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The Mechanism of Action of DNP on Phospholipid Bilayer Membranes

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Summary. The weak acid 2,4-dinitrophenol (DNP) acts as an uncoupler of oxidative phosphorylation in biological systems and, in consonance with the Mitchell hypothesis, also enhances the conductance of phospholipid bilayer membranes. Several models have been proposed in the literature to explain the molecular mechanism by which DNP exerts its electrical effects on the model membranes, none of which accounts for all of the data, and all of which ignore the possibility that the anion of DNP is also binding to the surface of the bilayer and modifying the charge density. Experimental evidence is presented in this report which suggests that when a bilayer membrane is formed from a neutral lipid, DNP does in fact adsorb to its surface and produce a substantial negative surface potential. When this phenomenon is taken into account, the model proposed by Lea and Croghan and by Finkelstein is capable of describing all of the effects of DNP on bilayer membranes. In this model, the permeant species is a negatively charged complex formed from the undissociated acid and its anion.

The weak acid 2,4-dinitrophenol (DNP) acts as an uncoupler of oxidative phosphorylation in mitochondria, chloroplasts and bacteria. Although its mechanism of action on these biological systems is unknown, some innovative support for the chemiosmotic hypothesis of Mitchell (1966) was obtained by Bielawski, Thompson and Lehninger (1966), who demonstrated that DNP enhances the conductance of a phospholipid bilayer. Many other uncouplers have since been shown to also increase the conductance of phospholipid membranes (e.g., Skulachev, Sharaf & Liberman, 1967; Hopfer, Lehninger & Thompson, 1968; Liberman & Babakov, 1968; Liberman, Mochova, Skulachev & Topaly, 1968; Liberman & Topaly, 1968*a*, *b*; Hopfer, Lehninger & Lennarz, 1970) but all attempts to formulate a model which would explain the effects of the uncouplers on bilayers in terms of a single permeation mechanism have met with a singular lack of success. The main reason for this, as LeBlanc (1971) has recently pointed out, is that there must be at least two classes of weak acid uncouplers and two different mechanisms of charge permeation.

For one class of uncouplers, exemplified by carbonylcyanide *m*-chlorophenylhydrazone (CCCP), the bilayer conductance is directly proportional to the uncoupler concentration, implying that the permeant species is simply the anionic form A^- of the weak acid HA. Many of the complex phenomena observed with this class of uncouplers, such as the maxima in the conductance *vs.* pH curves which occur to the alkaline side of the pK and the saturation in the current-voltage curves can be satisfactorily explained by taking into account the aqueous unstirred layers adjacent to the membrane (LeBlanc, 1971; Neumcke, 1971).

The other class of uncouplers includes, but is not restricted to, the substituted benzimidazoles. For these compounds, the bilayer conductance increases with the square of the uncoupler concentration, maxima are observed in the conductance *vs.* pH curves at the pK of the acid, and the current-voltage curves do not saturate (Liberman *et al.*, 1968; Liberman & Topaly, 1968*a*). Finkelstein (1970) analyzed the Liberman data and concluded that the weak acid HA was acting as a carrier for the anion A^-, the permeant species being the HA_2^- complex.

Lea and Croghan (1969) examined experimentally the properties of a bilayer exposed to 1 mM DNP and concluded that the permeant species with this uncoupler was also the HA_2^- complex. More recently, however, Hopfer *et al.* (1970) have obtained data with DNP on positive, neutral and negative membranes which appear to contradict the carrier model. They noted that if the carrier model is correct, maxima sould occur in the conductance *vs.* pH curves at the pK of the weak acid irrespective of the charge on the membrane and that the difference in the conductance observed on a neutral membrane and one with a constant surface charge should be independent of pH. Their experimental results disagreed with both of these predictions. Finally, we may note from the data of Bielawski *et al.* (1966) and Liberman *et al.* (1968) that the conductance does not depend on the square of the DNP concentration, as it should if the permeant species is the HA_2^- complex. Does this mean we must invoke yet a third permeation mechanism for DNP (e.g., Bruner, 1970) or, has some complicating factor, such as a change in the dielectric constant of the bilayer in the presence of DNP as suggested by Läuger (1970) been ignored in the analysis of the data?

We attempt to answer this question in the following manner. First, we show theoretically that all of the known effects of DNP on bilayer membranes are compatible with the carrier model proposed by Lea and Croghan (1969) and by Finkelstein (1970) once it is hypothesized that the anion of DNP may bind to the surface of the bilayer membrane and change the interfacial charge density. Next, we provide experimental data which corroborates this hypothesis.

The Carrier Model

Eqs. (1)–(5) summarize the carrier model of Lea and Croghan (1969) and Finkelstein (1970). We consider for simplicity only the case where the bilayer membrane separates identical solutions. The following two reactions are assumed to occur in the aqueous phases:

$$HA \rightleftharpoons H^+ + A^-, \quad K_1 \equiv \frac{[H^+][A^-]}{[HA]} \tag{1}$$

$$HA_2^- \rightleftharpoons HA + A^-, \quad K_2 \equiv \frac{[HA][A^-]}{[HA_2^-]}. \tag{2}$$

The concentration of HA_2^- is assumed to be much less than that of either HA or A^-, so the total concentration of uncoupler, $[A^{Tot}]$ may be approximated by:

$$[A^{Tot}] = [HA] + [A^-]. \tag{3}$$

By combining Eqs. (1)–(3) we obtain:

$$[HA_2^-] = \frac{K_1}{K_2}[A^{Tot}]^2 \frac{[H^+]}{(K_1 + [H^+])^2} \tag{4}$$

for the concentration of HA_2^- in the bulk aqueous phases.

The model assumes that the only permeant species is the HA_2^- complex[1], that the rate-limiting step is the movement of the complex through the membrane[2], and that space charge effects are negligible[3]. With these assumptions it follows that the conductance G measured in the limit of zero

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[1] This assumption may not be valid when the pH is far to the alkaline side of the pK and the concentration of the A^- form is therefore much larger than that of the HA_2^- form. Finkelstein (1970) may be consulted for further comments on this point.

[2] If the rate-limiting step was not the movement of the complex through the membrane, the current-voltage curves would exhibit a saturating shape. The current-voltage curves, however, were noted to be superlinear for all concentrations of DNP investigated.

[3] More explicitly, we assume that the concentration of the charged complexes within the membrane is less than 5×10^{-5} M (Neumcke & Läuger, 1970). Concentrations lower than this will not produce an electric potential sufficient to perturb the energy profile of an ion within the membrane. A variation in the energy profile or mobility of the ions within the membrane which does not depend on concentration (Hall & Mead, 1972) will not compromise the validity of the derivation, for we may simply regard $u_{HA_2^-}$ and $[HA_2^-]^*$ as being average values.

applied voltage, is given by:

$$G = \frac{F^2}{d} u_{HA_2^-} [HA_2^-]^* \tag{5}$$

where F is the Faraday, d the thickness of the membrane, $u_{HA_2^-}$ the mobility of the complex in the membrane, and the asterisk (*) denotes the concentration of HA_2^- within the membrane, which we may regard as uniform[3]. If the membrane bears a net surface charge σ there will be a potential ψ at the surfaces of the membrane relative to the bulk aqueous phases. The concentration of the permeant species within the membrane $[HA_2^-]^*$, will therefore be related via a partition coefficient k,[4] to its concentration in the aqueous phase immediately adjacent to the membrane:

$$[HA_2^-]^* = k[HA_2^-] \exp(F\psi/RT) \tag{6}$$

which is given by the Boltzmann relation[5]. Combining Eqs. (4)–(6) we obtain the following expression:

$$G = \frac{F^2}{d} u_{HA_2^-} k \frac{K_1 [A^{Tot}]^2 [H^+]}{K_2 (K_1 + [H^+])^2} \exp(F\psi/RT) \tag{7}$$

which relates the conductance to the two experimentally accessible variables, $[H^+]$ and $[A^{Tot}]$.

Let us first assume that no binding of the anion of DNP occurs to the bilayer and that the potential ψ is therefore independent[6] of both $[H^+]$ and $[A^{Tot}]$. It is then apparent from Eq. (7) that irrespective of the value of ψ the conductance should attain a maximum value when $[H^+] = K_1$. Hopfer *et al.* (1970) observed, however, that the conductance maximum was shifted about a pH unit to the acid side of the pK when measurements were made with 10^{-4} M DNP on a bilayer formed from a positive lipid. Next, Eq. (7) implies that if measurements are made on bilayers formed from a neutral and then a charged lipid at the same hydrogen ion and DNP concentrations, the ratio of these measurements should be independent of pH. Moreover, if the values of $\frac{F^2}{d} u_{HA_2^-} k$ are similar for the two membranes, the conductance ratio should reflect the value of the electric potential

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[4] Included in the partition coefficient are any electrostatic contributions due to dipoles at the membrane solution interface. The partition coefficient is assumed to be a constant.

[5] Neumcke and Läuger (1970) have shown that the Boltzmann expression for the ion concentration in the aqueous phase will be valid even when a potential is applied to the membrane.

[6] For the pH range under consideration, both the phosphate and amino groups on the phospholipid should retain their charges.

at the surface of the charged relative to the neutral membrane:

$$G^{\text{charged}}/G^{\text{neutral}}=\exp(F\psi/RT). \tag{8}$$

McLaughlin, Szabo, Eisenman and Ciani (1970), McLaughlin, Szabo and Eisenman (1971), Stark and Benz (1971), and MacDonald and Bangham (1971) have provided experimental evidence that the surface potential of a charged bilayer membrane is described to a good first approximation by the theory of the diffuse double layer and that the use of the simple Gouy equation is appropriate when only monovalent ions are present:

$$\sinh\left(\frac{F\psi}{2RT}\right)=\frac{136\sigma}{\sqrt{c}} \tag{9}$$

where σ is the charge density in electronic charges per Å^2, C is the total monovalent ion concentration in the bulk solution in moles per liter and $RT/F=25.3$ mV at 22 °C. The data of Hopfer *et al.* (1970) illustrate, however, that the ratio of the conductances produced by DNP on a neutral and positive bilayer membrane is much less than that predicted[7] by the combination of Eqs. (8) and (9) and, furthermore, that the ratio depends markedly on the pH. Finally, we note that Eq. (7) implies the conductance should increase with the square of the uncoupler concentration whereas the data of Bielawski *et al.* (1966) and Liberman *et al.* (1968) demonstrate the relationship is more nearly linear than quadratic.

These three apparent discrepancies between the predictions of a carrier model [Eq. (7)] and the experimental observations could all be due to the anion of DNP adsorbing to the bilayer membranes and changing their net charge densities σ and therefore their surface potentials ψ [Eq. (9)]. As $[A^-]$ depends on both $[A^{\text{Tot}}]$ and $[H^+]$ [Eqs. (1) and (3)], the value of ψ in Eq. (7) will be a function of pH when binding occurs and the maximum in the *G vs.* pH curve will be displaced to the acid side of the pK. Specifically, if a significant degree of binding occurs to a bilayer membrane formed from a positive lipid, but not to one formed from a neutral lipid at a DNP concentration of 10^{-4} M and an ionic strength of 10^{-2} M, this would explain why the *G vs.* pH curve is shifted about a pH unit to the acid side of the pK and flattened with lyslphosphatidyl glycerol (positive) bilayers, but occurs at about the pK of DNP with diglucosyldiglyceride (neutral) bilayers (Hopfer

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[7] Assuming a reasonable value for the charge density or area available per phospholipid molecule of about 35 to 70 Å^2. Conductance data obtained with other carriers on membranes formed from charged and neutral lipids can be described with Eq. (9) by assuming a charge density within this range (McLaughlin *et al.*, 1970, 1971; Stark & Benz, 1971), which encompasses the presumably correct value of about 60 Å^2 (Fettiplace, Andrews & Haydon, 1971).

et al., 1970). Since the net charge density of the lyslphosphatidyl glycerol bilayer would be reduced from its initial value of about 1 per 60 Å^2, this would also explain why the potential that Hopfer *et al.* (1970) calculated from an equation identical to Eq. (8) of this paper is much less than that predicted from the Guoy expression[7] [Eq. (9)], and furthermore, why the conductance ratio depends on the pH. Finally, it would explain why the G *vs.* $[A^{Tot}]$ relationship on an initial neutral bilayer is not quadratic for DNP concentrations between 10^{-4} and 10^{-3} M. If there is a large degree of adsorption to a positive membrane at a bulk concentration of 10^{-4} M, we expect theoretically that there will be a significant degree of adsorption to a neutral membrane at a bulk concentration between 10^{-4} and 10^{-3} M. It is well known, however, that theories, in contradistinction to women, are rarely appealing unless they are one's own, in which case they become positively seductive. We will therefore test experimentally a prediction of the theoretical hypothesis rather than reveling in the existing data with Eqs. (7), (9) and an adsorption isotherm.

Materials and Methods

The apparatus and procedure used to obtain conductance measurements were similar to those described by Szabo, Eisenman and Ciani (1969). Bilayers were formed on a hole about 1 mm^2 in area in a partition which separated two aqueous phases of identical composition and the conductance was determined by dividing the measured current by the applied voltage of 10 mV. The bathing solutions contained 10^{-1} M KCl, 2×10^{-3} M tris(hydroxymethyl)amino methane buffered to pH 7.0 ± 0.1 and various concentrations of DNP. The pH was measured before and after each experiment. The bilayers were formed from an amphoteric bacterial lipid, phosphatidyl ethanolamine, obtained from Supelco (Bellefonte, Pa.). The temperature was 22 ± 1 °C.

Results

The above theoretical analysis hypothesizes that the anion of DNP adsorbs to bilayer membranes, increases the negativity of the surface potential, and therefore depresses the conductance due to the negatively charged HA_2^- complex. If the hypothesis is valid, the adsorption of the anion should *enhance* the conductance due to a positive permeant species, such as the complex formed between the neutral carrier nonactin and the potassium ion. Specifically, the conductance due to the nonactin-K$^+$ complex has been shown to be given by the following equation (McLaughlin *et al.*, 1970):

$$G=\frac{F^2}{d}u_{is}k_{is}K_{is}^{+}[K^+][NON]\exp(-F\psi/RT) \qquad (10)$$

where F is the Faraday, d the thickness of the membrane, u_{is} the mobility of the charged complex in the membrane, k_{is} a partition coefficient[4] relating the (uniform[3]) concentration of the complex in the membrane to the concentration in the aqueous phase immediately adjacent to the membrane, K^{+}_{is} is the asociation constant for the formation of the complex and ψ is the surface potential. (Note that the term K^{+}_{is} $[K^{+}]$ $[NON]$ is merely the concentration of the permeant species in the bulk aqueous phase, and that the product of the last five terms is the concentration of the complex in the membrane, as given by the Boltzman relation[5] and the definition of the partition coefficient.)

Fig. 1 illustrates the effect of DNP on the conductance of a bilayer membrane formed from the amphoteric but neutral lipid, phosphatidyl

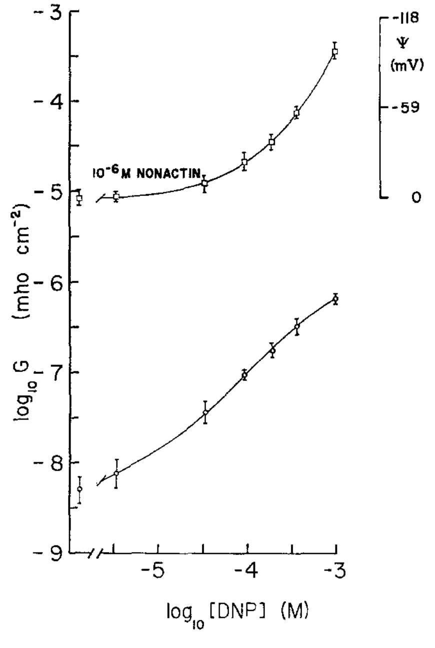

Fig. 1. *Lower:* The effect of DNP on the conductance of a bilayer membrane formed from the neutral lipid PE. The membrane was formed in a solution containing 10^{-1} M KCl, 2×10^{-3} M Tris buffered to pH 7.0 and the indicated concentration of DNP. *Upper:* The effect of DNP on the carrier-induced conductance of a bilayer membrane formed from PE. The bathing solutions are the same as for the lower curve, but with the addition of 10^{-6} M nonactin. Note that DNP produces a marked increase in the conductance due to the nonactin-K^{+} complex even though the conductance due to DNP is always orders of magnitude less than that due to the antibiotic. The increase in conductance is presumably due to the anion of DNP adsorbing to the surface of the membrane and producing a negative surface potential, as indicated by the ordinate on the right-hand side of the figure. The lengths of the vertical bars are twice the standard deviation of the five experiments

ethanolamine (PE). Note that DNP enhances the conductance due to the nonactin-K^+ complex (upper curve) even though the innate conductance due to some anionic form of DNP (lower curve) is always two orders of magnitude less than that produced by the antibiotic. The simplest interpretation of this increase in the nonactin-K^+ conductance[8] is that the anion of DNP is adsorbing to the membrane and producing a negative surface potential, the magnitude of which is given by the right-hand ordinate [*see* Eq. (10)].

The surface potential produced by DNP on this neutral lipid is of the magnitude required to reconcile the experimental data with the predictions of a carrier model, as a consideration of the dependence of conductance on uncoupler concentration illustrates. In Fig. 2, the measured value of the conductance produced by DNP[9] (lower curve) is "corrected" for the surface potential produced by the adsorption of the anion (upper curve). That is, each conductance measurement is multiplied by the appropriate value of $\exp(-F\psi/RT)$, as deduced from the upper curve in Fig. 1. When the measurements are "corrected" in this manner the slope of the conductance *vs.* uncoupler concentration curve should be a true indication of the stoichiometry involved. It is apparent in Fig. 2 that there is a quadratic relation between conductance and DNP concentration; on the log-log plot, the slope of the dashed regression line fitting the four data points between 10^{-4} and 10^{-3} M is 2.06. This quadratic relationship is consistent with the postulate that the permeant species is the HA_2^- complex [*see* Eq. (7)], as previously suggested by Lea and Croghan (1969) from an analysis of their *G vs.* pH data.

We have merely assumed up to this point that it is the A^- rather than the HA_2^- species which adsorbs to the bilayer. To test this assumption, experiments similar to those illustrated in Fig. 1 were performed at pH 6 rather than 7. For a given concentration of DNP, $[A^-]$ will remain constant within a few per cent as the pH is lowered from 7 to 6 because the pK of DNP is about 4.0. The concentration of the neutral species [HA] however, will increase about an order of magnitude and $[HA_2^-]$ should do likewise. It was observed experimentally that the effect of DNP on the nonactin-K^+ con-

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[8] We know that the increase in conductance produced by DNP on the nonactin-treated membrane is due to increased K^+ conductance for two reasons. First, the membrane remains permselective to K^+ ions in the presence of DNP, a 10-fold gradient of potassium producing a 59 mV potential. Second, doubling the nonactin concentration doubles the conductance, as predicted by Eq. (10).

[9] This curve agrees with that previously obtained by Liberman *et al.* (1968) at neutral pH on what was presumably a lecithin bilayer.

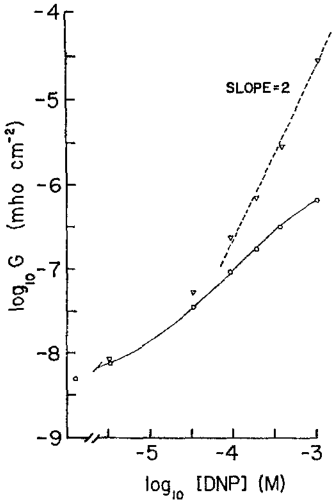

Fig. 2. *Lower:* The effect of DNP on the conductance of a bilayer membrane formed from the neutral lipid PE; data from Fig. 1. *Upper:* Data "corrected" for the effect of DNP on the surface potential of the bilayer membrane. Each data point is multiplied by the factor exp $(-F\psi/RT)$, as determined from the upper curve in Fig. 1. Note that the curve now has a slope of 2 on the log-log plot, consistent with the hypothesis the permeant species is the HA_2^- complex

ductance was identical at pH 6 and 7, an observation consistent with the notion that it is the A^- rather than the HA_2^- species which is adsorbing to the membrane[10].

Discussion

If it is accepted that the increase in the nonactin-K^+ conductance produced by the anion of DNP is a valid measure of the change in the surface potential of the bilayer, all of the data in the literature become qualitatively consistent with the carrier model and may be described by a combination of Eq. (7), the appropriate equation from the theory of the diffuse double layer [Eq. (9)] and an adsorption isotherm. It is not necessary to postulate that

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[10] For a concentration of DNP greater than 5×10^{-5} M, the conductance was found to be an order of magnitude higher at pH 6 than 7, in agreement with both previous work (e.g., Lea & Croghan, 1969; Hopfer *et al.*, 1970) and the prediction of Eq. (7). When the experimental data collected at pH 6 were "corrected" for the surface potential effects, a quadratic relationship again resulted, which extended to even lower values of [DNP] than illustrated in Fig. 2. This minor feature of the data was presumably due to the finite conductance of the A^- species being relatively less important at pH 6 than 7 (*see* Footnote 1).

the association of the anion of DNP with the bilayer involves covalent, or even specific ion pair formation. Rather, it seems more likely that it entails merely the nonspecific adsorption of an amphipathetic substance onto an oil-water interface, which apparently also occurs with negatively charged dye ANS (Lesslauer, Cain & Blasie, 1971). Pertinent to this argument is the observation that the effects of ANS on the electrical properties of bilayer membranes appear to be similar in *all* respects to those of DNP; they are simply manifest at lower concentrations. Specifically, ANS produces a negative surface potential on a PE bilayer at about an order of magnitude lower concentration that does DNP (McLaughlin *et al.*, 1971, *see* Fig. 6). Furthermore, if the conductance produced by ANS is "corrected" for the surface potential it produces, the dependence of G on [ANS] becomes quadratic, as in the case of DNP.

It is considered unlikely that the other uncouplers investigated by Liberman *et al.* (1968) adsorb significantly to neutral bilayer membranes because, with the exception of salicylate, their conductance *vs.* concentration curves are all approximately quadratic. Salicylate, as expected, did increase the nonactin-K^+ conductance of a PE bilayer when present at greater than millimolar concentrations (McLaughlin, 1972). Quite apart from any uncoupling action salicylate may exert on biological systems, it seems likely that its ability to adsorb to a phospholipid bilayer may explain the observation by Barker and Levitan (1971) that salicylate increases the K^+ and decreases the Cl^- permeability of a molluscan nerve when present at greater than millimolar concentrations.

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References

Barker, J. L., Levitan, H. 1971. Salicylate: effect on membrane permeability of molluscan neurons. *Science* **172**:1245.

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

Bruner, L. J. 1970. Blocking phenomena and charge transport through membranes. *Biophysik* **6**:241.

Fettiplace, R., Andrews, D. M., Haydon, D. A. 1971. The thickness, composition and structure of some lipid bilayers and natural membranes. *J. Membrane Biol.* **5**:277.

Finkelstein, A. 1970. Weak-acid uncouplers of oxidative phosphorylation. Mechanism of action on thin lipid membranes. *Biochim. Biophys. Acta* **205**:1.

Hall, J. E., Mead, C. A. 1972. The experimental energy profile of a carrier-ion complex in a black lipid film. *Biophys. Soc. Abs.* **44***a*.

Hopfer, U., Lehninger, A. L., Lennarz, W. J. 1970. The effect of the polar moiety of lipids on bilayer conductance induced by uncouplers of oxidative phosphorylation. *J. Membrane Biol.* **3**:142.

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

Läuger, P. 1970. Ion transport across lipid bilayer membranes. *In:* Physical Principles of Biological Membranes. F. Snell, J. Wolken, G. Iverson, and J. Lam, editors. p. 227. Gordon and Breach, New York.

Lea, E. J. A., Croghan, P. C. 1969. The effect of 2,4-dinitrophenol on the properties of thin phospholipid films. *J. Membrane Biol.* **1**:225.

LeBlanc, O. H. 1971. The effect of uncouplers of oxidative phosphorylation on lipid bilayer membranes: Carbonylcyanide *m*-chlorophenylhydrazone. *J. Membrane Biol.* **4**:227.

Lesslauer, W., Cain, J., Blasie, J. W. 1971. The location of 1-anilino-8-naphthalenesulfonate in lipid model systems. *Biochim. Biophys. Acta* **241**:547.

Liberman, E. A., Babakov, A. V. 1968. Diminishing characteristics and impedance of phospholipid membranes in presence of tetrachlorotrifluorobenzimidazole. *Biofizika* **13**:362.

Liberman, E. A., Mochova, E. N., Skulachev, V. P., Topaly, V. P. 1968. Effect of uncoupling agents of oxidative phosphorylation on bimolecular phospholipid membranes. *Biofizika* **13**:188.

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

Liberman, E. A., Topaly, V. P. 1968*b*. Transfer of ions across bimolecular membranes and classification of uncouplers of oxidative phosphorylation. *Biofizika* **13**:1025.

MacDonald, R. C., Bangham, A. D. 1972. Comparison of double layer potentials in lipid monolayers and lipid bilayer membranes. *J. Membrane Biol.* **7**:29.

McLaughlin, S. 1972. Local anaesthetics, salicylate and the surface potential of bilayer membranes. *Biophys. Soc. Abs.* **181***a*.

McLaughlin, S. G. A., Szabo, G., Eisenman, G. 1971. Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.* **58**:667.

McLaughlin, S. A. G., Szabo, G., Eisenman, G., Ciani, S. M. 1970. Surface charge and the conductance of phospholipid membranes. *Proc. Nat. Acad. Sci.* **67**:1268.

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

Neumcke, B. 1971. Diffusion polarization at lipid bilayer membranes in the presence of a homogeneous chemical reaction in the solutions. *T.I.T. Life Sci.* **1**:85.

Neumcke, B., Läuger, P. 1970. Space charge-limited conductance in lipid bilayer membranes. *J. Membrane Biol.* **3**:54.

Skulachev, V. P., Sharaf, A. A., Liberman, E. A. 1967. Proton conductors in the respiratory chain and artificial membranes. *Nature* **216**:718.

Stark, G., Benz, R. 1971. The transport of potassium through lipid bilayer membranes by the neutral carriers valinomycin and monactin. *J. Membrane Biol.* **5**:133.

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.