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Permeability of Housefly Flight-Muscle Mitochondria for Succinate

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It has been proposed that mitochondria from the flight muscle of the housefly (Van den Bergh & Slater, 1962) and blowfly (Sacktor & Childress, 1967) are impermeable to intermediates of the tricarboxylic acid cycle. We have found, however, that a very rapid oxidation of added succinate occurs in the absence of phosphate (about 450 ng-atoms of O/min per mg of protein). This result suggests that, in the absence of phosphate, succinate can permeate the mitochondrial membrane. The properties of this movement of succinate will be discussed.

The oxidation of succinate is loosely coupled, since no stimulation of oxidation by 2,4-dinitrophenol is observed in the absence of phosphate acceptor.

The oxidation of succinate is strongly inhibited by small amounts of phosphate (e.g. 86% inhibition by 3 mM-phosphate in the presence of 60 mM-succinate). It was found that the phosphate concentration inside the mitochondrion was lower when succinate oxidation was rapid than when it was inhibited.

The inhibition by phosphate could be overcome by mercurials (Tyler, 1968; Fonyo, 1968) such as mersalyl. This effect was dependent on the concentration of mersalyl. Low concentrations caused a temporary release of the phosphate-inhibited oxidation. A second addition of mersalyl restored the rapid oxidation after the inhibition had set in. Higher concentrations (>60 nmol/mg of protein) caused an irreversible inhibition after a temporary stimulation.

Since mersalyl inhibits, not only the movement of phosphate across the mitochondrial membrane (Fonyo, 1968; Tyler, 1968), but also the dicarboxylate-phosphate exchange (Meijer & Tager, 1969), it is suggested that the mercurial is able to overcome the phosphate inhibition of succinate oxidation by enhancing the net movement of dicarboxylate ions (succinate, fumarate and malate) across the mitochondrial membrane.

A Substoichiometric Assay for the Determination of Free Adenosine Triphosphate in the Presence of Reversibly Bound Adenosine Triphosphate

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The usual methods for determining ATP concentration suffer from the disadvantage that they measure total ATP and do not distinguish between free and reversibly bound ATP. A possible method for providing such a distinction was suggested from the enzymic-isotope-dilution principle developed by Newsholme & Taylor (1968). Assays based on this principle are substoichiometric: an enzyme converts only a small fraction (10–20%) of radioactive substrate into product and, as this is sufficient for determination of the substrate concentration, the conversion may not seriously disturb an equilibrium between free and bound substrate.

Glycerol kinase was selected for the assay of ATP by such a method because it obeys the enzymic-isotope-dilution principle for glycerol (Newsholme & Taylor, 1968). The assay medium was as described previously except it contained 0.9 mM-glycerol, 0.25 M-sucrose, 3.3 mM-Mg²⁺ and 0.13 mM-[¹⁴C]ATP (specific radioactivity 15.6 μ Ci/ μ mol) as final concentrations. A sample (100 μ l) of this was incubated with 20 μ l (5 μ g) of glycerol kinase for 15 s at 0°C; the reaction was stopped by addition of 100 μ l of ethanol containing 5 mM-ATP, 5 mM-ADP, 10 mM-EDTA and 10 mM-ethanedioxybis(ethylamine)tetra-acetate. T.l.c. on silica-gel-coated plastic sheets was used to separate ADP from ATP; the separated spots were cut out and radioactivity was measured as described previously.

Addition of 6, 12, 18, 24 and 36 nmol of unlabelled ATP (S) to the above system caused a decrease in the incorporation of radioactivity into ADP so that C_0/C_s ratios were 1.20, 1.40, 1.64, 1.85 and 2.30 for the above concentrations respectively. A plot of C_0/C_s against [S] is linear and C_0/C_s is unity when [S] is zero; these results are in agreement with the enzymic-isotope-dilution principle.