THE EFFECT OF 2,4-DINITROPHENOL ON THE ELECTRICAL RESISTANCE OF PHOSPHOLIPID BILAYER MEMBRANES

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Lipid bilayer membranes can be formed in apertures separating two aqueous phases (Mueller, Rudin, Ti Tien, and Westcott, 1962 a,b, 1963; Huang, Wheeldon, and Thompson, 1964; Huang and Thompson, 1965; Seufert, 1965; Huang and Thompson, 1966 a,b; Tien, 1966). The thickness, high electrical resistance and capacitance, and high water permeability of such bilayers closely resemble those of natural biological membranes (Thompson, 1964).

This communication reports the use of such phospholipid bilayer systems to test one of the elements of the chemiosmotic coupling hypothesis for oxidative and photosynthetic phosphorylation proposed by Mitchell (1961; 1966). Mitchell has postulated that uncoupling agents such as 2,4-dinitrophenol are not inhibitors of specific enzymatic reactions per se, but are lipid-soluble proton donor-acceptor systems that accumulate in the lipid phase of the H^+-impermeable mitochondrial membrane and prevent respiration-dependent formation of pH gradients across the membrane by acting as H^+ carriers. The effect of 2,4-dinitrophenol on the electrical resistance of phospholipid bilayer systems is described in this paper.

Methods and Materials. The bilayer membranes were formed as described elsewhere (Huang et al., 1964) in a 1.5 mm aperture in a thin Plexiglass septum separating two compartments containing 0.1 M NaCl. The membranes were generated at 36 ± 0.05 C° from a solution of purified egg phosphatidylcholine (5.0 mg), cholesterol (2.5 mg), and n-decane (2.0 ml). This system is similar to that described by Hanai et al., (1965). Bilayer electrical resistance was measured with an electrometer amplifier (type 201-C, E-H Research Laboratories) and a simple potentiometer circuit. Silver chloride electrodes with a saturated KCl bridge were employed.

Results. Data in Table 1 show the mean and range of the specific electrical resistance of control phospholipid bilayers measured at an applied potential of 20 mV. It is seen that the mean

Table I

The effect of DNP concentration on the specific resistance of lipid bilayers

2,4-dinitrophenol mM	Number of bilayers	Specific resistance (Ω cm^2) Mean	Range
0	20	1.43×10^7	$(0.62 - 2.15) \times 10^7$
1.0 ± 0.2	10	6.1×10^4	$(4.3 - 8.2) \times 10^4$

values for twenty membranes at or near equilibrium was 1.4×10^7 Ω cm^2. When the membranes were generated in identical systems containing 1.0 mM 2,4-dinitrophenol, there was a very large decrease in the electrical resistance. The mean value for 10 membranes generated in this manner was about 6.1×10^4 Ω cm^2, or

about 0.4% of the mean value for normal membranes. Approximately the same values for the electrical resistance were observed when 1.0 mM 2,4-dinitrophenol was added to both compartments after the membrane was formed. The large decrease in electrical resistance in the presence of 2,4-dinitrophenol apparently occurred very rapidly, since low resistance values were observed in the first measurements made after addition of 2,4-dinitrophenol ($\lesssim$60 sec).

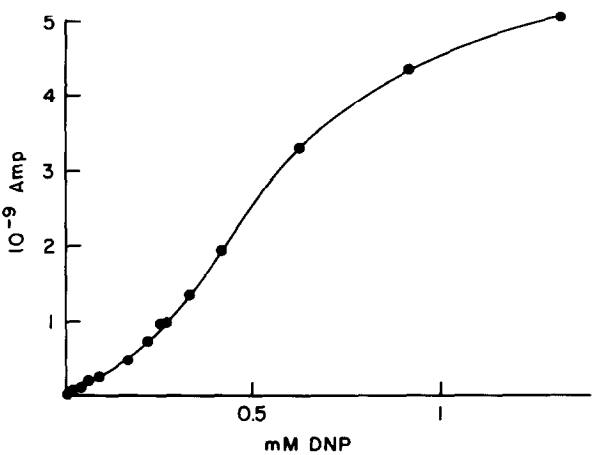

Fig. 1. The effect of concentration of 2,4-dinitrophenol on the resistance. The current passed at 20 mV applied voltage was measured.

Data collected in Fig. 1 show the effect of varying the concentration of 2,4-dinitrophenol on the resistance of a typical membrane which was generated in the absence of 2,4-dinitrophenol. Symmetrical additions of increasing amounts of 2,4-dinitrophenol were then made to both compartments. The current passed was found to increase in a non-linear manner with increasing concen-

trations of 2,4-dinitrophenol, up to about 1.0 mM. A half-maximal effect on the bilayer membrane was given by about 0.5mM and maximal by about 1.0mM. For comparison, data of Parker (1965) indicate that maximal stimulation of respiration in rat liver mitochondria is given by about 30 μM 2,4-dinitrophenol. However these differences are not unexpected and may be due to the differing solubility (cf. Hemker (1962); Parker (1965)) of 2,4-dinitrophenol in the synthetic bilayer and natural mitochondrial membranes, which differ considerably in lipid composition (Thompson, 1964), as well as the fact that much of the DNP added to the aqueous medium dissolves in the annular torus of lipid solution surrounding the aperture.

When 2,4-dinitrophenol in the aqueous phase exceeded about 1.0 mM, the electrical resistance of the bilayer membrane increased again significantly. This effect is reminiscent of the biphasic action of 2,4-dinitrophenol and other uncoupling agents on mitochondrial respiration (cf. Hemker, 1962); at low concentrations such agents show classical uncoupling and respiration-stimulating activity, whereas at higher concentrations they inhibit respiration

Discussion. It is not possible to determine from the data obtained to date in what manner the 2,4-dinitrophenol produces the large decrease in electrical resistance of phospholipid bilayers. It may cause an increase in the transport of protons alone, as Mitchell postulated. However, it is also possible that the 2,4-dinitrophenol causes an increase in the rate of transport of other cations such as Na^+, or even of anions. Appropriate experiments on the effect of 2,4-dinitrophenol on the transference numbers of H^+, Na^+, and Cl^- ions are under way. Similar large decreases in bilayer resistance have been observed when low

concentrations of cationic detergents are added to the aqueous phase (Seufert, 1965).

The mean specific resistance of about $6.1 \times 10^4 \Omega\ cm^2$ observed in the presence of 1.0 mM 2,4-dinitrophenol can be used to calculate the electrochemically equivalent flux of protons per unit area of membrane at any given potential gradient, assuming no other electrolyte passes the membrane. This would amount to a flux of about 1.4×10^{14} protons $sec^{-1}\ cm^{-1}$ at a potential difference of 1.0 volt, or about 3.5×10^{13} protons $sec^{-1}\ cm^{-1}$ at a potential of 250 mV, which Mitchell (1966) has suggested to be the actual potential across the mitochondrial membrane. It is of interest to compare the magnitude of the proton flux carried by dinitrophenol with the flux of protons potentially generated by electron transport, if it is assumed that the Mitchell hypothesis for H^+ pumping during electron transport is correct. From the known rate of oxygen uptake by liver mitochondria in the presence of succinate (State 3) of about 210 μatoms oxygen min^{-1} gm^{-1} protein, and the calculated area of the inner membrane of rat liver mitochondria of about 40 $m^2\ gm^{-1}$ protein (Mitchell, 1966, see p. 104), it can be calculated that the rate of H^+ ejection during succinate oxidation per unit area of inner mitochondrial membrane, assuming that 4 H^+ are transferred from the internal to the external compartments per atom of oxygen reduced (Rossi, et al., 1966), is about 2.4×10^{13} protons $sec^{-1}\ cm^{-2}$. It is clear that 1.0 mM 2,4-dinitrophenol acting on the lipid bilayer can cause charges to flow through the membrane at a rate somewhat greater than the calculated rate of ejection of H^+

during electron transport. This effect of 2,4-dinitrophenol is thus of the proper order of magnitude to be consistent with Mitchell's explanation of the mechanism of action of uncoupling agents.

Summary. The uncoupling agent 2,4-dinitrophenol at a concentration of 1.0mM decreased electrical resistance of synthetic lipid bilayers separating two aqueous components containing 0.1 M NaCl, to less than 0.4 per cent the control values. This observation is qualitatively and quantitatively consistent with Mitchell's hypothesis that uncoupling agents for oxidative phosphorylation act primarily to cause transfer of protons across the H^+-impermeable mitochondrial membrane.

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