UNCOUPLING OF OXIDATIVE PHOSPHORYLATION: ENERGY-DEPENDENT ANION TRANSFER, DISCHARGE OF MEMBRANE POTENTIAL OR ELECTROPHILIC ATTACK ON COUPLING INTERMEDIATE?

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The phenomenon of uncoupling of oxidative phosphorylation consists of switching off electron transfer from energy-conservation reactions. Several hypotheses have been suggested to explain the mechanism of uncoupling. The following three possibilities have been widely discussed recently.

1. Uncoupling consists of utilization of energy of oxidation for active transport of uncoupler anion across the mitochondrial membrane [1, 2].

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Abbreviations: CCCP, *m*-chlorocarbonylcyanide phenylhydrazone; FCCP, *p*-trifluororomethoxycarbonylcyanide phenylhydrazone; TMPD, N,N,N,'-tetramethyl-*p*-phenyldiamine; TTFB, tetrachlorotrifluoromethy-l benzimidazole.

2. Uncoupling is due to « short circuiting » between two membrane surfaces, *i.e.* to discharge of the membrane potential induced by the respiratory chain [3].

3. Uncoupling is the result of direct interaction between uncouplers and intermediates of an energy-conservation mechanism; the action of uncoupler is that of hydrolysing a high-energy precursor of ATP or of preventing its formation (for review, see refs. 4, 5).

The first of the above mentioned conceptions suggests that an uncoupler anion ($\varnothing^-$) is transferred into mitochondria by some lipid-soluble anion carrier localized in the mitochondrial membrane. It is postulated that this process is energy-linked. Inside the mitochondria $\varnothing^-$ combines with H^+ and diffuses passively outside as an uncharged lipid-soluble component $\varnothing H$.

The « anion » conception of uncoupling does not explain the mechanism of the uncoupling effect of weak lipid-soluble bases, such as laurylamine [6], decylamine, tributylamine (see below). It will also be shown that uncoupling may be brought about by some alkylating agents whose activity, evidently, depends not on their ionic properties, but on the property of electrophilic substitution.

According to its proposers, the « anion » hypothesis of uncoupling [2] is borne out by the fact that there is competition between oxidizable substrate and uncoupler when mitochondrial respiration is inhibited by the high uncoupler concentration. We investigated this phenomenon and found out that it is of very complex nature. Fig. 1 shows the response of various oxidative systems to the addition of FCCP. It is seen that low concentrations of FCCP stimulate State 4 oxidation of succinate and reduced TMPD in mitochondria. Increasing FCCP concentration causes inhibition of respiration. A FCCP-stimulated portion of succinate oxidase activity is the first to go out, then follows a decrease in the reduced TMPD oxidation. Finally, complete inhibition of succinoxidase activity in mitochondria occurs. Still greater FCCP doses inhibit succinoxidase reconstituted from complexes II, III and IV and cytochrome *c*. Cytochrome *c* oxidase activity of complex IV is also decreased. With moderately high uncoupler doses it is possible to demonstrate the competitive character of the interrelationship between the substrate (succinate) and the uncoupler in the recon-