(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.

1. von der Decken, A. and Hultin, T. *Exptl. Cell Research* **14** (1958) 88.
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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.

** Supported by a grant from *Ella och Georg Ehrnrooths stiftelse*.

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.

1. Baltscheffsky, H. *Exptl. Cell Research* **13** (1957) 630.

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

soluble cytoplasmic components have to be included in the system¹. It has been shown previously that microsomes, isolated from regenerating rat liver, work more effectively in incorporation systems of this kind than normal microsomes^{2,3}. The present report is concerned with the effects of regenerative growth induction on the soluble components of the incorporation system. As is well known, these components are required for the initial activation of the labeled amino acids at the expense of high-energy phosphates⁴. The complete soluble system used in these experiments contained the following essential components: (1) a mechanism for ATP-generation (in the present experiments composed of phosphoenolpyruvate, pyruvate kinase and adenylate kinase), (2) guanosine triphosphate⁵, (3) amino acid activating enzymes, associated in an hitherto unknown way with soluble RNA.

With standard amounts of liver microsomes as acceptor for activated amino acids, cell fluid from regenerating livers effected, per mg protein, a more rapid incorporation of ¹⁴C-L-leucine into protein than cell fluid from normal liver. The increase was preceded by a lag period of 12–14 h and culminated about 30 h after hepatectomy. As a rule, the activity then was 60–80 % higher in the case of regenerating livers.

In order to elucidate which of the components of the activation chain were affected by the regeneration, some of these components were studied separately. The higher efficiency of the cell fluid from regenerating liver was not due to increased contents of phosphoenolpyruvate, ATP or guanosine triphosphate, since these compounds were added separately to the incorporation system in adequate amounts. The concentrations of pyruvate kinase and adenylate kinase were not significantly higher in the preparations from regenerating liver. In several experiments, moreover, these enzymes (prepared from rabbit muscle) were added in excess to the incubation samples without influencing the regeneration effects. The amounts of amino acid activating enzymes were determined in dialyzed cell fluid from normal and regenerating livers by measuring the rate of ³²P-exchange between ³²P-pyrophosphate and ATP in the presence of L-leucine or an amino acid mixture. An increase of nearly 40 % was observed in the regenerating livers. The RNA-content showed a slight increase at the same time.

It is concluded, therefore, that the higher efficiency of the amino acid activating system, observed in regenerating liver, was primarily due to a higher activity of the amino acid activating enzymes proper.

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The Initial Reactions Involved in Respiratory Chain Phosphorylations

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Recent studies have indicated that the mitochondrial PO_4 -ATP ** exchange and DNP-activated ATPase reactions involve a reversible electron transport through the diaphorase-FAD of the respiratory chain^{1,2}. On the basis of these conclusions and of certain quantum-chemical considerations^{3,4} pointing to a possible participation of phosphorylated quinoid structures as intermediates in respiratory chain phosphorylations the following reaction sequence appears to be a satisfactory formulation of the mechanism of the first of the three phosphorylations occurring along the mitochondrial oxidation of DPNH by oxygen:

1) DPNH is oxidized by the diaphorase-FAD with the simultaneous cleavage of one of the P—O bonds of a molecule of inorganic phosphate and the attachment of the phosphoryl radical to the 2-keto group of the *iso*-alloxazine ring:

* On leave of absence from The Rockefeller Institute for Medical Research and the recipient of a travel grant from the National Research Council, U.S.A.

** Abbreviations: PO_4 , inorganic phosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; DPNH, reduced diphosphopyridine nucleotide; FAD, flavin adenine dinucleotide; DNP, 2,4-dinitrophenol.