THE EFFECT OF DINITROPHENOL ON THE PERMEABILITY OF THE MITOCHONDRIAL MEMBRANE

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According to the chemiosmotic hypothesis of energy-coupling in oxidative phosphorylation, uncoupling agents would short-circuit the mitochondrial membrane for H^+ ions, thus collapsing the pH gradient which is visualized to be the driving force for the energy conservation (Mitchell, 1966). Bielawski et al. (1966) have shown that dinitrophenol (DNP) lowers the electrical resistance of lipid bilayer membrane models. The demonstration that uncouplers can induce a permeabilization of isolated mitochondria to H^+ ions would be an important argument for assessing the validity of the chemiosmotic theory; however, unequivocal evidence is still lacking although some circumstantial evidence has been reported (Mitchell, 1966).

In the present communication, we report experiments with the classical uncoupler DNP that are relevant to this problem. They strongly indicate that DNP affects the permeability of isolated rat liver mitochondria; however, its effect is more complex than a simple equilibration of H^+ ions between the intra- and the extra-mitochondrial compartments.

METHODS

Mitochondria were prepared from the livers of Wistar strain albino rats by the standard sucrose procedure of Schneider (1957). The movements of H^+ and K^+ were monitored by Beckman glass electrodes coupled with a Beckman Expandomatic pH meter, equipped with a Texas Instruments multichannel recorder. The absolute amounts of H^+ and K^+ were determined with internal standards of HCl or KCl. Alternatively, K^+ was measured by flame photometry, as described in the legend to Figure 3. All the reagents used were of analytical grade.

RESULTS AND DISCUSSION

A mitochondrial suspension is added to a medium containing choline chloride (or sodium chloride) and buffered with Tris chloride or imidazole chloride. After some 50-60 seconds the pH trace reaches a stable steady-state; the addition of DNP after the steady-state has been reached causes an absorption of H^+ by mitochondria. The amount of H^+ absorbed decreases as the pH of the medium increases, as shown in Figure 1. However, at no pH, not even at 8.5 or above, does DNP cause a deflection of the pH trace

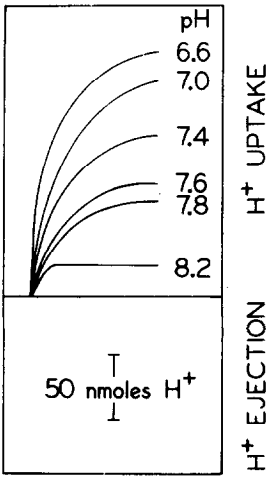

Fig. 1. Effect of DNP on the uptake of H^+ by mitochondria, at various pH's of external medium. To 3.8 ml system containing 100 mM choline-Cl and 3 mM Tris-Cl, at the pH's indicated, 5.4 mg mitochondrial protein were added. After equilibration, the reaction was started by adding 10^{-4} M DNP. Temperature 24° C.

in the direction of the ejection of H^+ from mitochondria, in contrast with what one would expect if DNP acts to equilibrate H^+ across the membrane in both directions. It can therefore be concluded that DNP affects the permeability of the mitochondrial membrane to H^+ only at acid pH. On the other hand, detergents such as Lubrol render mitochondrial dissociating groups accessible to quantitative titration (Rossi et al., 1966; Gear et al., 1967).

The experiments with phospholipid micelles reported by Chappell and Haarhoff (1966) and those on pH differentials created across the mitochondrial membrane by HCl

described by Mitchell (1966), have suggested that DNP may act cooperatively with valinomycin, and that the movements of H^+ and K^+ induced by these two agents are dependent on each other. On the basis of these findings, sequential additions of DNP and valinomycin were therefore tested, and the movements of both H^+ and K^+ recorded simultaneously, as shown in Figure 2. At pH 8.02, addition of valinomycin or DNP singly

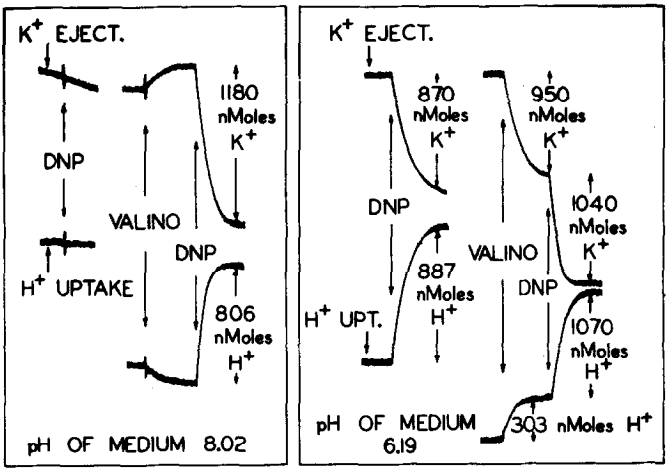

Fig. 2. Effect of DNP and valinomycin on H^+ and K^+ movements in isolated mitochondria. A: to a 3.8 ml system containing 100 mM choline-Cl buffered at pH 8.02 with imidazole-Cl, 10.3 mg mitochondrial protein were added. After 60 seconds, the first additions indicated were made. DNP was 10^{-4}M, valinomycin 10^{-8}M. B: details as in A, except that the pH of the medium was 6.19.

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has no detectable effect on the traces of H^+ and K^+. However, DNP added after valinomycin to the mitochondria, or valinomycin added after DNP, induces a large uptake of H^+ and a large output of K^+. At pH 6.19 on the other hand, DNP alone or valinomycin alone induced uptake of H^+ and ejection of K^+; their sequential addition produced no "extra" effect. These results therefore support the conclusion that DNP can let H^+ penetrate into the mitochondria provided K^+ can leave them in exchange. They also suggest that the mitochondrial membrane, which is known to be impermeable to K^+ at neutral pH, is freely permeable to it at acid pH; for this reason valinomycin is required at alkaline pH, and not at acid pH. Direct proof of the permeability of mitochondrial membrane towards K^+ at acid pH is shown in the experiment reported in Figure 3.

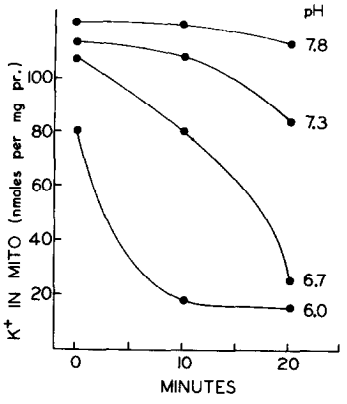

Fig. 3. K^+ release from mitochondria as function of the pH of external medium. Mitochondria were incubated at 25°C in 0.25 M sucrose buffered with imidazole-Cl at the pH indicated. At the times shown in the figure, aliquots of the complete system were centrifuged for 45 sec. at 16,000 rpm in a Beckman Microfuge. The pellets were resuspended in 5% TCA, containing 14.42 meq. Li_2SO_4 per liter. The precipitated protein was discarded by centrifugation, and the supernatants were analyzed for K^+ by flame photometry.

Mitochondria were suspended at 25°C in 0.25 M sucrose buffered at various pH's. At the times indicated, mitochondria were collected and analyzed for K^+ by emission photometry. The data show that endogenous K^+ leaks out rapidly from mitochondria at pH 6.0, whereas at pH 7.8 it is retained for at least 20 minutes. It may be concluded that DNP does not simply equilibrate protons across the mitochondrial membrane, rather it promotes an exchange between extramitochondrial H^+ and intramitochondrial K^+. Such an exchange can take place only if the membrane is permeable to K^+, a condition that exists at low pH (=6.0) in the absence of valinomycin, and which can be induced by valinomycin at higher pH zones.

Although it has been assumed in this paper that the events described represent transmembrane processes, i.e. permeability, it is of course entirely possible that they reflect exchange mechanisms at the membrane surface; such a possibility is currently under investigation in our laboratories. Detailed reports on this, as well as a comparative study of different uncouplers and of K^+/H^+ stoichiometry, will be published elsewhere.

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