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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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.

The electron-bypass and displacement concepts were investigated by measuring the sensitivity of an acetone-extracted heart-muscle preparation at different concentrations of inhibitors and in the presence of various concentrations of quinone.

Succinate oxidase activity of acetone-extracted heart-muscle preparation reconstituted with coenzyme Q-0 or methyl-coenzyme Q-0 was not completely abolished even by high concentrations of antimycin. High antimycin concentrations would, however, completely abolish coenzyme Q-2- or Q-3-stimulated succinate oxidase of acetone-extracted heart-muscle preparation. The pattern of inhibition appears consistent with the operation of an electron-bypass mechanism in coenzyme Q-0- and methyl-coenzyme Q-0-mediated electron transport and of an inhibitor-displacement mechanism of succinate oxidase stimulation in preparations treated with coenzyme Q-2 or Q-3.

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Inhibitory Effects of Uncoupling Agents on Systems I and II of Photosynthetic Electron Transport in Chloroplasts

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The materials carbonyl cyanide *m*-chlorophenyl-hydrazone (CCCP), dicoumarol, pentachlorophenol and 2,4-dinitrophenol have been reported to uncouple photophosphorylation and to inhibit the Hill reaction in chloroplasts, at similar concentrations (de Kiewiet, Hall & Jenner, 1965; Jagendorf & Neumann, 1965; Krogman, Jagendorf & Avron, 1959; Neumann & Jagendorf, 1964). Their effects on system I are more difficult to observe, since the absorption by the reagents themselves masks the reduction of NADP in the spectrophotometer. The following method makes use of the reduction of methyl viologen, observed by Arnon (1963).

Air-tight stoppered cuvettes contained broken chloroplasts equivalent to 0.05 mg. of chlorophyll, 1.5 μ moles of methyl viologen, 30 μ moles of cysteine and 0.3 μ mole of 2,6-dichlorophenol-indophenol in 3 ml. of 0.04 M-phosphate buffer, pH 6.8. They were illuminated in saturating red light for 20 sec. periods, and read in the spectrophotometer at 607 m μ against unilluminated controls. For system II, a Clark oxygen electrode was used to measure the Hill reaction with 2 mM-K₃Fe(CN)₆-1 mM-KCl, containing 0.05 mg. of chlorophyll in 3 ml. of the same buffer.

*K'*_i values for CCCP, dicoumarol, pentachlorophenol and dinitrophenol in the Hill reaction were

8.9, 20, 1.7 and 40 μ M respectively. In system I, the value for CCCP was 260 μ M, and no inhibition was observed with dicoumarol up to the limit of solubility, about 100 μ M at pH 7.1. Pentachlorophenol and dinitrophenol both caused a decrease in rate of some 40% at about 10 μ M, but this lower rate was not inhibited further at concentrations up to 0.5 mM. Since dinitrophenol can be reduced by system I (Wessels, 1960) the inhibition may be overestimated.

It is unlikely that these agents are acting on pigment system II, since system I would be thought similar, and it is hardly affected. There may be a specific inhibition of a reaction near system II that has no counterpart in system I. It is also possible that the action on system II is the same phenomenon as the uncoupling of phosphorylation on the hypothesis of Mitchell (1966), namely the destruction of some pH gradient or electrochemical gradient on which the Hill reaction might depend.

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The Mechanism of the Phosphoglycerate Mutase Reaction

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Muscle phosphoglycerate mutase was thought to catalyse the intermolecular transfer of phosphate from 2,3-diphosphoglycerate (2,3-diPGA) to 2- and 3-phosphoglycerate (2-PGA and 3-PGA), but Grisolia & Cleland (1968), who studied the effect of 2,3-diPGA concentration on the reaction rate proposed that the mechanism involved a phosphoenzyme as in the phosphoglucomutase reaction. However, such kinetic observations are compatible with both mechanisms (Britton & Clarke, 1968) and a phosphoenzyme has not been isolated. To distinguish between the mechanisms, induced-transport tests have been carried out (Britton & Clarke, 1968). These tests depend on the fact that when one substrate is converted into the other by the intermolecular transfer of phosphate from