability to water of oxidized cholesterol black membranes before and after treatment with prymnesin was studied using tritiated water. A typical example of such an experiment is shown in Fig. 6*A*. The main conclusion of this study was that an increase of 10^4-fold in membrane electrical conductance does not cause any discernible effect on membrane permeability to water which was around 6.0×10^{-4} cm/sec. This value is about 1.3 times smaller than the value reported by Tien and Ting (1968).

In this respect prymnesin resembles other "modifiers" which induce considerable changes in membrane electrical conductance but do not affect its water permeability (Finkelstein & Cass, 1968; Lippe, 1968). A reasonable way to express this phenomenon is to say that the prymnesin-induced increase of water permeability is negligible compared with the permeability to water of the untreated membrane.

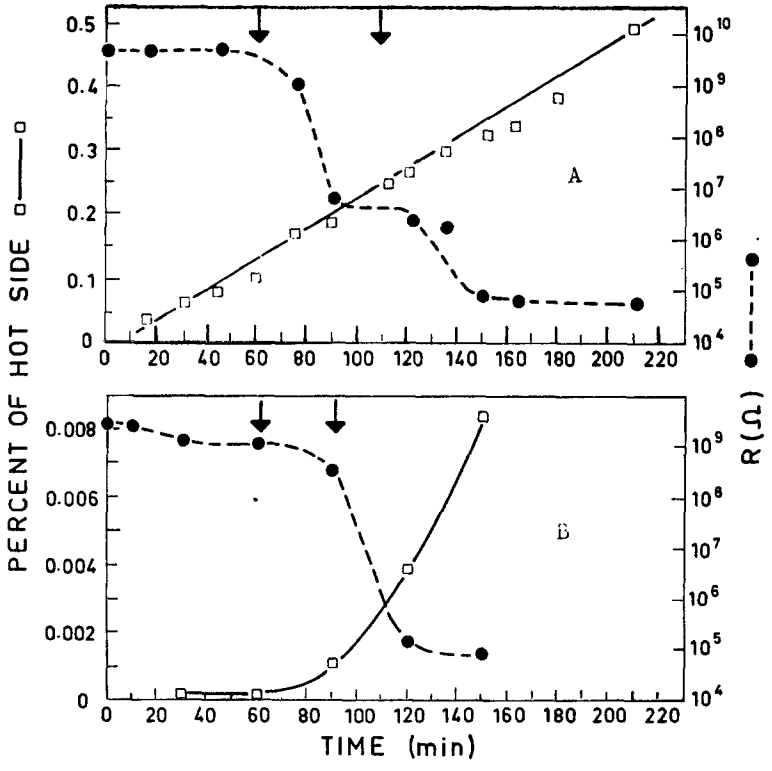

Fig. 6. Transport of radioactive water (*A*) and urea (*B*) across membranes before and after the addition of prymnesin (arrow) into the aqueous solution on one side of the membrane. The concentration of radioactivity in the outer cup (20 ml volume) is expressed as per cent of its concentration in the inner cup (5 ml volume). Membrane resistance was monitored continuously throughout the experiment

This can be shown to be true in the following way. Suppose that the electrical conductance of prymnesin-treated membrane, which was maximally about 10^{-2} mho/cm^2, was due to the formation of a single pore in the membrane. If the specific conductance of the pore solution was 10^{-2} mho/cm (i.e., about the specific conductance of 0.1 M KCl solution) and the membrane thickness 5×10^{-7} cm, the area of the pore should be 5×10^{-7} cm^2 per cm^2 of membrane in order to account for membrane conductance of 10^{-2} mho/cm^2; i.e., if 5×10^{-7}th of the black membrane area became porous it would account for the maximal effect of prymnesin on membrane conductance. The increase in membrane permeability to water due to such a pore will be given by:

$$\frac{\underset{\text{fraction of porous membrane}}{5 \times 10^{-7}} \times \underset{\text{diffusion coefficient of water}}{3 \times 10^{-5}}}{\underset{\text{thickness of membrane}}{5 \times 10^{-7}}};$$

i.e., 3×10^{-5} cm/sec. This value is approximately 5% of the measured water permeability of the untreated membrane. If unstirred layers were also affecting the measurement (Holz & Finkelstein, 1970) the water-permeability of the untreated membrane was even higher. Thus, there is no discrepancy between the pronounced effect of prymnesin on membrane permeability to ions and the lack of a clear-cut effect on the permeability to water.

On the other hand, prymnesin had a definite effect on membrane-permeability to urea. A typical experiment is shown in Fig. 6*B* where it can be shown that the permeability to urea increased from less than 1.2×10^{-7} cm/sec in membranes which had a conductance of 10^{-8} to 10^{-9} mho/cm^2 to 3.8×10^{-6} cm/sec in a prymnesin-treated membrane which had a conductance of about 10^{-4} mho/cm^2. The values of the permeability to urea are a little higher than those reported by Holz and Finkelstein (1970) for membranes treated with polyene antibiotics. Using the same considerations as mentioned above it can be shown that there is a fairly good correspondence between the increase in membrane conductance and the increase in permeability to urea if both were attributed to the formation of porous areas in the black membrane.

Asymmetry of Membranes Treated with Prymnesin on One Side Only

In this section we report some of the properties of membranes which were treated by prymnesin on one side, and which indicated that the system was asymmetric in its properties.

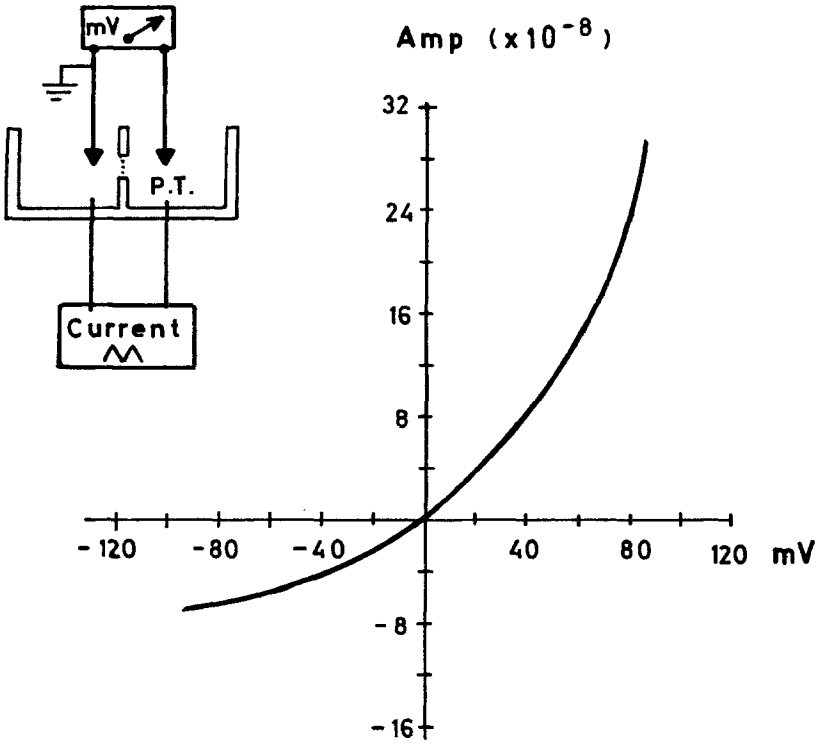

Fig. 7. Current-voltage relationship for prymnesin (10 Hu/ml)-treated membrane. 0.1 M KCl on both sides of the membrane. Continuous record on an X-Y plotter. The triangular shaped current source operated at a frequency of 0.03 cycles/sec. Membrane area about 0.01 cm^2

1. Current-Voltage Relationship. Fig. 7 shows a typical current-voltage curve for a prymnesin-treated membrane. It is clear that the curve is not symmetric near the zero-zero point. The conductance of the membrane is higher if the prymnesin-containing side is positive. The ratio between the currents in a membrane polarized by the same absolute values of voltages is increased as the voltage across the membrane increases (Fig. 8). For comparison it was found that the current-voltage curve of a monactin-treated membrane was symmetrical (Fig. 9).

2. pH Sensitivity. The sensitivity to pH of the prymnesin-treated membrane is remarkable, both in (a) its quantitative aspect (e.g., a change of two pH units in a particular range led to a change of three orders of magnitude in membrane conductance) and (b) in its asymmetric characteristics (i.e., lowering of pH led to an increase in membrane resistance, if it occurred in the side which contained the toxin, and to a decrease in membrane

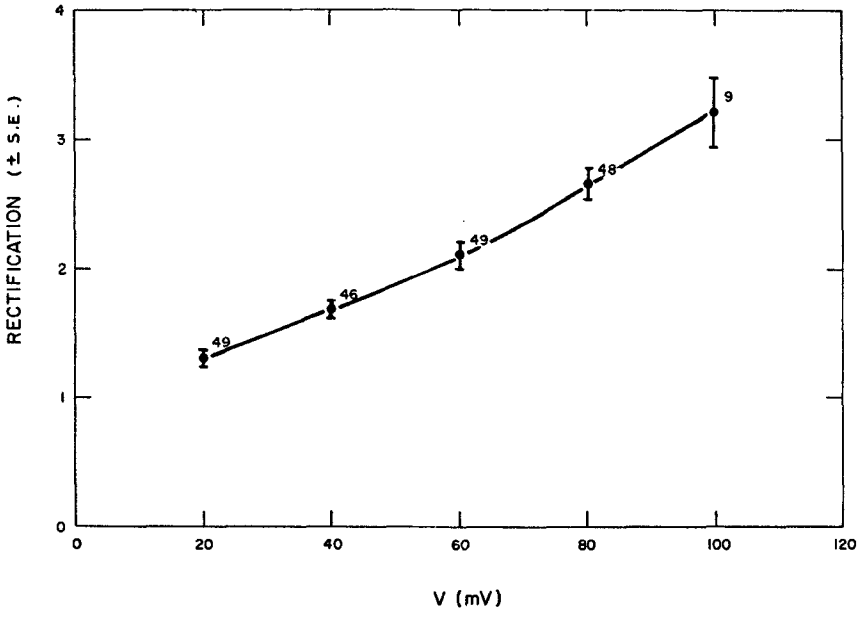

Fig. 8. Rectification by prymnesin-treated membrane as a function of membrane potential. Rectification defined as ratio between absolute values of currents when voltages of $+X$ and $-X$ are imposed on the membrane. Numerals near points denote number of measurements. Bars denote standard error limits

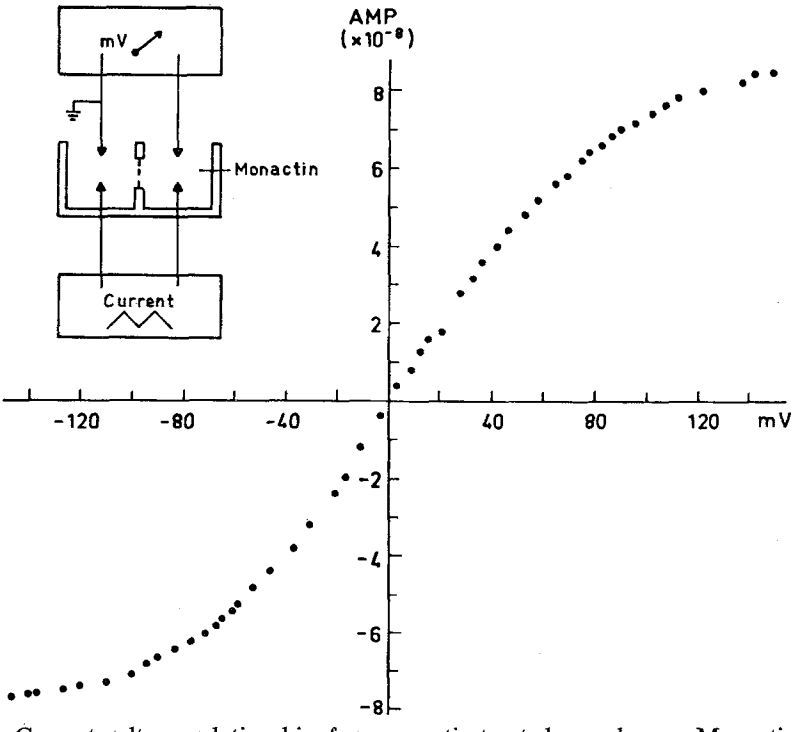

Fig. 9. Current-voltage relationship for monactin-treated membrane. Monactin at a concentration of 10^{-6} was added to one side of the membrane. 0.1 M KCl solutions. Other details as in Fig. 7

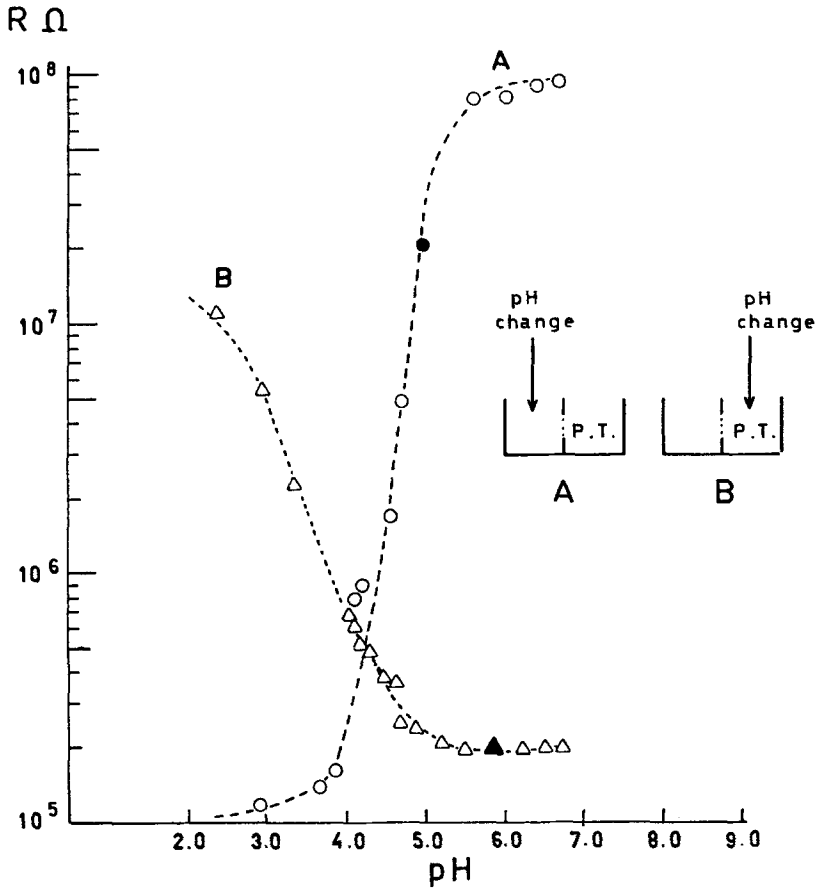

Fig. 10. Membrane resistance as a function of pH. Prymnesin (P.T.) was present on one side of the membrane. The initial conditions included identical solutions on both sides of the membrane at a pH indicated by a full point on each curve. The pH was varied subsequently on the side which did not contain prymnesin (curve *A*) or on the side which did contain prymnesin (curve *B*). The pH was varied by additions of small amounts of HCl or KOH, and was measured directly by glass electrodes. 0.1 M KCl solutions on both sides of the membrane

resistance if it occurred on the membrane side which did not contain the toxin [Fig. 10]). Because of these two opposing effects, the study of the dependence of prymnesin activity on the pH in a situation where the solutions on both sides of the membrane had the same pH did not show any striking features.

It is important to emphasize that: (a) no potential difference developed in response to formation of pH gradients across the membrane and (b) cation permselectivity as judged from the magnitude of the potential difference in a KCl concentration cell was independent of pH at least in the

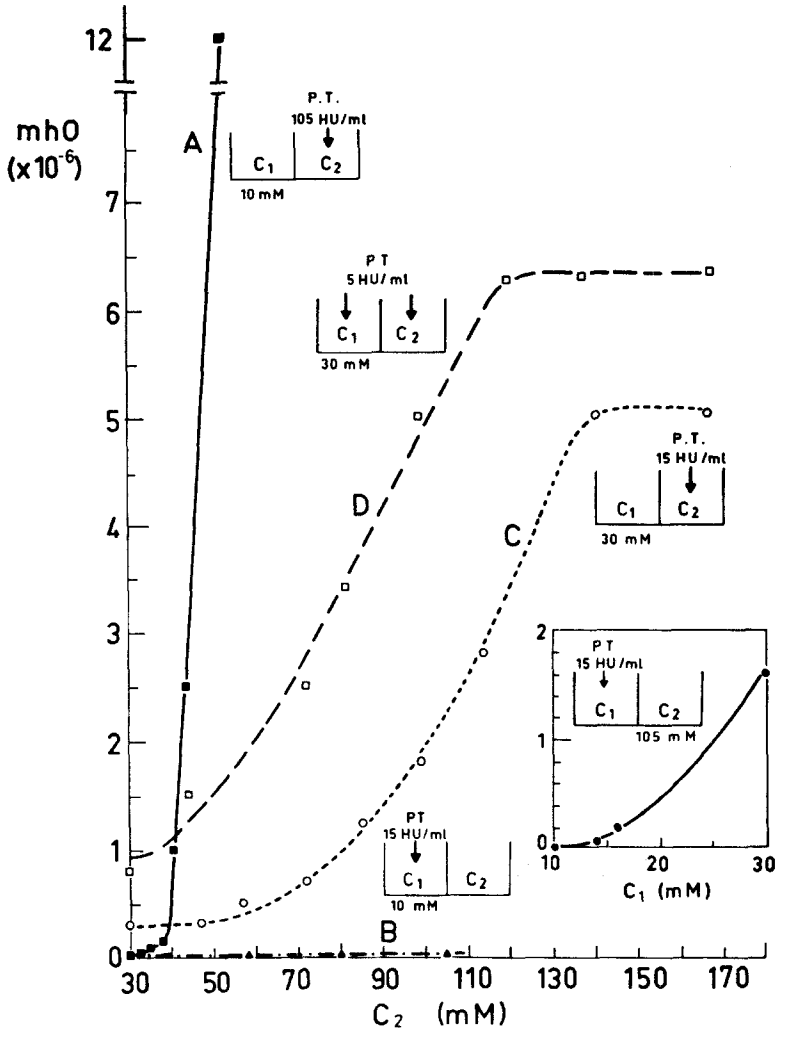

Fig. 11. Membrane conductance as a function of KCl concentration on one side of the membrane. Initial conditions: equal concentrations on both sides of the membrane, i.e. $C_1 = C_2$. The concentration in side 2 (C_2) was subsequently varied. Prymnesin was present in side 2 (curves *A* and *C*), side 1 (curve *B*) and in both sides (curve *D*). Note that the conductance was independent of KCl concentration in the side which did not contain prymnesin (curve *B*). The results of the continuation of the latter experiment are shown in the inset of the figure; i.e., increase in KCl concentration in the side which contained prymnesin resulted in a clear increase of membrane conductance

range of 3 to 8. These facts imply that the pH changes affect the membrane conductivity by altering its permeability to potassium and chloride rather than by varying the concentration of hydrogen or hydroxyl ions as charge carriers.

3. Salt Concentration and Membrane Conductance. The effect of increasing salt concentration on the conductance of the prymnesin-treated membrane is dependent upon the side in which the salt concentration is varied with respect to the side which contained the toxin (Fig. 11). The membrane conductance was independent of salt concentration on the side which did not contain the toxin (Fig. 11, curve *B*) but it was extremely sensitive to salt concentration in the side which contained prymnesin (Fig. 11, curves *A*, *C* and inset). A similar sensitivity was observed when the toxin was present on both sides of the membrane (compare curves *C* and *D* in Fig. 11).

Discussion

The results of the experiments reported in this article demonstrate clearly that prymnesin is a powerful modifier of bileaflet lipid membrane conductance. In the following, the properties of the prymnesin-treated membrane will be considered. Special attention will be given to the question of the mechanism for the prymnesin-induced increase in ion permeability.

A dose-response curve representing the prymnesin effect on membrane conductance (Fig. 3) shows the following features: (1) the slope of the log conductance-log concentration curve is greater than one; (2) the above slope is about twice as sharp if the toxin is applied to both sides of the membrane compared with the slope found when the toxin is applied to one side only. For typical carrier-mediated ion transport modifiers, the slope of the curve representing log membrane conductance as a function of log concentration is one (Szabo, Eisenman & Ciani, 1969). For some of the "pore"-forming modifiers the slope is high, indicating that aggregates of molecules are assembled within the membrane to form a conducting unit. Thus, for gramicidin the slope of the log conductance-log concentration curve is 2 (Goodall, 1970), suggesting that a dimer of gramicidin is forming the unit conductive channel in the membrane. For the polyene, nystatin, the slope of the log conductance-log concentration curve is about 10 if the nystatin is applied to the two sides of the membrane (Cass, Finkelstein & Krespi, 1970). Unlike prymnesin, nystatin does not affect membrane conductance if applied to one side of the membrane only. In nystatin it was, therefore, possible to study the dose-response relationship for the case where nystatin was added to one side of the membrane while the other side contained excess of the polyene. Under these conditions, the slope of the log conductance-log concentration curve was around 4; i.e., about half of the slope for the case where nystatin was added to both sides of the membrane (Cass *et al.*, 1970). In analogy to the interpretation of the phenomena in

the nystatin-treated membranes it is suggested that aggregates of 6 to 8 molecules of prymnesin are interacting in the membrane to form a conducting channel. Therefore, the conductance of the membrane should be proportional to the 6th to 8th power of the toxin concentration. When the toxin was added to one side of the membrane only, the limiting factor for the formation of channels in the membrane was the number of toxin molecules available for the formation of the part of the channel in the remote side of the membrane; i.e., the side of the membrane facing the solution which did not contain prymnesin. Under this condition, the membrane conductance depended only on a lower power of the toxin concentration (*see* Fig. 3).

Another analogy between nystatin and prymnesin is the reversed relationship between conductance and temperature found in membranes treated by these "modifiers" (Cass *et al.*, 1970, and Fig. 4).

The fact that the prymnesin-treated membrane is cation permselective (Fig. 5) and that its permselectivity is reduced by increasing salt concentration suggests very strongly that the toxin is forming pores in the membrane and that these pores contain fixed negative charges. It seems unlikely that a cation carrier would become also an anion carrier as the salt concentration in the aqueous solution increases. On the other hand, a salt concentration effect on the permselectivity of an ion exchanger is an anticipated and well understood factor (Teorell, 1953) as indicated already in this paper. Electrophoretic studies of the toxin indicate that it is indeed negatively charged (Shilo, 1971).

An interesting aspect of the prymnesin-treated membrane is its asymmetric properties with respect to changes in the solution on either side of the membrane or with respect to the direction of electrical current (rectification). This should be compared with the symmetric property of the monactin-treated membrane (Fig. 9). Monactin is a typical cation carrier-"modifier" (Szabo *et al.*, 1969). The main problem in that context is the distinction between the following two possibilities: (1) asymmetry which reflects asymmetry of the individual pores; (2) asymmetry which reflects the fact that variation in the solution on the side which contains prymnesin can affect also the interaction between prymnesin and the membrane whereas variation in the solution on the side which does not contain prymnesin can affect only the conductance of the individual pores. For instance, the effect of pH changes on conductance of prymnesin-treated membranes (Fig. 10) can be interpreted in principle along the following two lines: (1) asymmetry of pores in the membrane (i.e., the side of the pore which faces the prymnesin-containing side of the membrane responds

to pH changes differently from the other edge of the pore); (2) the pore itself is symmetric (i.e., it responds to pH changes in a similar fashion on both sides); however, variations in pH on the side which contains prymnesin affect also the number of pores in the membrane.

The same considerations apply to the analysis of the asymmetric dependence of membrane conductance on salt concentration (Fig. 11). Obviously, it is also possible that combinations of both factors are responsible for the observed asymmetric phenomena.

In principle, one could possibly resolve the question of whether or not the pore itself is asymmetric by measuring the properties of a unit conductive channel as was done in the case of gramicidin (Hladky & Haydon, 1972) or EIM (excitability inducing material)-treated membranes (Ehrenstein, Lecar & Nossa, 1970; Bean, 1972). Unfortunately, we were not able to detect any discrete changes in membrane conductance in the course of the action of prymnesin on the black membrane which could be attributed to the opening of a single pore in the membrane. Thus, there was no way of looking at the conductance of single pores in the prymnesin-treated membrane.

Outwardly it seems that the rectification by the membrane (Fig. 8) indicates clearly that the pores in the membrane are asymmetric. Yet even in this case it is possible to argue that the voltage across the membrane can modify the number of toxin molecules within the membrane and consequently the number of pores within it. Thus, rectification would not be the property of each pore, according to such an argument, but it may express the fact that the number of pores are altered by varying the membrane polarization. The extreme voltage-dependent conductance of membranes treated with monazomycin were attributed to alteration in the number of monazomycin molecules inside the membrane as modified by the potential differences at the membrane-water interfaces and within the membrane (Muller & Finkelstein, 1972). Yet, the fact that prymnesin is negatively charged and that the conductance of the membrane is lower when the prymnesin-containing side is negative is not compatible with the latter idea. Moreover, the time course for a change of the conductance from a low to a high level or vice versa in response to a stepwise change in voltage across the membrane was instantaneous (less than 10 msec). This observation is not compatible with the idea that the conductance changes are brought about by construction or dismantlement of pores. The latter processes would seem to take longer (*see* Fig. 1). In the case of monazomycin, the time course of the increase in conductance due to stepwise changes in polarization was indeed much longer (Muller & Finkelstein, 1972). Thus,

the rectifying properties of the prymnesin-treated membrane suggest that the conductive channel formed in a thin lipid membrane by prymnesin applied to one side of the membrane is asymmetric in character. This implies in analogy that if a membrane-protein is formed in the cytoplasm of a cell it can bind to the lipid matrix of the cell membrane and endow it with asymmetric properties.

The studies reported in this paper suggest also that prymnesin pores may interact between themselves. Thus, as the concentration of the prymnesin and consequently of membrane conductance increased beyond a critical level, the membrane ruptured. Rupture of membranes is a major manifestation of the toxic activity of prymnesin in nature. This is suggested by the fact that proteins and nucleic acids leak out of cells treated with the toxin (Shilo, 1971). On the other hand, it is plausible that the prelytic reversible effects of the toxin on biological cells (Dafni & Shilo, 1966) expressed by increased membrane permeability to ions are analogous to the effects of prymnesin on thin lipid membranes as reported in this paper.

An intriguing speculative question is whether prymnesin in a lipid layer represents a prototype of proteolipidic ionic routes in cellular membranes. It suggests that "appropriate" extracts of proteolipids from membranes may contain building blocks for "pores" which hopefully may be reconstituted in thin lipid membranes.

A.M. was the recipient of a training award from the Israel National Council for Research and Development.

References

Bean, R. C. 1972. Multiple conductance states in single channels of variable resistance lipid bilayer membranes. *J. Membrane Biol.* **7**:15

Cass, A., Finkelstein, A., Krespi, V. 1970. The ion permeability induced in thin lipid membranes by the polyene antibiotic nystatin and amphotericin B. *J. Gen. Physiol.* **56**:100

Dafni, Z., Shilo, M. 1966. The cytotoxic principle of the phytoplagellate *Prymnesium parvum*. *J. Cell Biol.* **28**:461

Ehrenstein, G., Lecar, H., Nossa, R. 1970. The nature of the negative resistance in bimolecular lipid membrane containing excitability-inducing material. *J. Gen. Physiol.* **55**:119

Finkelstein, A., Cass, A. 1968. Permeability and electrical properties of thin lipid membranes. *J. Gen. Physiol.* (*Suppl.*) **52**:145s

Goodall, M. C. 1970. Structure effects in the action of antibiotics on the ion permeability of lipid bilayers. III. Gramicidins "A" and "S", and lipid specificity. *Biochim. Biophys. Acta* **219**:471

Hladky, S. B., Haydon, D. A. 1972. Ion transfer across lipid membranes in presence of gramicidin A. I. Studies of the unit conductance channel. *Biochim. Biophys. Acta* **271**:294

Holz, R., Finkelstein, A. 1970. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *J. Gen. Physiol.* **56**:125

Lippe, C. 1968. Thiourea and potassium permeability of phospholipid bilayer membranes as affected by enniantin. *Nature* **218**:196

Moran, A., Ilani, A. 1970. Surface tension of an artificial bileaflet membrane in comparison to parent lipid solution-water interfacial tension. *Chem. Phys. Lipids* **4**:169

Moran, A., Ilani, A. 1971. Effect of prymnesium toxin on the electrical conductance of an artificial bileaflet membrane. *Israel J. Med. Sci.* **7**:333

Moran, A., Ilani, A. 1972. Prymnesium toxin and the conductance of bileaflet membranes. *Israel J. Med. Sci.* **8**:574

Mueller, P., Rudin, D. O., Tien, H. T., Wescott, W. C. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* **67**:534

Muller, R. U., Finkelstein, A. 1972. The effect of surface charge on the voltage-dependent conductance induced in thin lipid membranes by monazomycin. *J. Gen. Physiol.* **60**:285

Shilo, M. 1971. Toxins of *Chrysophyceae*. *In:* Microbial Toxins. S. Kadis, A. Ciegler, and S. J. Ajl, editors. p. 67. Academic Press Inc., New York, London

Szabo, G., Eisenman, G., Ciani, S. 1969. The effects of the macrotetralide actin antibiotics on the electrical properties of phospholipid bilayer membranes. *J. Membrane Biol.* **1**:346

Teorell, T. 1953. Transport processes and electrical phenomena in ionic membranes. *In:* Progress in Biophysics and Biophysical Chemistry. J. A. V. Butler and J. T. Randall, editors. Vol. 3, p. 305. Academic Press Inc., New York

Tien, H. T., Carbone, S., Davidowicz, E. A. 1966. Formation of "black" lipid membranes by oxidation products of cholesterol. *Nature* **212**:718

Tien, H. T., Ting, H. P. 1968. Permeation of water through bilayer lipid membranes. *J. Colloid Interface Sci.* **27**:702

Ulitzur, S., Shilo, M. 1970. Procedure for purification and separation of *Prymnesium parvum* toxins. *Biochim. Biophys. Acta* **201**:350