Identification of an Electron Transfer Particle from Rat Liver[1]

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Recent studies in our laboratory on changes in electron transport processes during experimental thyrotoxicosis (Moury, 1964) have led us to the isolation of a fraction with properties similar to the electron transfer particle (ETP) which has been isolated from beef heart by Crane et al. (1956). While it has been proposed that these particles from heart are outer membrane fragments of the mitochondria, essentially free of cristae (Green, 1962), attempts to isolate similar particles from liver mitochondria by methods applicable to heart mitochondria have previously been unsuccessful.

White male rats were sacrificed by decapitation and the liver removed, chilled in the homogenizing medium (0.25M sucrose-0.005M Ethylenediaminetetraacetic Acid (EDTA), pH = 7.4) and homogenized in a Potter-Elvejem homogenizer with a loosely fitting teflon pestle. Homogenates were filtered through a double layer of cheesecloth and centrifuged successively at 200 X g, 1,000 X g, 3,000 X g, 8,700 X g and 78,000 X g.

Reduced nicotinamide adenine dinucleotide (NADH)- and succinic-cytochrome c reductase activities were determined by modifications of assays described previously (Green et al., 1955; Mackler and Green, 1956). Cyto-

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chrome c oxidase activity was measured polarographically as described (Wharton and Griffith, 1962), and NADH and succinic oxidase activities were determined polarographically by modifications of previously described spectrophotometric assays (Green et al., 1955; Mackler and Green, 1956). Tetrachlorohydroquinone (TCHQ) oxidase was determined polarographically by a modification of the method of Jacobs and Crane (1961). All assays were performed at 38°C and the degree of "openess" of the NADH and succinic oxidase systems to exogenous cytochrome c was determined. Coenzyme Q (Co Q) was determined after the method of Crane and Dilley (1963) and protein by the biuret reaction (Gornall et al., 1949). Cytochrome a and combined cytochromes b, c and c_1 were estimated by difference spectra after reduction with sodium dithionite.

RESULTS AND DISCUSSION

When rat liver homogenates are centrifuged as described, the ETP sediments with the microsomes in the 78,000 X g fraction. The ETP of rat liver has relatively high succinoxidase and NADH oxidase activities which are unaffected by exogenous cytochrome c (Figures 1 and 2), and has low succinic-cytochrome c reductase and cytochrome c oxidase activities (Figure 3). These data are in excellent agreement with those obtained on beef heart ETP (Crane et al., 1956). J. M. Machinist, in our laboratory, has also shown that ETP from beef heart mitochondria possess a TCHQ oxidase system which has lost the protamine requirement as compared to heavy mitochondria (Jacobs and Crane, 1962). No protamine is required with rat liver ETP, whereas, the mitochondrial fraction shows a protamine requirement for maximum TCHQ oxidase activity (Figure 4). The NADH-cytochrome c reductase activity found in the 78,000 X g fraction (Figure 5) is not inhibited by antimycin A and is, therefore, microsomal rather than mitochondrial in nature.

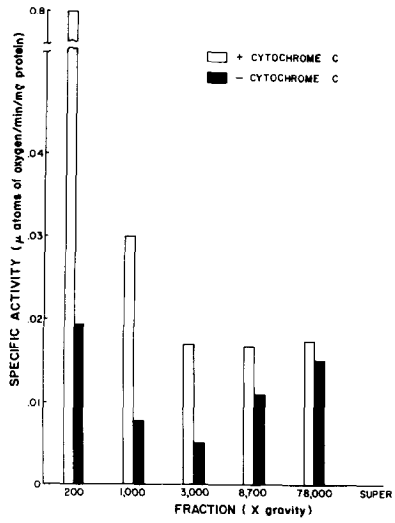

FIGURE 1

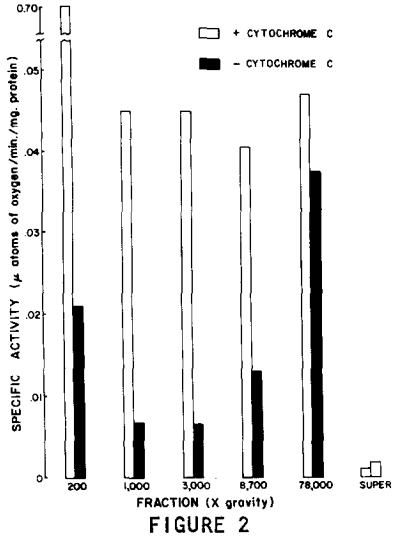

FIGURE 2

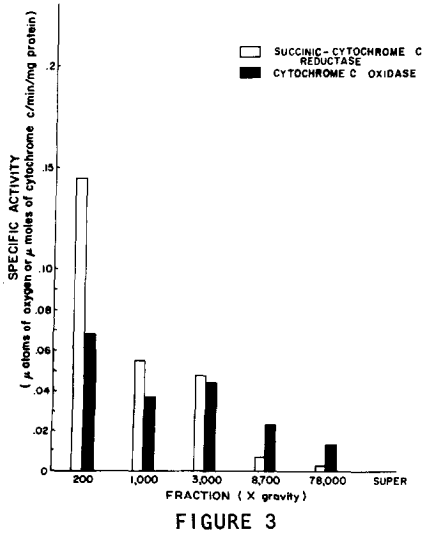

FIGURE 3

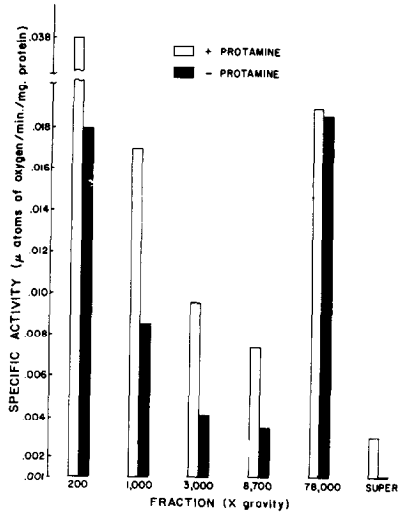

FIGURE 4

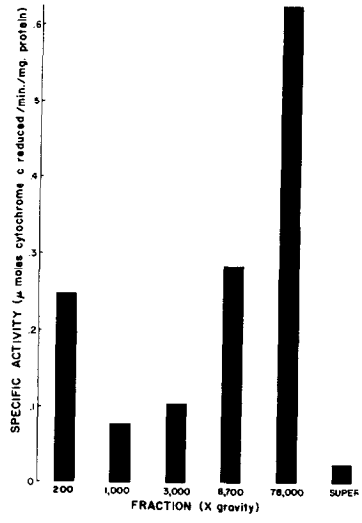

FIGURE 5

Distribution of the specific activities of several electron transport enzyme systems among fractions from rat liver. 1. - succinoxidase; 2. - NADH oxidase; 3. - Cytochrome c oxidase and succinic-cytochrome c reductase; 4. - Tetrachlorohydroquinone oxidase; 5. - NADH-cytochrome c reductase.

Table I shows that there is an enrichment of the cytochromes b, c, and c_1 and especially of cytochrome a in the 78,000 X g fraction in a manner comparable to the increase of succinoxidase in this fraction.

Fraction	Cytochrome a (595 mμ)	Cytochromes b, c and c_1 (555-7 mμ)
200 X g	.0070	.0085
1,000 X g	.0007	.0011
3,000 X g	.0005	.0014
8,700 X g	.0023	.0033
78,000 X g	.0027	.0047

Table I. Content of cytochrome a and cytochrome b, c and c_1 in fractions from rat liver. Values are expressed as change in optical density per mg of protein as determined by difference spectra after reduction with dithionite.

The lack of success in isolating ETP from liver by the same procedures used for the isolation of the ETP from heart presented a problem in the interpretation of the ETP as a fundamental structure since one would expect to be able to isolate them from all mitochondria. If the ETP is of mitochondrial origin then it appears that the greater fragility of liver mitochondria causes the ETP to be disengaged during mitochondrial preparation so that subsequent attempts to isolate ETP from these mitochondria prove futile.

Leonhauser et al., (1962) have previously demonstrated the existence of Co Q in the microsomal fraction of liver. It seems, therefore, that there should be a reevaluation of the amount of Co Q in microsomes since it has been shown that while the concentration of Co Q is the same in both heavy heart mitochondria and heart ETP (Crane et al., 1957), succinoxidase activity is four times greater in the ETP (Crane et al., 1956).

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