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The Action of a Mitochondrial Protein Fraction on Mitochondrial ATP-ase and Oxidative Phosphorylation

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A protein fraction has been extracted from rat liver mitochondria which stimulates "latent" mitochondrial ATP-ase activity without exhibiting ATP-ase itself and which stimulates the oxidation of succinate in a mitochondrial system containing AMP as the terminal phosphate acceptor. The fraction, in 0.1 M Tris (tris(hydroxymethyl)aminomethane), pH 7.4, has its spectroscopic peak in the Soret region at 414 mμ; in the visible region at 548 mμ and 576 mμ. The mitochondrial ATP-ase stimulating activity is proportional to the concentration of the fraction added.

The mitochondrial fraction (fraction 60) is prepared as follows. Rat liver mitochondria are prepared essentially according to Schneider and Hogeboom [1] using 0.25 M sucrose-0.001 M versene, pH 7.4, as the homogenizing medium. The mitochondrial pellet is washed twice in 0.25 M sucrose and then suspended in Na_2CO_3 which has been adjusted to pH 8.4 with HCl and diluted to 0.1 M. The suspension is then shaken for 12—18 hours at 0—4° C, followed by centrifugation in a Spinco Model L Centrifuge for 7 979 400 *g*-minutes (3 hours, 21 000 RPM, head $\neq$21). The resulting pinkish-yellow supernate is then adjusted to pH 6.5 with acetic acid. The slight cloudy precipitate which forms is centrifuged down. Chilled acetone is added to the supernate in a dropwise fashion with constant stirring to 10 % and maintained in an acetone-dry ice bath made by producing a thick slurry of 9 % acetone and powdered dry ice. The precipitate is spun down in an International Refrigerated Centrifuge at the temperature of the acetone-dry ice bath. Additional fractions are collected at 20, 30, 40 and 60 % acetone, adjusting the acetone-dry ice bath to 19, 29, 39 and 59 % acetone at each subsequent precipitation. The centrifuge is also adjusted accordingly until its lower limit is reached. The pink precipitate resulting from the 40—60 % fraction, called fraction 60, is completely soluble in Tris buffer, pH 7.4, resulting in a cherry red solution. From approximately 100 g of rat liver 10 ml of a solution with an optical density reading at 280 mμ (Beckman DU spectrophotometer) of 1.7 through a 1 cm path length have been obtained. Preliminary experiments indicate that fraction 60 may also be prepared by the above method from "acetone mitochondria" which have been stored *in vacuo* at —15° C for several weeks.

The preparation of a mitochondrial fraction with an action on mitochondrial ATP-ase processes provides a basis for hypotheses which implicate the fraction in oxidative phosphorylation. A protein factor, called mitochrome-I, has been reported briefly [2-4] which appears to have an action similar to fraction 60. Attempts to prepare mitochrome-I, however, have not been successful in our hands. Furthermore, since the only datum of a chemical nature reported for mitochrome-I is its approximate molecular weight, a direct comparison of the two factors has been impossible.

It must be added that fraction 60 is probably not homogeneous. Attempts are in progress to further purify the active principle of fraction 60 by zone electrophoresis and chromatography on cellulose ion exchange columns, as well as to gain some insight into its role in the transfer of high energy phosphate.

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