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# The Effect of 2,4-Dinitrophenol on the Properties of Thin Phospholipid Films

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*Summary.* The properties of a system consisting of a thin phospholipid film separating two electrolyte solutions containing 1 mM 2,4-dinitrophenol have been studied. Both the variation of electrical conductance as a function of pH, keeping the pH the same on both sides of the membrane, and the nonlinear variation of electrical potential difference as a function of pH difference across the membrane have been explained in terms of lipid-soluble complexes of the type $XP_2^-$, where $X$ is a cation and $P$ dinitrophenate. The maximum conductance was found to be $1.4 \times 10^{-5}$ mhos $cm^{-2}$ at pH 4.2.

The chemiosmotic hypothesis was put forward by Mitchell (1961, 1966) to explain the coupling between electron transport and ATP formation in mitochondria. The protons which remain behind when electrons are transported through the cytochrome system from substrate hydrogen to oxygen reverse ATP hydrolysis. Mitchell suggested that agents such as 2,4-dinitrophenol (DNP) which uncouple phosphorylation from electron transport do so by forming lipid-soluble proton donor-acceptor systems in the membrane which conduct protons across it, thereby short-circuiting the proton potential. Recently, attempts have been made to test this hypothesis by studying the effect of DNP on thin lipid films.

Thus Bielawski, Thompson and Lehninger (1966) have shown that the conductance of thin lipid films separating salt solutions is greatly increased by DNP. Hopfer, Lehninger and Thompson (1968) and Liberman and Topaly (1968) have suggested that under certain conditions the increase of electrical conductance is almost entirely due to proton transport. Many substances are known that increase the electrical conductance of lipid films, and the evidence indicates that some of these substances act as carriers which form charged complexes in the film (Liberman & Topaly, 1968; Eisenman, Ciani & Szabo, 1968; Finkelstein & Cass, 1968; Kilbourne, Dunitz, Pioda & Simon, 1967; Tosteson, Andreoli, Tieffenberg & Cook, 1968).

This paper is concerned with a study of the effect of DNP on the system consisting of a thin lipid film together with its circular border, separating two electrolyte solutions. Both the variation of film conductance as a function of pH, keeping the pH the same on both sides of the membrane, and the variation of potential difference as a function of the pH difference across the film have been interpreted in terms of lipid-soluble DNP complexes. A preliminary account of this work has been given by Croghan, Lea and Lelièvre (1969).

## Methods

### *General*

Thin lipid films were formed, by the brush technique of Mueller, Rudin, Ti-Tien and Wescott (1963), on the ends of polythene tubes (*see* Fig. 1) from solutions of purified egg lecithin and cholesterol in n-decane (0.25% w/v lecithin, 0.125% w/v cholesterol). The films were formed in a solution containing 200 mM KCl and 1 mM DNP. The experiments were arranged so that up to five tubes could be used simultaneously. The growth of the area of black films during drainage was followed by measurement of their capacitances.

In experiments involving changes in the pH of the external solution ($pH_2$), the pH in the external solution was measured directly using electrodes E.I.L. types GM23 and RSM23. In order to adjust $pH_2$ to any given value within the range pH 3.6 to 8, the solution from one of two reservoirs was allowed to run into it. One reservoir contained a solution of 200 mM KCl and 1 mM DNP at pH 3.6; the other contained a similar solution adjusted to pH 8.5 with KOH. Thorough mixing of the solutions in the outer chamber was achieved by means of a magnetic stirrer. A constant level was maintained by means of a suction pipette. As a consequence of the small bore of the tubes used for the formation of the films and the material used, fluctuations in the level of solution in the outer chamber left the level of solution in the tubes unchanged. This simple but important artifice enabled us to change the solution in the outer chamber without risk of bulging or damaging the films. The shape of the ends of the polythene tubes ensured that the films formed on the ends of the tubes and not in the tubes.

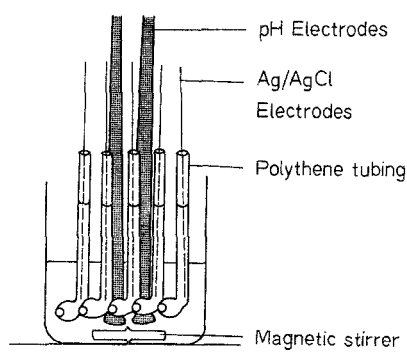


Fig. 1. Experimental arrangement showing the polythene tubes on which the films were formed

### *Measurement of Transmembrane Potential Difference, Conductance and Capacitance*

Ag-AgCl electrodes, one on each side of the membrane, were used for measurement of the transmembrane potential difference (p. d.) and, during conductance measurements, for passage of a small current as well. The p. d. between the two electrodes was measured by the use of an electrometer (E.I.L. Vibron Model 33B or Keithley Instr. Inc. Model 603). The sign of the p. d. is defined as that of the potential of side 2 (outside) with respect to side 1 (inside). The conductance $G$ (mhos), was calculated from the steady value of the change in p.d. resulting from the passage of a current of $I=10^{-9}$ amps between the two electrodes. The behavior of the films was not found to deviate significantly from Ohm's law even with currents up to 10 times the value used for the measurements of conductance described here. Capacitance was measured by including the two electrodes in an A.C. bridge circuit.

## Results and Discussion

### *Variation of Conductance with Film Area*

The conductance $G$ (mhos) and the capacitance $C$ (μF) of 100 films have been measured; the results are summarized in Table 1 where they are divided into groups according to the size of hole on which the films were formed.

Fig. 2 shows that the conductance is approximately a linear function of the capacitance and thus of the film area. The small intercept is due to

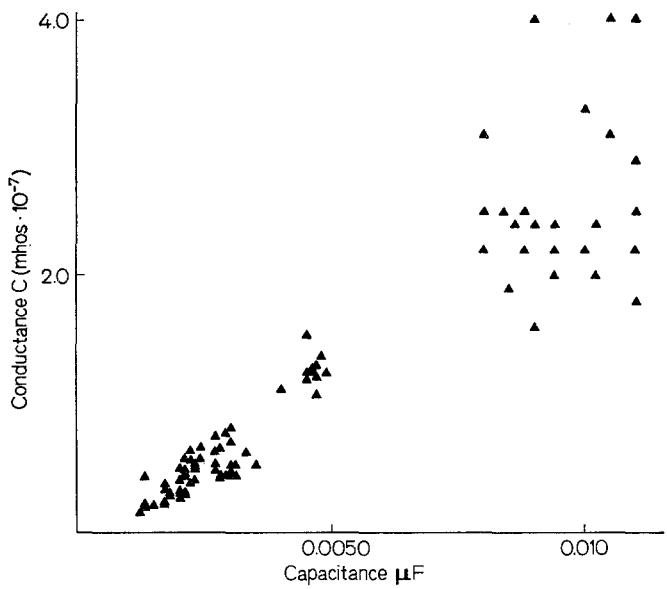


Fig. 2. The relation between film conductance and capacitance

Table 1. *Measurements of conductance and capacitance of black films formed in solutions containing 200 mM KCl and 1 mM DNP at pH 3.6*

| Hole diameter (mm) | Mean $C$ (μF) | $G$ (mhos $\times 10^{-7}$) | $C$ (μF) | $G$ (mhos $\times 10^{-7}$) | $C$ (μF) | $G$ (mhos $\times 10^{-7}$) | $C$ (μF) |
|---|---|---|---|---|---|---|---|
| 0.9 | 0.0018 | 0.67 | 0.0024 | 0.56 | 0.0022 | 0.50 | 0.0023 |
| | | 0.64 | 0.0022 | 0.41 | 0.0023 | 0.33 | 0.0020 |
| | | 0.33 | 0.0017 | 0.22 | 0.0017 | 0.50 | 0.0020 |
| | | 0.38 | 0.0022 | 0.29 | 0.0018 | 0.29 | 0.0021 |
| | | 0.44 | 0.0013 | 0.38 | 0.0017 | 0.22 | 0.0013 |
| | | 0.44 | 0.0021 | 0.31 | 0.0018 | 0.45 | 0.0021 |
| | | 0.18 | 0.0013 | 0.20 | 0.0013 | 0.15 | 0.0012 |
| | | 0.18 | 0.0013 | 0.24 | 0.0017 | 0.27 | 0.0020 |
| | | 0.22 | 0.0017 | 0.41 | 0.0020 | 0.49 | 0.0021 |
| | | 0.31 | 0.0020 | 0.31 | 0.0021 | 0.20 | 0.0015 |
| 1.21 | 0.0026 | 0.63 | 0.0033 | 0.58 | 0.0021 | 0.54 | 0.0027 |
| | | 0.52 | 0.0023 | 0.54 | 0.0023 | 0.58 | 0.0024 |
| | | 0.75 | 0.0027 | 0.78 | 0.0029 | 0.82 | 0.0030 |
| | | 0.71 | 0.0030 | 0.65 | 0.0028 | 0.63 | 0.0027 |
| 1.32 | 0.0029 | 0.44 | 0.0029 | 0.45 | 0.0028 | 0.45 | 0.0028 |
| | | 0.53 | 0.0031 | 0.44 | 0.0028 | 0.53 | 0.0035 |
| | | 0.44 | 0.0031 | 0.45 | 0.0030 | 0.48 | 0.0030 |
| | | 0.48 | 0.0027 | 0.53 | 0.0030 | — | — |
| 1.66 | 0.0046 | 1.11 | 0.0040 | 1.25 | 0.0045 | 1.19 | 0.0045 |
| | | 1.28 | 0.0046 | 1.38 | 0.0048 | 1.21 | 0.0047 |
| | | 1.25 | 0.0049 | 1.25 | 0.0046 | 1.08 | 0.0047 |
| | | 1.31 | 0.0047 | — | 0.0045 | — | 0.0044 |
| | | 1.54 | 0.0045 | — | 0.0044 | — | 0.0048 |
| | | — | 0.0044 | — | 0.0044 | — | 0.0044 |
| | | — | 0.0042 | — | 0.0043 | — | 0.0043 |
| 2.45 | 0.0095 | 2.5 | 0.0080 | 3.3 | 0.0100 | | 0.0100 |
| | | 4.0 | 0.0105 | 4.0 | 0.0110 | 3.1 | 0.0105 |
| | | 2.9 | 0.0110 | 2.5 | 0.0110 | 2.4 | 0.0102 |
| | | 2.0 | 0.0102 | 1.6 | 0.0090 | 2.2 | 0.0110 |
| | | 1.8 | 0.0110 | 4.0 | 0.0090 | 2.2 | 0.0094 |
| | | 2.2 | 0.0100 | 3.1 | 0.0080 | 2.5 | 0.0088 |
| | | 2.4 | 0.0090 | 1.9 | 0.0085 | 2.5 | 0.0084 |
| | | 2.4 | 0.0094 | 2.2 | 0.0080 | 2.2 | 0.0088 |
| | | 2.0 | 0.0094 | 2.4 | 0.0086 | — | — |

the inclusion in the capacitance measurements of the capacitance of the measuring circuit. These results show that so far as DNP-treated films are concerned, the edge effects due to electrical leakage between the toroidal rim of the film and the support or conduction through the toroidal rim itself are negligible.

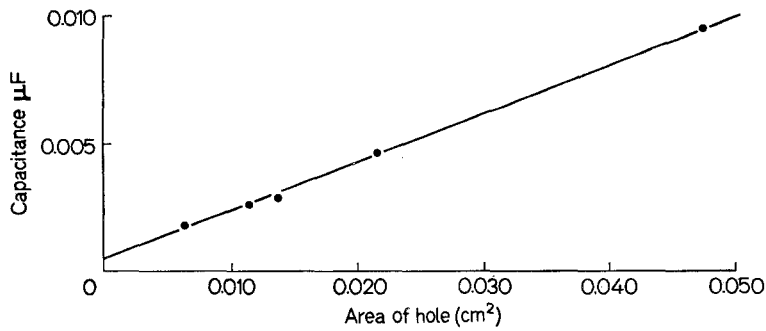


Fig. 3. The relation between film capacitance and hole area. Each point represents the mean of a number of measurements

Fig. 3 shows the mean capacitance for each group of data plotted as a function of hole area. The slope of the regression line is 0.19. This value has been used for calculating nominal film areas from measured capacitances in the conductance experiments described below.

### *Variation of Film Conductance with pH*

The conductance has been measured in films formed in solutions containing 200 mM KCl and 1 mM DNP at various pH values. In these experiments the pH was the same on both sides of the membrane. The results are summarized in Fig. 4. Each point represents the mean value of $G$ for

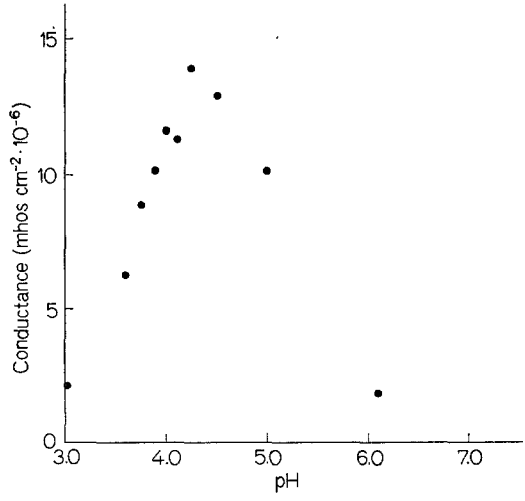


Fig. 4. The relation between film conductance and pH. The pH was the same on each side of the film

five membranes. It can be seen that the maximum conductance occurs at pH 4.2. This is to be compared with the value of 4 given by Hopfer et al. (1968) and that of 4.1 given by Liberman and Topaly (1968). The maximum conductance was $1.4 \times 10^{-5}$ mhos $cm^{-2}$.

Several alternative models have been considered: (1) The only ions present in the film are dinitrophenate ($P^-$) and hydrogen ($H^+$) ions. (2) The only ions present in the film are complexes soluble in the hydro-carbon part of it. The conductance of such a system is derived in the Appendix. The conductance has been considered for two types of complex: (a) Cationic complexes of the type $X(HP)_n^+$ where $X$ is a cation or hydrogen ion and $HP$ is dinitrophenol. This type of complex would be analogous to those apparently formed by uncharged macrocyclic compounds (Liberman & Topaly, 1968; Eisenman et al., 1968; Kilbourne et al., 1967). (b) Anionic complexes of the type $XP_2^-$.

Of these models, only (2b) can explain the observed relation between film conductance and hydrogen ion concentration in the aqueous solutions.

The expression for the conductance $G$ in this case may be written:

$$G = A' \left( \frac{A_0 K_D}{H_0^+ + K_D} \right)^2 (H_0^+ + \alpha' K_0^+) \tag{1}$$

where $A'$ is a constant, $A_0$ is the total concentration of DNP in the system, $K_D$ is the dissociation constant of DNP, $H_0^+$ and $K_0^+$ are, respectively, the concentrations of $H^+$ and $K^+$ ions in the aqueous solutions, and $\alpha'$ is a constant defined in the Appendix.

The condition for maximum $G$ is:

$$H_0^+ = K_D - 2\alpha' K_0^+. \tag{2}$$

The experimentally determined maximum is at pH = 4.2. Thus $\alpha' = 1.2 \times 10^{-4}$. The low value for $\alpha'$ may be taken to imply that the complex $KP_2$ is important only at high pH.

The theory given in the Appendix may be readily extended to include complexes of the type $X_i(L_j)_n$, where $X_i$ is a cation and $L_j$ a ligand. In this case

$$G = L_j^n \sum_i A_{ij} K_{ij} X_i. \tag{3}$$

This equation gives the relation between $G$ and the concentration of ligand:

$$d \ln G = n \, d \ln L_j. \tag{4}$$

Data fitting Eq. (4) have been obtained with a number of substances (Liberman & Topaly, 1968; Eisenman et al., 1968; Finkelstein & Cass, 1968; Tosteson et al., 1968) and can be used to derive $n$, the number of moles of ligand per mole of complex, which with different substances has a value of 1 or 2.

### *Variation of Transmembrane Potential with pH Difference Across the Membrane*

With the internal solution at pH 3.6, the pH of the external solution ($pH_2$) was adjusted to various values and the transmembrane p.d., $V$, was measured. The results are summarized in Fig. 5. Each point represents the

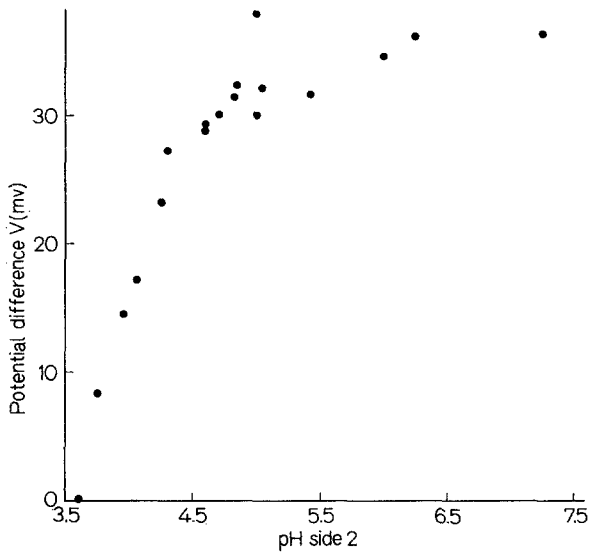


Fig. 5. The relation between transfilm p.d. and the pH difference. The pH on side 1 remained constant at pH 3.6. The sign of the potential is defined as that of side 2 with respect to side 1

mean value of $V$ for several membranes, in most cases three to five. Although the membrane appears to behave approximately as a hydrogen electrode in the region of pH 3.6, it is clear that it departs from this behavior as $pH_2$ is increased. Similar results were obtained when NaCl or $Na_2SO_4$ at the same ionic strength was used instead of KCl.

There are two alternative explanations for this behavior: (1) The membrane is a barrier permeable only to hydrogen ions and dinitrophenate ions. In this case, the transmembrane p.d. can be described by the Gold-

man equation (Goldman, 1943; Hodgkin & Katz, 1949; Sandblom & Eisenman, 1967).

$$V=\frac{RT}{F}\ln\frac{P_{02}^{-}+\alpha H_{01}^{+}}{P_{01}^{-}+\alpha H_{02}^{+}} \tag{5}$$

where $P_0^-$ and $H_0^+$ are the concentrations of dinitrophenate and hydrogen ions in the bulk solution outside the membrane on sides 1 and 2, and $\alpha$ is the ratio of the permeability coefficients for hydrogen and dinitrophenate ions. Eq. (5) has been fitted to the experimental data (Fig. 5) by the method of least squares. The value for $\alpha$ was found to be $0.36\pm0.14$. It thus appears that the variation of $V$ with $pH_2$ can be explained in terms of permeability to $H^+$ and $P^-$. (2) The membrane constitutes a distinct phase and regard must be paid to the distribution of ions between the aqueous and membrane phases. The p.d. is written:

$$V=\frac{RT}{F}\ln\frac{P_{02}^{-\prime 2}(H_{02}^{+\prime}+\alpha' K_0^{+\prime})}{P_{01}^{-\prime 2}(H_{01}^{+\prime}+\alpha' K_0^{+\prime})} \tag{6}$$

where primed concentrations refer to the aqueous solutions immediately outside the lipid phase, and $\alpha'$ is a constant defined in the Appendix.

Eq. (6) which describes the p.d. across a film containing the complexes $HP_2+KP_2$ has been derived in the Appendix without using arbitrary assumptions as to electroneutrality or gradients of concentration or electrical potential. This equation, together with the unstirred-layer Eqs. (24), (25), and (29), has been fitted to the experimental data. These equations involve, in addition to the concentrations of hydrogen and dinitrophenate ions, two parameters: $\beta$, the ratio of the mobilities of dinitrophenate and hydrogen ions in aqueous solutions, and $a$ which is given by

$$a=\frac{Pd'}{D} \tag{7}$$

where $P$ is the permeability coefficient of the film to the undissociated DNP, $d'$ is the thickness of the unstirred layer, and $D$ is the diffusion coefficient in aqueous solution. Given that $\beta=0.1$, the fit permits the evaluation of the concentrations immediately outside the film and the parameter $a$. The best fit was found for $a=1.0$; the concentrations are summarized in Table 2.

Taking $D=10^{-5}\,\mathrm{cm^2\,sec^{-1}}$ and $d=100\,\mu$, then from Eq. (7) the permeability coefficient $P_{HP}$ was estimated to be roughly $10^{-3}\,\mathrm{cm\,sec^{-1}}$,

Table 2. *Calculated concentrations (mM) of various species in the aqueous solutions immediately outside the lipid phase. Bulk $pH_1$ 3.6, bulk $pH_2$ 7.0*

| Component | Bulk solution 1 | Outside lipid phase 1 | Outside lipid phase 2 | Bulk solution 2 |
|---|---|---|---|---|
| A | 1 | 0.69 | 1.32 | 1 |
| HP | 0.71 | 0.49 | 0.18 | 0 |
| $P^-$ | 0.29 | 0.20 | 1.14 | 1 |
| $H^+$ | 0.25 | 0.25 | 0.016 | 0 |

a value in good agreement with those obtained by Dainty and Ginzburg (1964) for the permeability of a series of alcohols across cell membranes.

It thus appears that both the variation of conductance with pH and the variation of transfilm p.d. with pH difference across the film can be explained in terms of anionic complexes soluble in it. Moreover, there is some evidence for the existence of complexes of a type similar to that envisaged for DNP (Sidgwick & Brewer, 1925). A variety of other uncoupling agents exhibit similar conductance behavior (Hopfer et al., 1968; Liberman & Topaly, 1968).

From the measured conductances, it is clear that the total ion concentration within the film must be extremely low (probably $\sim 10^{-7}$ M). It is clearly unnecessary to postulate that the complexes need high stability constants on high lipid-water partition coefficients.

The preferred model provides a possible explanation of the uncoupling effect of DNP. If the transmembrane potential differs from the value predicted by Eq. (16), a net current of anionic complexes will flow across the membrane, tending to short-circuit the $H^+$ electrochemical p.d. envisaged by Mitchell (1961, 1966).

## Appendix

On the following assumptions, expressions are derived for the conductance and transfilm p.d. of a thin lipid film in salt solutions containing uncoupling agents. (1) The only charged species present in significant amounts in the lipid phase are cationic (model 2a) or anionic (model 2b) complexes. There is no difficulty regarding the absence of counterions since the Debye length for such a phase (probably $\sim$ 2000 A) is considerably greater than the thickness of the film. (2) All ionic species present in the system are in equilibrium across each phase boundary. Equating the electrochemical potentials on each side of the phase boundary:

$$C_{im} = k_i C_{i0} \exp(-z_i F E_p / R T) \tag{8}$$

where $C_{i0}$ is the concentration of ion $i$ just inside the aqueous phase (0), and $C_{im}$ is the concentration just inside the lipid phase ($m$), $k_i$ is a partition coefficient, and $E_P$ is the electrical p.d. between a point just inside the lipid phase and a point just inside the aqueous phase (phase boundary potential).

### *Expression for Electrical Conductance of a Lipid Film*

The conductance $G$ of a film is determined by the concentrations and mobilities of the various ions within the film. In the case where the solutions on the two sides of the film have the same composition, the relation is

$$G=\sum_i \frac{(z_i F)^2 u_i C_{im}}{d} \tag{9}$$

where $u_i$ is the mobility of ion $i$, and $d$ is the thickness of the film.

The problem is to evaluate $C_{im}$. This may be done in the following way: $C_i$ is distributed between the film and the aqueous phase according to the relation

$$C_{im}=k_i C_{i0} \exp(-z_i F E_p/R T). \tag{10}$$

The black film may be regarded as a conductor separating two capacitors (the "double layer" capacitors), one on each side of the film, so that the relation between the phase boundary potential $E_p$ and the net charge density in the film $\rho$ is

$$E_p=\rho d/C \tag{11}$$

where $C$ is the double layer capacitance.

On substituting values of $10^{-5}$ coulombs $cm^{-3}$ for $\rho$ (an approximate figure estimated from the maximum conductance measured, assuming a mobility coefficient for the complex, 0.1 % of that of small ions in aqueous solution), 10 $\mu$F $cm^{-2}$ for $C$, and $10^{-6}$ cm for $d$, it can be seen that $E_p$ is of the order of $10^{-6}$ V and that the exponent in Eq. (8) is of the order of $10^{-4}$. Thus, to a high degree of accuracy

$$C_{im}=k_i C_{i0} \tag{12}$$

where $k_i$ is the partition coefficient.

From Eqs. (9) and (12), the conductance $G$ is given by

$$G=\sum_i A_i C_{i0} \tag{13}$$

where

$$A_i=(z_i F)^2 \frac{u_i k_i}{d}.$$

### *Model (2b)*

The concentration of dinitrophenate ions $P^-$ in the aqueous solution may be written:

$$P_0^- = \frac{A_0 K_D}{H_0+K_D} \tag{14}$$

where $A_0$ is the total concentration of DNP and $K_D$ its dissociation constant.

The concentration of the complexes $HP_2^-$ and $KP_2^-$ in aqueous solution may be written:

$$[HP_2^-]_0=K_1 H_0^+ P_0^{-2}, \tag{15}$$

$$[KP_2^-]_0=K_2 K_0^+ P_0^{-2} \tag{16}$$

where $K_1$ and $K_2$ are, respectively, the stability constants for the complexes $HP_2$ and $KP_2$.

From Eqs. (13), (14), (15), and (16)

$$\begin{aligned} G &= A' P_0^{-2} (H_0^+ + \alpha' K_0^+) \\ &= A' \left( \frac{A_0 K_D}{H_0^+ + K_D} \right)^2 (H_0^+ + \alpha' K_0^+) \end{aligned} \tag{17}$$

where $A'$ is a constant and $\alpha' = \frac{K_2}{K_1} \cdot \frac{k_2}{k_1} \cdot \frac{u_2}{u_1}$.

### *Expression for Electrical p.d. Across a Lipid Film*

The p.d. ($V$) between the two aqueous solutions can be written

$$V = E_{p1} + E_M - E_{p2} \tag{18}$$

where $E_M$ is the p.d. between two points just inside the lipid phase on each side of the film, and $E_{P1}$ and $E_{P2}$ are the two phase boundary potentials. It has already been demonstrated that the phase boundary potentials are negligible [Eqs. (10) and (12)].

The assumption that there are ions of one sign only in the membrane phase means that the Nernst-Planck equation at zero current

$$0 = \sum_i u_i \left( \frac{d C_{im}}{dx} + C_{im} z_i \frac{F}{RT} \frac{dE_M}{dx} \right) \tag{19}$$

can be integrated without the assumption of a constant field to give

$$E_m (= V) = -\frac{RT}{zF} \ln \frac{C_{im2} + \alpha C_{jm2}}{C_{im1} + \alpha C_{jm1}} \tag{20}$$

where $C_{im}$ and $C_{jm}$ are the concentrations of ions $i$ and $j$ just inside the lipid phase on sides 1 and 2, and $\alpha$ is the ratio of the mobilities of the two ions in the lipid phase. Two terms are given corresponding to the two complexes considered.

This equation may be expanded, using Eqs. (12), (15) and (16) to give:

$$V = \frac{RT}{F} \ln \frac{P_{02}^{-2} (H_{02}^+ + \alpha' K_{02}^+)}{P_{01}^{-2} (H_{01}^+ + \alpha' K_{01}^+)} \tag{21}$$

where $\alpha'$ is as defined above after Eq. (17).

Now the permeability of the film to the uncharged form of a carrier is likely to be quite high. Thus, if the concentration of the uncharged species is different on the two sides of the film, there will be a net transport of this species across it. Then, due to the presence of unstirred layers, the concentration of the uncharged species and ions in equilibrium with it may not be the same immediately outside the lipid phase as in the bulk solutions. These considerations do not apply to the conductance experiments reported here, but they may affect the p.d. experiments.

It seems clear that the permeability of the film to the uncharged species must be so much greater than the permeability to ions that, for the purpose of determining the

effect of the unstirred layers on the p.d. experiments, the movement of ions through the lipid phase can be ignored.

In the case of DNP, the treatment is complicated by the equilibrium between $HP$, $P^-$ and $H^+$ concentrations. The situation is considered for the case where the total DNP concentration is the same in both bulk solutions.

Assuming no electrical field in the unstirred layers (high concentration of KCl present) and zero current flow,

$$d H_0^+ - \beta d P_0^- - \gamma d O H_0^- = 0 \tag{22}$$

where $\beta$ and $\gamma$ are ratios of ion mobilities. In the system considered, there is negligible error in ignoring $OH^-$.

Then on side 1,

$$H_{01}^+ - H_{01}^{+\prime} = \beta (P_{01}^- - P_{01}^{-\prime}) \tag{23}$$

where non-primes refer to the bulk solution and primes to the aqueous solution immediately outside the lipid phase. This equation can be written

$$P_{01}^{-\prime} H_{01}^+ - K_D H P_{01}' = \beta P_{01}^{-\prime} (P_{01}^- - P_{01}^{-\prime}) . \tag{24}$$

Similarly on side 2 ,

$$P_{02}^{-\prime} H_{02}^+ - K_D H P_{02}' = \beta P_{02}^- (P_{02}^- - P_{02}^{-\prime}) . \tag{25}$$

Also for a steady state flux of DNP across the unstirred layer and assuming that the mobilities of $P^-$ and $HP$ are the same,

$$d P_{01}^- + d H P_{01} = d P_{02}^- + d H P_{02} . \tag{26}$$

Then,

$$A_0 - (P_{01}^{-\prime} + H P_{01}') = (P_{02}^{-\prime} + H P_{02}') - A_0 . \tag{27}$$

The flux of DNP across the unstirred layers must equal the flux of $HP$ across the lipid film. Then,

$$\frac{D}{d'}\left(A_0 - (P_{01}^{-\prime} + H P_{01}')\right) = P(H P_{01}' - H P_{02}') = \frac{D}{d'}\left((P_{02}^{-\prime} + H P_{02}') - A_0\right), \tag{28}$$

$$\text{or } A_0 - (P_{01}^{-\prime} + H P_{01}') = a(H P_{01}' - H P_{02}') = (P_{02}^{-\prime} + H P_{02}') - A_0 \tag{29}$$

where $D$ is the diffusion coefficient of $P^-$ and $HP$ in the unstirred layer, $d'$ the thickness of the unstirred layer, $P$ the permeability coefficient of the membrane for $HP$, and $a = Pd'/D$.

The four unstirred layer Eqs. (24), (25), and (29) together with (6) were fitted to the experimental data to give values for $P_0^{-\prime}$, $HP_0'$, $H_0^{+\prime}$ and $a$.

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## References

Bielawski, J., T. E. Thompson, and A. L. Lehninger. 1966. The effect of 2,4-dinitrophenol on the electrical resistance of phospholipid bilayer membranes. *Biochem. Biophys. Res. Commun.* **24**:948.

Croghan, P. C., E. J. A. Lea, and J. Lelièvre. 1969. The effect of 2,4-dinitrophenol and acetic acid on properties of black phospholipid films. *J. Physiol.* **200**:114P.

Dainty, J., and B. Z. Ginzburg. 1964. The permeability of the protoplasts of *Chara australis* and *Nitella translucens* to methanol, ethanol and isopropanol. *Biochim. Biophys. Acta* **79**:122.

Eisenman, G., S. M. Ciani, and G. Szabo. 1968. Some theoretically expected and experimentally observed properties of lipid bilayer membranes containing neutral molecular carriers of ions. *Fed. Proc.* **27**:1289.

Finkelstein, A., and A. Cass. 1968. The permeability and electrical properties of thin lipid membranes. *J. Gen. Physiol.* **52**:145 S.

Goldman, D. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**:37.

Hopfer, U., A. L. Lehninger, and T. E. Thompson. 1968. Protonic conductance across phospholipid bilayer membranes induced by uncoupling agents for oxidative phosphorylation. *Proc. Nat. Acad. Sci., Wash.* **59**:484.

Kilbourne, B. T., J. D. Dunitz, L. A. R. Pioda, and W. Simon. 1967. Structure of the $K^+$ complex with nonactin, a macrotetrolide antibiotic possessing highly specific $K^+$ transport properties. *J. Mol. Biol.* **30**:559.

Liberman, E. A., and V. P. Topaly. 1968. Selective transport of ions through bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **163**:125.

Mitchell, P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* **191**:144.

— 1966. Chemi-osmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev.* **41**:445.

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

Sandblom, J. P., and G. Eisenman. 1967. Membrane potentials at zero current. *Biophys. J.* **7**:217.

Sidgwick, N. V., and F. M. Brewer. 1925. Co-ordinated compounds of the alkali metals. Part II. *J. Chem. Soc.* **127**:2379.

Tosteson, D. C., T. E. Andreoli, M. Tieffenberg, and P. Cook. 1968. The effects of macrocyclic compounds on cation transport in sheep red cells and thin and thick lipid membranes. *J. Gen. Physiol.* **51**:373.