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 atebrin, a drug known to interfere with flavins probably in a competetive 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 atebrin 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 atebrin 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 atebrin.

The significance of these findings will be discussed.

1. Siekevitz, P., Löw, H., Ernster, L. and Lindberg, O. *Biochim. et Biophys. Acta (In press.)*
2. Löw, H., Siekevitz, P., Ernster, L. and Lindberg, O. *Biochem. et Biophys. Acta (In press.)*
3. Haas, E. *J. Biol. Chem.* **155** (1944) 321.
4. Berger, M., Strecker, H. J. and Waelsch, H. *Nature* **177** (1956) 1234.

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 ^{14}C-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 ^{14}C-labeled azo dyes. This effect is most easily demonstrated by adding soluble proteins to the microsomal systems metabolizing ^{14}C-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-aminofluorene-9-^{14}C. In the regenerating livers, finally, the concentration of cytochrome b_5