

(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*<sub>5</sub> 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.
2. Hultin, T. *Exptl. Cell Research* **13** (1957) 47.

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

Herrick Baltscheffsky\*\*

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

A new method for obtaining submitochondrial particles capable of respiration and oxidative phosphorylation has been briefly reported<sup>1</sup>. After grinding liver mitochondria from rat or guinea pig with Al<sub>2</sub>O<sub>3</sub> and centrifuging the ground material at 25 000 × *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 × *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 × *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 activity<sup>1</sup>. 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<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 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<sup>++</sup> 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

Alexandra von der Decken  
and Tore Hultin

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

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