On the Relation of Mitochondrial ATPase to Diaphorase Flavin

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Recent studies ^{1,2} of the relationship between oxidative phosphorylation and 2,4-dinitrophenol (DNP) stimulated adenosinetriphosphatase (ATPase) have indicated that the mitochondrial enzyme sequence which can be stimulated by DNP to break down ATP includes one link which is a part of the electron transport chain, viz. the diaphorase flavoenzyme. Further support for this conclusion could be obtained by showing that atebtrin, a drug known to interfere with flavins probably in a competitive manner ³, stimulates the DNP-activated ATPase by about 40 % in low concentrations, while in higher concentrations it almost completely inhibits this reaction. Typical data are shown in Table 1. Due to a certain structural

Table 1. The effect of atebtrin and promazines on DNP-ATPase

Compounds	Concentration, mM	Relative activity %
None	—	100
Atebrin	1.5	149
Atebrin	8.0	11
Promazine	0.1	135
Promazine	0.5	11
Chloropromazine	0.05	142
Chloropromazine	0.2	15
Acetylpromazine	0.2	134
Acetylpromazine	1.0	6

Each incubation tube contained the following: 5 mM ATP, 5 mM tris buffer pH 7.5, 0.1 mM DNP and 0.25 M sucrose. Final volume 2.0 ml. The tubes were incubated at 30°C for 20 min.

relationship between atebtrin and promazine derivatives and to the fact that these are known to influence oxidative phosphorylation in isolated mitochondria ⁴ the effect of a series of promazine compounds was also tested. As is shown in Table 1 these gave an effect similar to that of atebtrin.

The significance of these findings will be discussed.

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The Potency of the Microsomal Material to Metabolize and Bind Carcinogens during Liver Regeneration

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As a result of partial hepatectomy pronounced modifications are induced in the microsomal material of liver cytoplasm ¹. After a lag period of about 12–14 h there is a fairly rapid increase of the RNA/protein ratio. About simultaneously the activity of the microsomes in amino acid incorporating systems shows a rapid increase. The culmination generally occurs about 30 h after the operation.

Concurrently with this activation other components of the microsomal material decrease. The activity of glucose-6-phosphatase, measured per mg microsomal protein, is reduced by approximately 15 %. The concentration of DPNH-cytochrome c reductase is reduced to approximately the same degree. Liver microsomes are capable of demethylating the carcinogenic azo dye methylaminoazobenzene in the presence of TPNH under aerobic conditions ². The capacity of the microsomal material for carrying out this oxygen transfer, is reduced during regeneration, usually by about 20 %. Under the same conditions of oxygen transfer, reactive metabolites of the azo dye are produced. When ¹⁴C-labeled azo compounds are used in the system, the isotope becomes bound to the microsomal proteins. Microsomes from regenerating liver have a considerably reduced capacity for producing reactive metabolites from ¹⁴C-labeled azo dyes. This effect is most easily demonstrated by adding soluble proteins to the microsomal systems metabolizing ¹⁴C-labeled carcinogenic azo compounds. Reactive metabolites are secondarily emitted from the microsomes, and became trapped by the soluble proteins. Subsequently the specific activities of the soluble proteins may be determined. Similar experiments have been carried out with 2-amino-fluorene-9-¹⁴C. In the regenerating livers, finally, the concentration of cytochrome b₅

(determined on deoxycholate extracts by dithionite reduction) is diminished by about 30%.

It is generally considered that the endoplasmic reticulum of the cytoplasm is the main origin of the microsomal material. Since RNA is concentrated to the nucleoprotein granules, which are attached to the delicate membranes of this network, while the enzymes and cytochrome b_5 apparently are associated with the membranes, the experiments suggest a richer attachment of nucleoprotein granules to the membranes during liver regeneration. Apparently, however, the composition of the membranes may become modified at the same time.

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On Submitochondrial Particles with High DPNH-oxidase* and Low Succinic Oxidase Activity

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A new method for obtaining submitochondrial particles capable of respiration and oxidative phosphorylation has been briefly reported¹. After grinding liver mitochondria from rat or guinea pig with Al_2O_3 and centrifuging the ground material at $25\,000 \times g$ for 10 min, in order to get down the alumina and heavier mitochondrial structural fragments the particles are in the supernatant. After centrifugation for 1 h at $100\,000 \times g$ practically all the DPNH-oxidase activity of this supernatant is in the sediment, which is then suspended in a suitable medium.

Two slightly different methods of preparation (I and II) have been used. In I the medium for the last centrifugation before grinding and for the subsequent operations is 30 mM phosphate buffer of pH 7.0, in II 0.25 M sucrose. Significant differences between the preparations so obtained are: II gives a several

* Abbreviations: DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ITP, inosine triphosphate; EDTA, ethylenediaminetetraacetate; M, moles per liter.

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fold higher final yield and the stimulation of its DPNH-oxidase by added cytochrome c is lower (usually less than 50 %, compared to more than 200 % for I). In II the supernatant after the $25\,000 \times g$ centrifugation consists of two separate layers, the bottom one being small in volume and red-colored.

2 mM amytal inhibits practically all the DPNH-oxidase activity in the absence of added cytochrome c, which eliminates the possibility that a significant part of this respiration is due to microsomal contamination.

The submitochondrial particles have a high DPNH-oxidase activity compared to their succinic oxidase activity¹. Also, the DPNH-cytochrome c reductase activity is much higher than the succinate-cytochrome c reductase activity. The ratio for five different preparations varied between 7.7 and 17, mean value 10 (I gave somewhat higher ratios than II). In the heavier structural fragments the ratio is much lower. The almost complete separation obtained, of the system responsible for the oxidation of DPNH from that responsible for the oxidation of succinate is thus possibly due to a tighter binding of the latter to these fragments.

The rate of oxidation of DPNH is stimulated by $Na_4P_2O_7$, EDTA and ATP, active where ADP and AMP are not. ITP also stimulates this respiration, but only in higher concentration and to a lesser degree than ATP. These stimulations are inhibited by amytal.

The TPNH-oxidase and the TPNH-cytochrome c reductase activities of the particles are low compared to the corresponding DPNH-activities, but are several fold increased by addition of DPN.

The particles have ATP-ase activity. It is increased up to 100-fold by added Mg^{++} whereas 2,4-dinitrophenol only has a slight (about 2-fold) stimulating effect.

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Activity of Amino Acid Activating Enzyme Systems during Liver Regeneration

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In order to attain an incorporation of labeled amino acids into proteins by cell-free liver preparations, microsomal material and certain