Competition between Anions in Mitochondria and Evidence for Compartmentation

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The mitochondrial uptake of anions from the medium depends on four factors besides the applied concentrations; these are (1) the mitochondrial K^+ content, which limits their maximum anion capacity, (2) the presence of a store of anions ready to exchange, which is lost or becomes unavailable during energy depletion, (3) anion interactions and (4) the rate of metabolic consumption of the substance. Under conditions in which metabolism is inhibited the competition or facilitation of anions can be studied. Facilitation of citrate or oxoglutarate uptake by malate (Chappell, 1964) is largely taken care of by endogenous substrate when undepleted mitochondria are used. This leaves the interanion competition to be evaluated. Generally, more charged anions are better competitors than less charged anions, providing that the latter do not also discharge energy as, for example, is the case with 2,4-dinitrophenol. However, β -hydroxybutyrate resists competition from malate, citrate, phosphate and malonate, though succinate has some effect (K_1 6.8mM). β -Hydroxybutyrate does not affect malate oxidation though it slightly inhibits succinate oxidation (K_1 15.5mM). It appears that β -hydroxybutyrate occupies a compartment separate from the locus of accumulation of the malate group though it admits succinate. Also, more malate or phosphate is necessary to halve succinate uptake than the succinate or phosphate required to halve malate uptake (Harris & Manger, 1968). It appears as if one succinate pathway or compartment is not involved with members of the malate group, whereas a limited interaction occurs in another pathway or compartment. A possible conclusion from the results is that succinate enters both the ' β -hydroxybutyrate' compartment and the 'malate' (citrate, phosphate, malonate) compartment, but the β -hydroxybutyrate cannot enter the latter compartment and the malate group cannot enter the first compartment. Other evidence that there is an exclusion of β -hydroxybutyrate from the 'malate' compartment is obtained from experiments in which valinomycin is used to induce a massive K^+ uptake. Whereas most substrate anions are taken up along with K^+ , β -hydroxybutyrate is not.

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Chappell, J. B. (1964). *Biochem. J.* **90**, 225.

Harris, E. J. & Manger, J. R. (1968). *Biochem. J.* **109**, 239.

A Ubiquinone-Mediated Pathway of Electron Transport in an Antimycin-Inhibited Heart-Muscle Preparation

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Solvent-extraction procedures have shown that removal of the endogenous ubiquinone from respiratory-enzyme particles leads to the loss of succinate and NADH oxidase activities.

Succinate oxidase activity may be restored by the addition of ubiquinone homologues. Ambe & Crane (1960), using the succinate dehydrogenase complex, demonstrated an antimycin-insensitive stimulation of succinate-cytochrome *c* reductase activity by short-chain ubiquinones. Ambe & Crane (1960) suggested an electron-bypass mechanism of electron flow around the antimycin-sensitive site to account for these findings.

More recently, work by Takemori & King (1964) on the effects of coenzyme Q-2 (i.e. ubiquinone with two isoprene units/mol.) on antimycin-inhibited succinate-cytochrome *c* reductase particle has been interpreted by Reiske (1967) in terms of the displacement of the inhibitor by the added quinone and not as the result of an electron-bypass mechanism.

The antimycin sensitivity of the electron-transport process was investigated with an ox heart-muscle preparation. It was found that the succinate oxidase activity of acetone-extracted ox heart-muscle preparation could be restored by the addition of ubiquinone homologues.

Where short-chain ubiquinone homologues were used to restore succinate oxidase activity, the reconstituted system was found to be no longer fully inhibited by antimycin.

When the acetone-extracted preparation was preincubated with ubiquinone homologues and then washed free of unbound quinone, the succinate oxidase of the reconstituted particle was found to be completely inhibited by antimycin.

The coenzyme Q-0 and methyl-coenzyme Q-0-mediated antimycin-insensitive rate of succinate oxidation was found to be the same in both extracted and unextracted heart-muscle preparations. This suggests that this inhibitor-insensitive pathway of electron flow does not involve the endogenous ubiquinone site of the respiratory chain.