

# Water Permeation Across Ultrathin (Black) Lipid Membranes

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## FOREWORD

This is one of a continuing series of reports designed to present accounts of progress in saline water conversion and the economics of its application. Such data are expected to contribute to the long-range development of economical processes applicable to low-cost demineralization of sea and other saline water.

Except for minor editing, the data herein are as contained in a report submitted by the contractor. The data and conclusions given in the report are essentially those of the contractor and are not necessarily endorsed by the Department of the Interior.

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## I. SUMMARY

The black lipid membranes (BLM) of Rudin-Mueller-Tien type have been studied with the objective to determining the mechanism of water transport and to relating the effect of structure or composition of the latter to their water/salt transport properties. The effects of physical parameters such as pressure, concentration, and electrical potential differences on the transport of water and various solutes were also investigated. Results and conclusions from these studies are:

A. The water permeability coefficients,  $P_0$ , were determined for BLM formed from oxidized cholesterol, cholesterol-DAP (dodecyl acid phosphate), cholesterol-HDTAB (hexadecyltrimethylammonium bromide, and chloroplast extracts.

B. The temperature dependence of  $P_0$  for BLM generated from chloroplast extracts was also measured in the range of 17.5-34.0°C. Experimental activation energy was calculated to be  $4.2 \pm 0.5$  Kcal/mole. From the temperature-permeability coefficient data, various derived quantities from absolute reaction rate theory have been evaluated.

C. With the chloroplast BLM it has been demonstrated that photoelectric effects are possible. Further, it has been observed that transport of water can be initiated by light. The best value obtained to date is  $70 \mu\text{l/hr-cm}^2$ .

D. The most important conclusion drawn from these studies is that BLM constituted from photoactive pigments are a unique model system for study which could aid us in the understanding and exploitation of the photolysis of water.

## II. DESCRIPTION OF RESEARCH ACCOMPLISHMENTS

Permeability and osmotic properties of chloroplast and oxidized cholesterol bimolecular (black) lipid membranes have been the subject of continued work in the BLM Research Laboratory of the Department of Biophysics at Michigan State University under the direction of Professor H. Ti Tien.

In recent years, considerable efforts have been made to develop and utilize bimolecular lipid membranes (BLM) for membrane phenomena (1). BLM's have been used as models for the plasma membrane of erythrocyte, the nerve membrane of axon, the cristae membrane of mitochondrion, the thylakoid membrane of chloroplast, and the rod outer segment sac membrane retina (2). Results of recent findings are summarized under the following headings:

- A. Composition of lipid solutions for black lipid membrane Formation
- B. Water permeability of BLM
- C. Derived thermodynamics quantities
- D. The mechanism of water permeation
- E. Light-induced water flow across chloroplast BLM
- F. Miscellaneous results

### A. Composition of Lipid Solutions for BLM Formation

Depending upon the properties under investigation, various lipid solutions have been established for the formation of bimolecular lipid membranes (BLM). The composition of the lipid solutions which produced stable BLM is given in Table 1.

Table 1. Composition of lipid solutions for the formation of BLM

	<u>Lipid solution (w/v)</u>	<u>Aqueous phase</u>	<u>Remarks</u>
A.	Diocetyl phosphate (.08%) + cholesterol (.8%) in n-dodecane	0.1N NaCl	non-ionic
B.	Dodecyl acid phosphate (.35%) + cholesterol (.93%) in n-dodecane	0.1N NaCl	anionic
C.	Cholesterol (1%) in n-dodecane	0.001N NaCl +0.008% HDTAB*	cationic
D.	Oxidized cholesterol (4%) in n-octane	0.1N NaCl	very stable
E.	Spinach chloroplast extract (5%) in n-octane	0.1M KCl	light sensitive

\* Hexadecyltrimethylammonium bromide

Lipid solutions A, B, and C were established in the hope that a physical parameter could be found relating the formation and the stability of the black lipid membranes, the effects of surface-active compounds on membrane formation were explored. It was known from earlier observation that freshly crystallized cholesterol would not produce a stable black film. It was decided therefore to adopt cholesterol dissolved in n-dodecane as a standard for the study of

water permeation. In regard to surface-active agents, the choice had to be made in view of the fact that a vast number of such compounds are available. Therefore only three were chosen. These were DODP [ $C_{18}H_{37}O)_2PHO$ ] and DAP (equimolar of mono- and di-ester of dodecanol of orthophosphoric acid). These compounds are classified as oil-soluble surfactants. Also chosen was HDTAB [ $C_{16}H_{33}N(CH_3)_3Br$ ] which belongs to the water-soluble class. The choice of these surfactants was partially based on the fact that the first black lipid membrane observed was made from a solution containing phospholipids. As typified by lecithin, both the phosphate and quaternary ammonium groups were present. Further, the decision was made so that these surfactants represented three of the four major types. Thus, DAP is negatively charged when ionized in solution (anionic surfactant), HDTAB bears a positive charge when ionized (cationic surfactant), and DODP represents the so-called non-ionic surfactants. Initially, a wide range of concentration parameters for organic and aqueous phases were examined. After considerable tests mainly by trial and error, stable black lipid films could be formed from cholesterol in dodecane, which required the presence of a surface-active agent either in the organic or aqueous solution. It should be emphasized that, although only a few surfactants have been tried, numerous other surface-active agents including proteins and polymeric materials could be used. Without much doubt, some of these materials should also give similar results in producing stable black lipid films. Lipid solutions D and E were used mainly for investigating the water permeability of the BLM. The BLM formed from lipid solution E exhibit photoelectric phenomena and are therefore of special interest.

In spite of the fact that black lipid membranes could be generated from various lipid solutions, it should be stated that there is little direct evidence to indicate the precise composition of black lipid membrane. At present, the use of direct chemical analysis for black membrane composition is ruled out owing to very small areas and thickness of the membrane.

#### B. Water Permeability of BLM

Previously the permeability coefficients of BLM have been measured by several groups of investigators (1,2). The values reported vary from 4.9 to 104  $\mu$ /sec. It is not certain from these studies whether this large discrepancy is owing to the difference in the experimental techniques or the composition of the lipid solution used for BLM formation or both. Our measurements with the BLM generated from various lipid solutions should therefore be of some interest.

Regarding the mechanism of water permeation through the BLM, it is generally recognized that only a very limited amount of information can be derived from measurements of water permeability coefficient at a single temperature. Hence there is an obvious need to carry out studies at various temperatures. Further, one would like to raise the question of the nature of the interfacial region (i.e., the zone near the solution/membrane interface) upon the water permeability. Therefore, we have measured the permeability coefficients of water through the BLM as a function of (i) the temperature and (ii) the ionic composition of

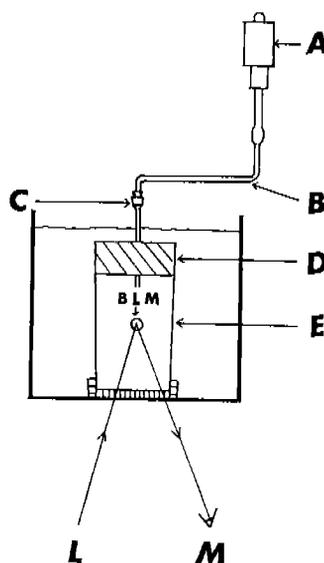
the bathing medium. The permeability-temperature data permit an evaluation of the various derived quantities from the absolute reaction rate theory, from which certain speculations concerning the mechanism of water permeation through the BLM are now possible. The permeability coefficient data determined as a function of solute used provide an opportunity to examine the effect of ions on water permeation and possibly on the structure of the BLM/solution interface.

1. Experimental

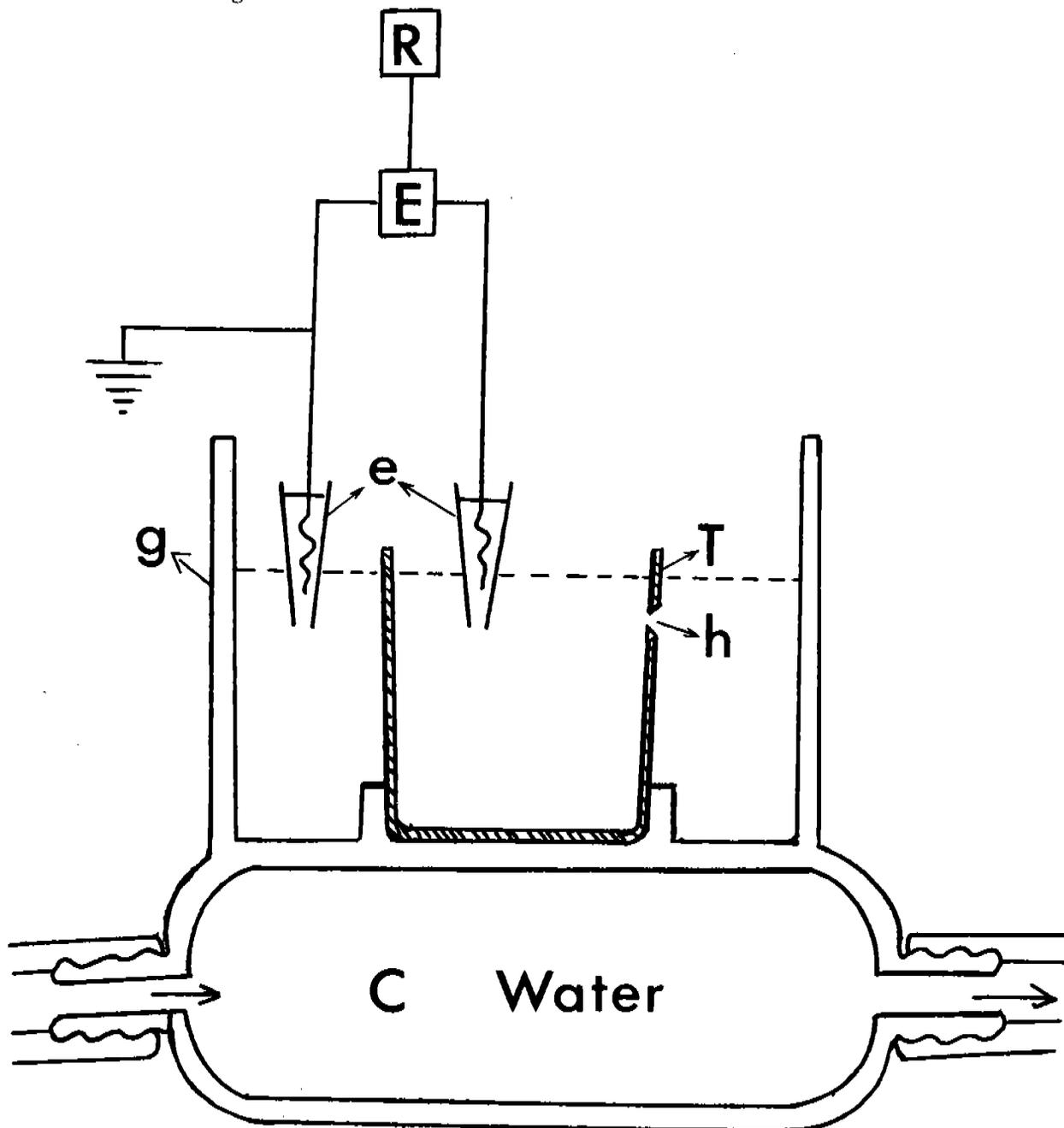
a. Materials. Cholesterol and hexadecyltrimethyl-ammonium bromide (HDTAB) were obtained from Eastman Kodak Co. (Rochester, N.Y.) and samples of dodecyl acid phosphate (DAP) were provided by Hooker Chemical Corp. (Niagara Falls, N. Y.) Reagent grade solvents and inorganic salts were used as received. Double-distilled water from an all-glass still was used throughout in this work. Spinach leaves used for chloroplast extract were obtained from local food markets.

b. Lipid solutions for BLM formation. (i) Oxidized cholesterol solution - a 4% solution of cholesterol in n-octane was oxidized with molecular oxygen at the reflux temperature for 6.5 hours. The supernatant was used for the membrane formation, (ii) DAP-Cholesterol solution - this solution was simply prepared by mixing 7 parts of a 5% solution of DAP in n-dodecane and 93 parts of a 1% solution of recrystallized cholesterol in n-dodecane, (iii) HDTAB-Cholesterol system - This membrane solution had two parts; the lipid phase was a 1% solution of cholesterol in n-dodecane, and the aqueous phase contained 0.008% of HDTAB, and (iv) chloroplast lipid solution- this solution was prepared by homogenizing 200 g. spinach leaves in 200 ml of 0.5M sucrose and 0.05M  $\text{KHCO}_3$  at pH 7.5. The mixture was then filtered through 4 layers of cheesecloth and centrifuged. The residue was extracted with 2:1 petroleum-ether and methanol. The extract after evaporation was taken up in a small volume of n-dodecane-n-butanol solution (3:1 V/V).

c. Apparatus. A simple arrangement was used in the present study (Fig. 1).



The cell consisted of a closed inner chamber (10 ml. Teflon beaker), the volume of which could be adjusted by the use of a calibrated digital pipette (Manostat Corp. New York). The closed inner chamber containing the membrane aperture ( $\sim 1\text{mm}$ ) was immersed in an outer glass chamber of a much larger volume. The outer chamber was stirred magnetically. In some of the later experiments, the inner chamber was replaced by a length of Teflon capillary tubing (Hamilton Co. Whittier, California), one end of which was connected directly to the pipette via luer locks. The temperature of the cell was controlled by flowing thermostated water through a coil placed in the outer chamber. The temperature was maintained within  $\pm 0.05^\circ$ . The electrical properties of the membrane were measured in an apparatus illustrated in Fig. 2.



d. Procedure. All membranes studied were generated by the brush technique (2) in a 0.1 N NaCl solution, except for BLM formed from the HDTAB-cholesterol system where the concentration of NaCl was  $10^{-3}$  N. After the membrane had become black, a known quantity of concentrated salt solution was added to the outer chamber and thoroughly mixed. The resultant efflux of water from the Teflon chamber (or capillary tubing) caused the membrane to bulge inward with time. By adjusting the digital counter dial (connected to a glass plunger), the volume change as a result of water flow could be determined. The criterion used in setting the dial was based upon the visual observation of a chosen pattern of the light reflected from the membrane. For Chl-BLM, the light used to observe the membrane was transmitted through a narrow-band interference filter (5461 Å). Similar arrangement was used for the light-initiated water flux studies. The illumination of the Chl-BLM was provided by a 650-W "Sun Gun" (Sylvania Electrical Products, Inc.). The light was focused by a pair of condensers and projected onto the membrane. In order to prevent the local heating, the light was filtered through a 3-cm saturated  $\text{CuSO}_4$  solution and a heating-absorbing glass before reaching the membrane.

## 2. Theoretical Considerations

In this section we shall summarize the basic equations and define the terms used in this study. We shall treat the permeation of water through a BLM as a typical rate process. The driving force for the steady-state process in our work is provided by an osmotic pressure gradient.

a. The rate of volume change in terms of the osmotic pressure gradient. As has been shown earlier by Lewis and Randall (3), the osmotic pressure of a concentrated solution is best expressed in terms of the mole fraction, i.e.,

$$\pi = - \frac{RT}{\bar{V}} \ln a \sim - \frac{RT}{\bar{V}} \ln N \quad (1)$$

where  $\bar{V}$  is the molar volume of water,  $a$  and  $N$  are the thermodynamic activity and mole fraction of water in the solution. In the generally accepted theory, diffusion may be viewed fundamentally as a reaction that equalizes the activity of solutes (including the solvent) which are free to move in solution. In the present case when two solutions are separated by a BLM, the solute or the water molecules enter the membrane at both interfaces. We shall designate the two co-existing interfaces as a "biface". To relate the rate of volume change and osmotic pressure gradient across a membrane permeable only to water, it can be shown that

$$J = \frac{dV}{A dt} = P' \frac{\Delta\pi}{RT} \quad (2)$$

where  $j$  is the net flow of water assuming that the BLM is ideally impermeable to the solute (to be justified later),  $A$  is the area of the BLM,  $\Delta\pi$  is the osmotic gradient across the BLM, which is given by

$$\Delta\pi = \nu RT(\phi_i C_i - \phi_o C_o) \quad (3)$$

in that  $\nu$  is the number of particles of the solute on dissociation,  $\phi$  is the standard osmotic coefficient,  $C$  is the effective concentration, and the subscripts "o" and "i" refer to the outside and inside of the membrane, respectively. The symbol  $P'$  in eq. (2) is the so-called permeability coefficient with units in  $\text{micron}^3/\text{micron}^2\text{min-atm}$ . In practice the permeability coefficient is usually expressed in  $\mu/\text{sec}$  as  $P_o$  ( $=0.925 \text{ RTP}'$ ) which are the units used in this study.

b. The Arrhenius activation energy,  $E_a$ . It is assumed in our case that permeation of water through an unmodified BLM takes place by a molecular process which can be described by an Arrhenius equation of the form:

$$\ln P_o = A - \frac{E_a}{RT} \quad (4)$$

where  $A$  is a constant,  $E_a$  is the activation energy involved in the rate-controlling step in the process,  $R$  and  $T$  are the gas constant and absolute temperature, respectively. It would be expected that, if the assumption were correct, a standard  $\ln P_o$  vs.  $1/T$  plot should give a straight line. The slope and intercept of the plot yield the activation energy,  $E_a$  and the constant  $A$  (frequency factor).

c. The derived quantities from the Eyring absolute reaction rate theory. The fundamental equation of the theory of absolute rate processes (4)

$$k_i = \kappa \frac{kT}{h} \exp\left(-\frac{\Delta F^*}{RT}\right) \quad (5)$$

will be applied to the permeation of water through BLM. In Eq. (5)  $k_i$  is a specific reaction rate constant,  $\kappa$  is the so-called transmission coefficient being assumed to be unity,  $k$  is the Boltzmann constant,  $h$  is Planck's constant, and  $\Delta F^*$  is the free energy of activation. By means of familiar relationships, it can be shown that Eq. (5) may be written as

$$D_i = \lambda^2 \left(\frac{kT}{h}\right) \exp\left(-\frac{\Delta F^*}{RT}\right) \quad (6)$$

where  $k_i = D_i/\lambda^2$ .  $D_i$  is the diffusion coefficient and  $\lambda$  is the distance between two successive equilibrium positions of a permeating water molecule. It is further assumed that permeation of water through the membrane involves no volume change. Therefore, the diffusion coefficient may be expressed by

$$D_i = e\lambda^2 \left(\frac{kT}{h}\right) \exp\left(\frac{\Delta S^*}{R}\right) \exp\left(-\frac{E_a}{RT}\right)$$

where  $e$  is the base of the natural logarithms. Eq. (7) is obtained by means of common relationships

$$\Delta F^* = \Delta H^* - T\Delta S^* \quad (8)$$

and

$$E_a = \Delta H^* + RT \quad (9)$$

when  $\Delta H^*$  and  $\Delta S^*$  are the respective enthalpy and entropy of activation.

### 3. Results and Discussion

The osmotic permeability coefficient,  $P_o$ , of bilayer lipid membrane (BLM) generated from oxidized cholesterol in n-octane as a function of temperature was measured. In the system under study, the BLM is pictured as analogous to an ultrathin layer of liquid hydrocarbon interposed between two aqueous phases. The evidence that the interior of the BLM at least, is "hydrocarbon-like" has been provided by several workers (for references to the BLM literature, see Refs. 1,2). For example, the DC resistance of unmodified BLM in 0.1N NaCl is usually in the order of  $10^6 - 10^8 \Omega - \text{cm}^2$ . The dielectric breakdown strength of the BLM is about  $5 - 10 \times 10^5 \text{ V-cm}^{-1}$ . These properties are very similar to those of liquid hydrocarbon in bulk. The very high D.C. resistance implies that the BLM is practically impermeable to charge carriers such as monovalent ions generally present in the aqueous solution used in the membrane formation. This impermeability to charge carriers may be shown by a simple calculation. For a  $1 \text{ cm}^2$  BLM with a D.C. resistance  $10^7 \Omega$  and an applied potential of 0.1 volt, only about  $4 \times 10^{-10}$  equivalent of charge move through the BLM per hour. It seems therefore that for an unmodified BLM, it behaves essentially like a semi-permeable membrane. In the discussion that follows this liquid-hydrocarbon-like picture of BLM will be our frame of reference.

a. The Osmotic permeability-coefficient,  $P_o$ . We see that the units of  $P_o$  ( $\mu/\text{sec}$ ) have the dimension of a velocity. We may consider that, therefore, the reciprocal of  $P_o$  is a measure of the resistance offered by the BLM to water permeability. The total resistance encountered by a water molecule in transferring from one side of the membrane to the other side may be viewed as consisting of five separate resistances in series. These are two resistances at the two membrane/solution interfaces, one due to the membrane, and two bulk-phase resistances. Since the self-diffusion coefficient of water is several orders of magnitude larger than the other coefficients, we need only consider the resistances due to the membrane and the membrane/solution interfaces. Therefore, this total resistance may be expressed as

$$\frac{1}{P_o} = R_{sm} + R_m + R_{ms} \quad (10)$$

where the subscripts s and m refer to the solution and membrane, respectively. According to an analysis given by Zwolinski et al. in terms of the Eyring absolute reaction rate theory (4) there is a specific rate constant,  $k_1$ , associated with each step (see Fig. 3).

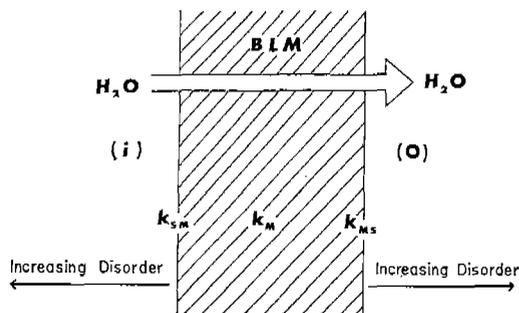


Fig. 3. Mechanism of water permeation.

Here  $k_m$  refers to the rate constant in the BLM, while  $R_{sm}$  and  $R_{ms}$  are the rate constant for diffusion in the solution/BLM and BLM/solution interfacial region, respectively.

Although the magnitude of  $P_o$  provides us with some clues concerning the permeation barrier, it is not possible however to assess the relative contribution due to each resistance. Hence, nothing more can be said about the rate-controlling step and the mechanism of water permeation through the BLM. It is of interest to note, however, that the permeability coefficient of water in liquid hydrocarbon as estimated from the solubility and diffusion data of Schatzberg (5) is much higher than the value obtained in this work. For instance, at  $35^\circ$  a value of  $78 \mu/\text{sec}$  is estimated for water permeability through a  $40\text{\AA}$  thick layer of n-hexadecane using the data of Schatzberg as compared with  $15.7 \mu/\text{sec}$  for a BLM formed from oxidized cholesterol in n-octane at the same temperature. Obviously, the two systems are not comparable in many respects. One outstanding difference is that the BLM is believed to have a highly organized structure at the biface as a result of molecular orientation and the presence of high interfacial pressure ( $\sim 50 \text{ dynes-cm}^{-1}$ ), whereas such orderliness is lacking in the case of liquid hydrocarbon in bulk. The lower  $P_o$  value for BLM is seen to be consistent with the idea that the structure of BLM is more condensed and orderly. The ordering effect of hydrocarbon and polar groups at the bifacial region will be elaborated later.

c. Activation energy of water permeation and derived thermodynamic quantities.

1. The activation energy,  $E_a$ . The experimental energy of activation as calculated from the slope of  $P_0$  vs.  $1/T$  plot. Since the plot is practically linear the value of  $E_a$  is seen to be independent of temperature for the temperature range examined (22.5-44°) unless otherwise noted. In Table 2 a list of  $E_a$  for three other systems is compiled. It is seen that  $E_a$  for BLM is only about 1/3 of that for natural membranes but somewhat higher than the values for self-diffusion in water (6) and in n-hexadecane (5). The intermediate value for BLM is interpreted to mean that a water molecule must pass over a higher energy barrier in moving from one equilibrium position to the next than that in moving through the water or liquid n-hexadecane systems.

2. The derived quantities,  $\Delta S^*$ ,  $\Delta H^*$ , and  $\Delta F^*$ . The application of the theory of absolute reaction rates to diffusion processes through membranes has been made by several investigators. In particular, Zwolinski et al. have extended the theory to the diffusion and permeability of membranes (4). We shall follow their treatment in analyzing our water permeability data through BLM.

As indicated in Fig. 3, there are three rate constants which are involved in the permeation of water across the BLM. From the nature of the interface involved, we may assume that  $k_{ms}$  is much larger than the  $k_{sm}$  and  $k_m$  associated with the solution/membrane interfacial region and the BLM itself, respectively. Zwolinski et al. have derived an equation relating  $P_0$  and other parameters which is given by

$$\frac{1}{P_0} = \frac{2\lambda}{D_{sm}} + \frac{t}{D_m K} \quad (11)$$

where  $t$  is the thickness of the membrane,  $K = k_{sm}/k_{ms}$ ,  $D_{sm}$  and  $D_m$  are the respective diffusion coefficient for the solution/membrane interface and the membrane. Further,  $D_i = k_i \lambda^2$  where  $i$  refers to either  $sm$  or  $m$ . Referring to Fig. 3, three cases may be considered, namely, (i) the membrane limiting, (ii) the interface limiting, (iii) the membrane and interface limiting. We shall consider each case separately in the following paragraphs.

a. Membrane limiting case - The rate-controlling step is diffusion in the BLM. Therefore,  $K_{ms}$  is much greater than  $k_m$ . In this case the first term on the right-hand side of eq. (11) is negligible, we have

$$P_0 = \frac{\lambda^2 k'}{t} \quad (12)$$

where  $k' = k_{sm} K$ ,

By combining eqs. (6) and (12), we may write

$$P_o = \frac{kT}{h} \left(\frac{\lambda}{t}\right)^2 \exp\left(\frac{\Delta F^*}{RT}\right) \quad (13)$$

where  $\Delta F^*$  is the free energy of activation for permeability, which is interpreted to mean the difference in free energy of the permeating water molecule between its initial position in the aqueous solution bathing the membrane and the top of the highest energy barrier the water molecule must overcome within the BLM. The calculated  $\Delta S^*$ ,  $\Delta H^*$ , and  $\Delta F^*$  according to eq. (8) and (13) are given in Table 2.

Table 2. Experimental Activation Energies and Derived Quantities from Transition State Theory of Rate Processes for Various Systems.

System	$\Delta E_a$ (Kcal/mole)	$\Delta S^*$ (Cal/mole/deg.)	$\Delta H^*$ (Kcal/mole)	$\Delta F^*$ (Kcal/mole)
Water (28)	4.6	2.5	4.0	3.9
n-Hexadecane (27)	3.4	-0.2	2.8	3.4
Cellular membranes (26)	15-22	16-39	14-21	9.7-10.1
Case I (t = 40A)	6.8	-13.3	6.2	10.4
BLM				
Case II ( $\lambda = 1A$ )	6.8	-13.7	6.2	9.6
Case III ( $\lambda = 1A$ )	6.8	29.1	6.2	-2.4
Lecithin BLM <sup>a</sup>	12.0	7.0	11.4	9.3

<sup>a</sup>Temperature range - 22-38°C.

b. Interface limiting case. In this case  $k_m \gg k_{ms}$ , eq. (11) becomes

$$P_o = \frac{\lambda k_{sm}}{2} \quad (14)$$

By plotting in  $(P_o h/kt)$  vs.  $1/T$ , the various derived quantities were evaluated, which are also summarized in Table 2.

c. Membrane and interface limiting. In this case since  $k_{ms} \approx k_m$ , the permeability coefficient is given by

$$P = \frac{k_{sm} \lambda}{t} \quad (15)$$

The derived quantities are given in Table 2.

On the basis of  $\Delta F^*$  calculation, we can rule out Case (c) since a negative  $\Delta F^*$  would mean that the activated state actually possesses less energy. This is a highly unlikely event (in terms of resistance,  $R \leq 0$ , therefore  $P \rightarrow \infty$ ).

As mentioned above, the magnitude of the permeability coefficients will not provide information concerning the rate-determining step involved in the process. The temperature dependence of the permeability coefficient by itself also does not seem to be able to decide the controlling step regarding the mechanism of permeation, as can be seen from the calculated values in Table 2, which are remarkably similar. As was pointed out by Zwolinski et al. if  $k_m$  is only slightly larger than  $k_{ms}$ , the  $\ln(P_h/kt)$  vs.  $1/T$  plot would exhibit a definite curvature toward the abscissa axis. This case can be ruled out also since the plot was essentially linear. Thus we have only two cases to decide, i.e. whether the rate-controlling step is due to the membrane or the solution/membrane interfacial region. In the case where the interfacial region is the slowest step, any modification of the interfacial region should have a significant effect on the magnitude of  $P_0$ . In later sections we shall provide additional experimental evidence to examine the effect of several molecular properties at the biface on BLM to water permeability, which may provide us with a basis to understand the mechanism of water permeation through the BLM.

3. The entropy of activation. It can be seen from Eq. (7) that the entropy of activation  $\Delta S^*$ , may be determined if one assumes a reasonable value of  $\lambda$ . In our calculations, the molecular jump,  $\lambda$ , was assumed to be either 3A (about 1-2 molecular diameters of water). In the third column of Table 2,  $\Delta S^*$  for various systems is listed. The most striking feature of the tabulated values is that for Cases I and II, rather large negative entropies of activation were obtained. The values given are very approximate since both  $\lambda$  and BLM thickness,  $t$ , have to be assumed. In Case II the permeation of water is governed by diffusion through the solution/membrane interfacial region, the  $P_0$  is therefore independent of the membrane thickness. According to the theory of absolute rates (4), the negative  $\Delta S^*$  implies one of two events: (a) the bonding of activated complex with the membranes, i.e., the permeating water molecules are being partially immobilized in the membrane structure or in the interfacial region (iceberg formation); or (b) permeation through the membrane was not the rate-controlling step in the process. Experimental evidence discussed below seems to support the latter case.

4. The effect of molecular properties at the biface. Up to now we have been mainly concerned with the BLM itself. As mentioned in the preceding paragraphs, the permeability-temperature data alone is incapable of deciding the mechanism of water permeation (with perhaps one exception noted above). Since the interior of the BLM could not be easily modified (apart perhaps by introducing unsaturation in the hydrocarbon chains), the effect most amenable to change will be at the solution/membrane interface. Since we were able to form BLM from several surface-active agents in combination with cholesterol,  $P_0$ 's were therefore measured with BLM-forming compounds bearing either positive or negative charges when ionized in solution. The systems studied together with  $P_0$  data are given in Table 3.

Table 3. Bimolecular Lipid Membranes (BLM) Generated from Three Different Lipid Solutions and Their Permeability to Water at 22.5°.

BLM formed from	Aqueous Phase	$P_o$ ( $\mu\text{-sec}^{-1}$ )
Oxidized Cholesterol in n-octane	0.1 N NaCl	$8.4 \pm 0.5$
Oxidized Cholesterol + $2.5 \times 10^{-5}$ M Valinomycin	0.1N KCl	1.0
Oxidized Cholesterol + $2.5 \times 10^{-5}$ M Valinomycin	0.1N KCl	9.5

Although the chemical composition of the BLM remains unknown, these membranes were believed to be composed of predominantly cholesterol molecules. The much more surface-active agents (HDTAB, DAP, or "impurities" associated with oxidized cholesterol) constituted a very small fraction of the BLM structure. The main effects of these agents lie, probably in their ability to stabilize the BLM structure (2).

2. The effect of solute at the biface. A dramatic effect on  $P_o$  was observed when different solute was used to generate the osmotic gradient. The data are presented in Table 4. Here a three-fold increase in  $P_o$  was obtained when the aqueous solution was changed from NaCl to either NaI or CsCl.

Table 4. The Effect of Solute Used on the  $P_o$  for BLM Generated from Oxidized Cholesterol in n-Octane at 22.5°.

Aqueous Solution	$P_o$ micron/sec.)	D.C. Resistance (ohm-cm <sup>2</sup> ) <sup>b</sup>
NaCl	8.4	$5 \times 10^8$
NaI	24.7	$2 \times 10^6$
CsCl	25.9	$8 \times 10^6$
NaCl + dextran (16,800)	24.8	---
NaI and (sucrose)	17.0	---
Sucrose	53	---

#### D. The mechanism of water permeation.

To attempt an interpretation of the data obtained in this study, it is in order to consider the most likely pathways for water movement through the solution/BLM/solution system, and the nature of liquid/liquid interfaces relevant to our discussion.

1. The Nature of Water Flow through BLM with and without "pores". Basing upon tritiated water and osmotic flux measurements, it has been suggested that "pores" may exist in the BLM (7). If pores or channels (presumably for water flow only) do exist in the BLM, the rate of self-diffusion for water should be proportional to the total area of the pores.

It is reasoned therefore that  $P_d$  (self permeability coefficient) and  $P_o$  would be different with the latter being considerably larger as a result of a hydrostatic pressure or osmotic gradient. Later experiments have shown (8), however, that  $P_d \approx P_o$  suggesting that apparent difference between  $P_d$  and  $P_o$  reported by earlier workers was due to the existence of stagnant or inadequately stirred layers at the biface (9). In view of the fact that the interior of BLM is liquid hydrocarbon-like as well as from the evidence resulting from recent studies, the possibility of the existence of "pores" in the BLM is untenable. The fact that  $P_o \approx P_d$  implies that the same pathway must be involved in transporting the water molecules across the BLM system. Previously, a simple solubility-diffusion mechanism had been proposed (10). This is based upon the results that the magnitude of  $P_o$  found experimentally is roughly the same order as that calculated from the solubility and diffusion coefficient data (5).

## 2. The energy barrier mechanism and the nature of water/hydrocarbon interface.

Alternatively, we suggest an energy barrier mechanism for water permeation across the BLM system. The energy barrier idea was first used by Langmuir and Schaefer (11) and later by others (12) in reference to monolayer penetration by water and simple gas molecules at the air/water interface. Since the existence of "pores" in the BLM appears to be ruled out, it seems that the only other way for the water molecules to get across the BLM system (solution/BLM/solution biface) is by "barging" their way through. This means the creation of "holes" along the pathway as the molecules permeate across the system. In terms of reaction rate theory, only those water molecules striking the interfacial region with sufficient energy will get across the BLM at the biface, implying that the permeating water molecules are at an activated state.

Information concerning the nature of liquid/liquid interfaces relevant to our discussion is very limited. Franks and Ives (13) suggest that the water/hydrocarbon interface is covered by a layer of icebergs which are known to surround hydrocarbon molecules in aqueous solution as suggested earlier by Frank and Evans (14). Further work on hydrocarbon/water interfaces has been reported by several other investigators (15). It is generally accepted that the interaction between saturated hydrocarbons and water involves dispersion forces (16). Although no information is available about the BLM systems, from the afore-mentioned studies, it seems probable that the presence of polar (or ionic) groups at the biface would enhance the orderliness of the interfacial region, that is, that the bifacial region tends to be more organized than bulk phases. The exact thickness of this interfacial region is not known and could be anywhere from one molecular layer to several hundred. The important point for our argument is, however, that this interfacial region is more orderly than the bulk solution. Perhaps a reasonable view to adopt is that there is a gradual change of structure from the

BLM/solution interface in which the orientation of the water molecules becomes increasingly disordered as the distance from the interface is increased (see Fig. 3).

Applying these considerations to the water permeation through the BLM system, our attention will be focused upon the bifacial region rather than the BLM itself. If the rate limiting step were due to the membrane (i.e., liquid hydrocarbon with or without pores) one would expect the experimental activation energy,  $E_a$ , to be similar or less to that for diffusion. This is not supported by the data collected in Table 2. In fact,  $E_a$  for permeation of water in the liquid hydrocarbon ( $C_{16}$ ) is somewhat lower than for free diffusion in water, whereas  $P_o$  value for the BLM system is twice the value for n-hexadecane. The higher value for the BLM system, according to the reaction rate theory, means that the permeating water molecules must overcome a higher energy barrier. This additional energy height could come from the orderliness of the solution/membrane interfacial region.

If the interfacial region were the rate-controlling step, then any disturbance of the region should have noticeable effect on  $E_a$  which in turn should be reflected in  $P_o$ . The data presented in Table 4 are therefore of interest. The permeability coefficients for water are seen to be three times larger when either NaI or CsCl was used as the solute. This marked change of  $P_o$  may be explained in terms of the Gurney's order-disorder concept (17). According to this view, liquid water possesses distinctive structural features near its freezing point. Depending upon the size of ionic solute introduced, those that can fit into the structural "vacancies" will not disrupt the water structure.  $Cs^+$  and  $I^-$  are classified as structure-breakers. Assuming that the results are applicable to the present situation, the introduction of these large ionic solutes ( $Cs^+$  and  $I^-$ ) tends to break up the ordered interfacial region thereby lowering the interfacial resistance for water permeation. It may be argued that  $Cs^+$  or  $I^-$  could also modify the BLM structure, hence a lower energy barrier for water permeation. The evidence we have against this suggestion is that the D.C. electrical resistance of the BLM was lowered somewhat when these ions were used as the bathing medium. On the other hand we have found that  $I_2$  greatly reduced the D.C. resistance of the membrane while  $P_o$  remained practically unchanged. In this connection it is interesting to note that a proteinaceous material of uncertain composition known as "EIM" lowers the D.C. resistance of the BLM by several orders of magnitude when added to the aqueous phase (1,2). On the other hand it has been found that the permeability coefficient for water was little affected (8). It has been suggested that, upon introduction of EIM, the structure of the membrane is altered insofar as ionic transport is concerned. It would seem, therefore, from these results that ionic movement across the BLM is governed by the nature of the membrane whereas the water permeation is primarily determined by the orderliness of the interfacial region. If this explanation is correct,

we would predict that any species whose presence in water causes the breaking up of the water structure at the biface will promote water permeation while the electrolyte transport may or may not be affected.

#### E. Light-Induced Water Flow Across Chloroplast BLM

Of greater interest from the viewpoint of desalination are our recent findings of light-induced water transport across ultrathin lipid membranes constituted from chloroplast extracts (abbreviated as Chl-BLM). Briefly, when a Chl-BLM is illuminated by light (400-800nm) water is observed to flow across the membrane to the compartment where electron acceptors are located. This new phenomenon and other properties of chloroplast BLM are detailed below.

1. Experimental Procedure. Experimental methods for the present work were essentially those used in previous studies (1,2). They consisted in photo-emf, photoelectric spectrum, and water permeability measurements of BLM separating two aqueous phases. The BLM-forming solutions were prepared from either chloroplast lamellae extract dissolved in a mixture of n-decane and n-butanol (3:1, v/v). The asymmetrical conditions across the BLM were created by the addition of a modifier to one side or different modifiers to the opposite sides of the bathing solutions. Because these types of BLM's studied were sensitive to hydrogen ions (i.e. a membrane potential of 50-58 mV per ten-fold difference of hydrogen ion concentration could be produced in the pH range 4-6), they were all formed in 0.1 M acetate buffer at pH 5.0 unless otherwise noted. In the buffer medium, the introduction of modifiers to one or both sides of the bathing solutions usually generated a dark membrane voltage. The magnitude of the dark voltage varied from a few to tens of millivolts depending upon the modifier used. For example, the presence of  $Fe^{+3}$  and hydroquinone (HQ) in the opposite sides of the bathing solutions gave rise to a dark membrane voltage of about 5mV. The side containing  $Fe^{+3}$  was negative. For BLM excitation, two different light sources were used: a 150-watt projection lamp (Model DFG, General Electric) for open-circuit photo-emf studies and a 1000-watt xenon arc lamp (Hanovia) for obtaining photoelectric spectra.

2. The effect of modifiers on chloroplast BLM. In the absence of modifiers, the photo-emf's of the BLM were small but easily measurable. The photo-emf of the BLM could be dramatically increased by 2-3 orders of magnitude when the modifiers were present in the bathing solutions. Furthermore, the time constant of photoresponse was greatly reduced and the system appeared to be reversible to exciting light. The photoeffects in the presence of  $Fe^{+3}$  (an electron acceptor) were independent of the direction of light but the polarity of the photo-emf was determined by the location of  $Fe^{+3}$ , being always negative with respect to the ironfree side. The open-circuit photo-emf ( $E_{Op}$ ) as a function of light intensity (I) has been found to follow the simple equation

$$E_{op} = A \log \left( 1 + \frac{I}{B} \right) \quad (16)$$

where A and B are constant for a given BLM at a particular temperature. Under conditions of low light intensity ( $B \gg I$ ), as would be expected  $E_{op}$  was shown to be directly proportional to I. It should be mentioned that eq. (16) is also obeyed by most semiconductor photo-voltaic cells. Depending upon the modifier present the time constants for the rise and decay of the photo-emf have been observed ranging from less than 50 msec to several tens of seconds. Also, the shape of the curves could be quite different. In general, however, the presence of a reducing agent or the absence of a good electron acceptor greatly increased the time constants. It is interesting to note that the resistance of the BLM, which was about  $10^6$ - $10^7$  ohms-cm<sup>2</sup>, was not significantly altered by the presence of modifiers or illumination. For obtaining photoelectric spectrum, the BLM was illuminated with monochromatic light over the spectral region 300-800 nm, and photo-emf's were monitored. As was noted earlier, the photoelectric spectra are similar to the absorption spectra of their respective lipid solutions used. It is worth mentioning that the chloroplast BLM exhibited red fluorescence when excited by light of shorter wavelengths. The results show that the photo-emf's reported here are primarily due to light absorbed by the pigments in the BLM and not due to the modifiers present in the bulk solutions. It may be mentioned, however, that certain BLM's (e.g. formed from oxidized cholesterol or phospholipids) which are otherwise not photo-active can be sensitized by certain inorganic ions and organic dyes dissolved in the aqueous solution. We have observed photoeffects similar to those reported here in oxidized cholesterol BLM sensitized by a variety of dyes such as methylene blue, methyl red, thionine, rhodamine B, and methyl viologen.

3. Light initiated water movement. In the case of light-initiated water flow, a typical curve of volume change vs. time can be described as follows: the initial slope was 0.04  $\mu$ l/min. due to the osmotic gradient. Upon illumination, the slope was changed to 0.07  $\mu$ l/min. When the light was turned off, the initial rate of flow was again observed (see Fig. 4). If an electrical potential difference was applied across such a BLM, electro-osmosis was observed. It has been found that the volume flow of water as a function of applied voltage increases with increasing applied voltage. The direction of the water flow depended upon the polarity of the field. The net flux of water was in the compartment where the potential was negative.

The best value obtained to date is 70  $\mu$ l per hour per square centimeter of membrane area. The introduction of electron acceptors to the membrane system, it should be mentioned, produces an open-circuit photo-emf of greater than 100 mV. In essence, the membrane system thus constituted behaves like a photovoltaic device, the novelty being that it comprises of biological materials immersed in an aqueous environment.

This interesting phenomenon of light-induced water flow can be decreased or abolished completely if an inhibitor of uncoupler of photophosphorylation is present. It is found, however, that high concentrations of uncouplers such as 2,4 dinitrophenol (DNP) and p-trifluoromethoxy-carbonylcyanidephenylhydrazone (FCCP) tend to rupture the BLM. When  $10^{-5}$ M of DNP or  $10^{-7}$ M of FCCP is added to the aqueous phase, both the photovoltaic effect and light induced water flow are abolished. Another compound, DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) at  $10^{-7}$ M is only partially effective.

The findings described above can be explained in terms of semiconductor physics and classic electrokinetics. When a beam of light excites the BLM, electrons and holes are produced. If it is assumed that the electrons and holes thus produced have different lifetimes and mobilities, a separation of charges in the BLM would result leading eventually to a potential difference across the membrane. Since the chloroplast BLM is shown to be negatively charged, it is therefore poorly permeable to anions. The passage of current through it will produce local concentration changes at the biface. The concentration gradient thus produced could result in an osmotic water flow, in addition to the movement of hydration water associated with current-carrying cations (possibly also hydrated electrons within the membrane). The light-induced voltage across the BLM is believed to be the primary driving force responsible for the water flow. Externally applied voltage should therefore accomplish the same purpose as has been experimentally demonstrated.

The development of membrane potential across Chl-BLM may take place in two steps: (i) electronic charge carriers are first generated by light, and the field across the BLM causes the charges of different sign moving toward the opposite side of the biface, thereby one side of the biface acquires an oxidizing and the other side a reducing character. Instead of producing an unknown entity [H] the potassium ion may be reduced. If so, the pH on the side where  $K^+$  is neutralized would be higher. Since Chl-BLM is highly sensitive to  $H^+$ , the resulting pH difference could then give rise to the observed membrane potential. Fig. 5 gives a pictorial description of the suggested redox reactions across the BLM.

#### F. Miscellaneous Results.

(1) Active water transport across oxidized cholesterol-DAP black lipid membranes: In biological systems, active transport is defined where the transfer of material requires work on the part of the cells. The membranes are believed to be intimately involved in active transport. In an attempt to demonstrate that active transport can also take place in artificially constituted black lipid membranes, we have incorporated ATPase (generously supplied by Dr. R. W. Albers of NIH) into oxidized cholesterol-DAP BLM. Using the short circuit method of Ussing and Zerahn (18), the influx of water is measured. Other experimental details are

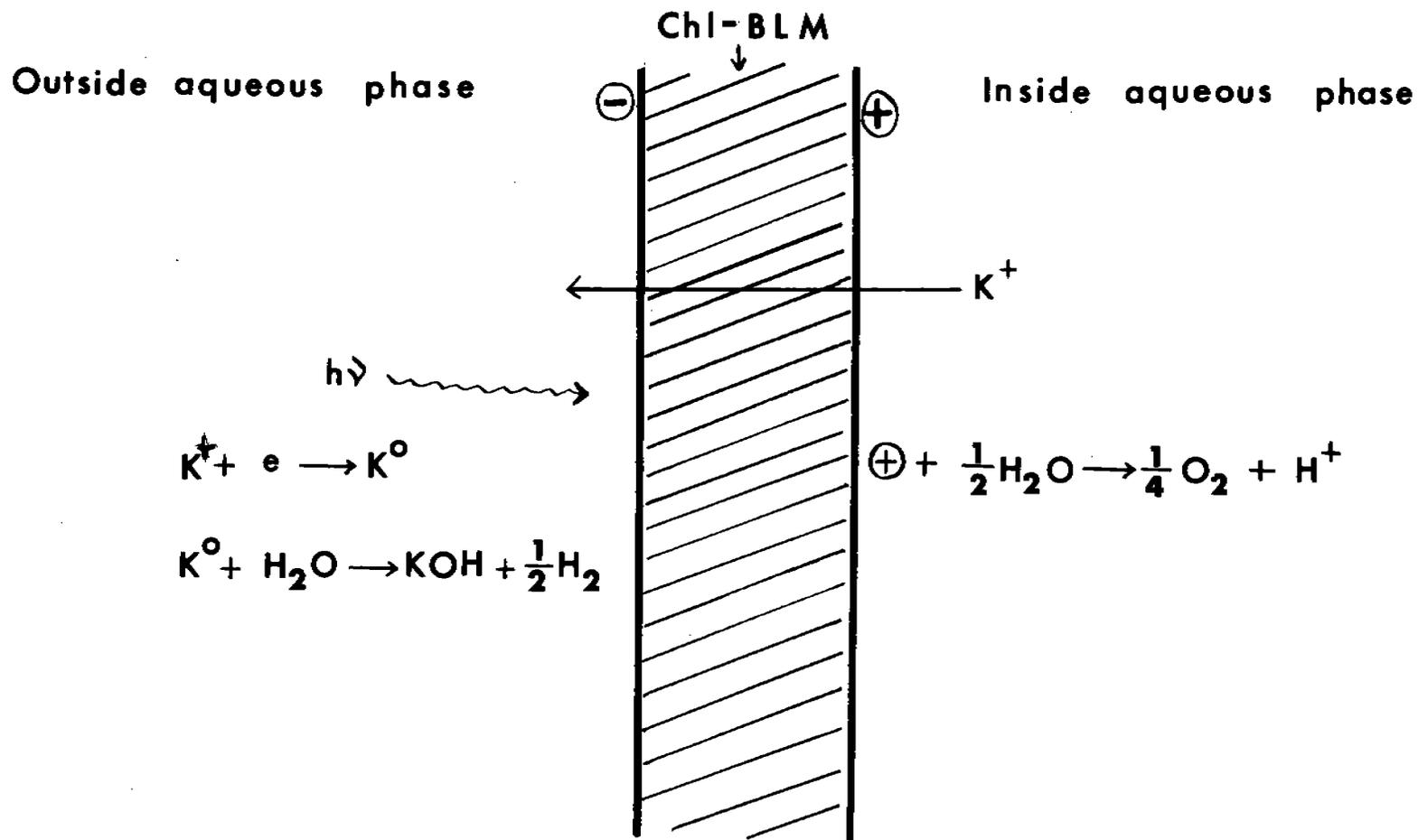


Figure 5. A possible mechanism for the photolysis of water in a Chl-BLM. It should be noted that, instead of  $K^+$ , other electron acceptors such as ferric ion as mentioned in the text can serve the same purpose.

similar to those given by Jain, Strickholm, and Cordes (19). Evidence for active transport of water transport across ATPase modified BLM has also been obtained in our experiments.

(2) Light-induced water transport in a larger cell: A new chamber capable of accommodating a membrane several square cm have been designed. It was used with a sensitive pressure transducer (Pace-model P-90D) as a detector. The use of this set-up for the photolysis of water is being actively pursued.

(3) Energy transduction and energy storage via a photoactive ultrathin lipid membrane: The observation of light-induced water flow across Chl-BLM and a large photo-emf ( $>135$  mv) indicated the need for a basic approach in order to explain the underlying mechanism. A new set-up consisting of a flash tube, amplifier and assorted electronic components has been used to detect very fast light response in the membrane. Some preliminary experiments using single flashes (about 1  $\mu$ sec) show that there are at least two components; a very fast one with no detectable latency and a relatively slow one in the msec range. The mode of generation of photopotentials is under investigation.

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#### IV. PUBLICATIONS AND OTHER ACCOMPLISHMENTS

1. H. T. Tien, S. P. Verma, H. P. Ting, and N. Kobamoto, "Properties of black lipid membranes (BLM) of chloroplast extracts", Proc, 3rd International Biophysics Congress, Boston, 1969. p. 56.
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Other activities include demonstrating and instructing the BLM techniques to visiting scientists and students--Apart from interested persons from Michigan State University, a number of scientists from outside have visited the Principal Investigator's laboratory from time to time to familiarize themselves with the BLM techniques which might have been helpful in their own research.

#### V. RECOMMENDATIONS

The observed light-induced effect in the photoactive black lipid membranes is most exciting since it appears to meet all the requirements of an energy-transducing membrane. Many attempts have been made in the past in devising means of using solar energy directly as light. The membrane system we have developed is clearly capable of achieving the following:

- (i) Conversion of light into electrical energy
- (ii) Mediation of redox reactions across the membrane, and
- (iii) Initiation of water flow as a result of item (ii).

It is therefore recommended that a comprehensive program of approximately three years duration is in order. The proposed project will attempt to provide new insights into fundamental mechanisms of salt and water transport across such membranes. A research proposal detailing our program was submitted to OSW on February 8, 1971. Below is a recapitulation of the proposal.

Recently, ultrathin lipid membranes of less than 100 Å in thickness have been formed from chloroplast extracts and commercially available pigments in this laboratory. Experiments indicate that these membranes possess very high salt-rejection capacity and

excellent permeability to water. In addition, when the membrane is illuminated by light, a net transfer of water is observed. The proposed research is concerned with the fundamental physical chemistry of light-initiated water transport through these membranes. The aims include extensive experimental and theoretical investigations on the mechanisms of water permeation of these structures in relation to membrane biophysics and desalination by electro-osmosis.

The financial support is sought to aid in the continuation and expansion of experimental and theoretical work on light-induced water transport across ultrathin lipid membranes. These membranes, a new type of interfacial barrier, serve as unique model systems for study which would aid in the understanding and exploitation of the photolysis of water.

To translate these new findings from pure laboratory curiosities to any potential membrane process for water purification, it would be necessary to have answers to the following questions:

- (1) When actinic light is applied to the membrane, what charge carriers will flow and in which directions?
- (2) How will the composition of the membrane and/or added modifiers alter the magnitude of photo-emf?
- (3) What will be the effect of the external load on the membrane?
- (4) What are the basic parameters such as membrane area, temperature, current output, and power output of the system?
- (5) What is the cost of the use of the membrane system for desalination?

Our basic aims are to answer some and all of these questions which we feel will provide rational basis for further development in the application of our findings. We plan to investigate, in particular, the phenomenon of light-initiated water transport across these membranes, which hitherto has not been known.

The theoretical tools for investigation will be solid-state physics, classical and non-equilibrium thermodynamics which hitherto have not been applied to these systems. Experimentally, the recommended research would be centered on the following:

- i) measurement of water flux through these black lipid membranes using light,
- ii) measurement of water flux through these black membranes under an osmotic, hydrostatic pressure, and/or potential gradients,
- iii) preparation and testing of light-sensitive membranes of larger area for water purification, and
- iv) investigation of the various photochemical effects of these ultrathin membrane systems in relation to energy storage and desalination.

The long range aims of the recommended program include both experimental and theoretical studies of light-initiated water transport with special attention to the structural aspects of the membranes and the energetics of water permeation through a liquid hydrocarbon barrier which is about two molecules in thickness.