The Oxidation of TPNH by Rat Liver Microsomes and its Stimulation by Inducing Agents

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A number of enzymatic reactions related to metabolic detoxication have been shown to be associated with the microsomal structures of the liver. The reactions are aerobic but take place only in the presence of reduced pyridine nucleotides, primarily TPNH. The activity of these enzymes temporarily increases after the intraperitoneal injection of small amounts of methylcholanthrene (MC)[1]. It has been shown that TPNH can be oxidized by rat liver microsomes in the presence of *i.a.* cytochrome c, dichlorophenolindophenol or tetrazolium salts. Cytochrome c, however, is not present in the microsomes, which instead contain another related compound, cytochrome b_5. Preparations of cytochrome b_5 become reduced in the presence of liver microsomes and TPNH.[2]

In our experiments the oxidative demethylation of *p*-methylaminoazobenzene by liver microsomes of young rats was stimulated about 3 times as a result of MC treatment. The reduction of cytochrome b_5 in the presence of TPNH by the same microsomal preparations was measured by following the development of the difference spectrum in the region of 557 mμ. Normal microsomes usually showed an activity of the order 3×10^{-3} μmoles of reduced cytochrome b_5 per mg protein per minute. The cytochrome b_5 reduction was significantly increased in the microsomes from MC-treated animals. Also the TPNH-diaphorase (measured by dichlorophenolindophenol or neotetrazolium) was stimulated, while on the other hand the TPNH cytochrome c reductase showed a significant decrease. The content of cytochrome b_5 in the microsomes did not change appreciably under these conditions.

After partial hepatectomy the activity per mg protein of oxidative demethylase and the content of cytochrome b_5 in rat liver microsomes decrease by about 50 %[3]. Hepatectomized rats were treated with MC 24 hours after the operation. In comparison with non-treated, hepatectomized rats the demethylase activity increased about 4 times. At the same time the reduction of cytochrome b_5 by TPNH was stimulated. The TPNH diaphorase activity increased by 15—25 %, while the TPNH cytochrome c reductase again showed a decrease. In the regenerating livers the cytochrome b_5 content showed a positive response to MC.

The experimental results may imply that an oxidation of TPNH by cytochrome b_5 is somehow involved in the microsomal reactions stimulated by the MC-treatment.

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2. Strittmatter, P. and Velick, S. F. *J. Biol. Chem.* **221** (1956) 277.
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Ethanol and Liver Metabolism

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During the oxidation of ethanol to acetic acid *ca.* 1/3 of its energy content is liberated in the liver. If it is assumed that the need of energy is constant in the liver, and that the energy derived from ethanol can be utilized as well as that of other substrates, it would seem reasonable to postulate that the breakdown of other substrates would decrease correspondingly during the oxidation of ethanol.

During the artificial perfusion of an isolated normal rat liver the addition of ethanol to the perfused blood decreased the amount of CO_2 liberated, whereas the fasted liver with a low initial CO_2 production was unaffected. The oxidation of ethanol changed the type of metabolism in a normal liver from a complete oxidation of substrate to CO_2 to a partial oxidation to intermediates, whereas the metabolism of the fasted liver, where the partial oxidation already was considerable, was unaffected. In the normal liver the partial oxidation was increased by ethanol and an accumulation of organic acids proportional to the decrease of the complete oxidation was observed. During the perfusion of normal liver 2.27 mg of CO_2 per min was produced. When ethanol was added to the blood the production of CO_2 decreased to 1.48 mg per min and additional energy was produced from the partial oxidation to acetic acid of 1.26 mg of ethanol per min. If it is assumed that the CO_2 production in the normal liver is derived solely from complete oxidation of carbohydrates the energy production would be 5.7 cal per min. In the case where ethanol was added to the blood the calculated energy production would be 6.6 cal per min.