SYNTHESIS OF MICROSOMAL MEMBRANES AND THEIR ENZYMIC CONSTITUENTS IN DEVELOPING RAT LIVER

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The limiting membrane of the endoplasmic reticulum (ER) has a variety of enzymatic activities which are ascribed to some of its constitutive proteins (Siekevitz, 1963). The location of the enzymes in the membrane is related to the function of the ER system; moreover, enzyme activity is influenced by the position and relations of the enzyme within the membrane structure (Ernster et al., 1962). The question arises whether during biogenesis of the ER membrane, the synthesis of the membrane-bound enzymes is closely connected with, and perhaps dependent on, the synthesis of other membrane components, such as phospholipids and probably non-enzymic protein. The developing rat liver appears to be a suitable system for answering this question. Available data indicate that the fetal hepatic cell has a poorly developed ER (Howatson and Ham, 1954; Peters et al., 1963) and is deficient in some enzymes (Nemeth, 1954; Strittmatter, 1963). This paper describes the differential rates of synthesis of membrane-bound enzymes in the ER of the newborn rat.

Experimental. Rats of CFN strain (Carworth Farms, Inc., New City, N.Y.) were used. The standard diet (supplied ad libitum) was Lab Chow (Ralston Purina Co., St. Louis, Mo.). Liver microsomes from fetus, newborn and non-starved adult rats (90-day old), were prepared as described by Ernster et al. (1962). (Because of the high glycogen contents of these livers some microsomes are lost in the preliminary low speed centrifugation.) Separation of smooth and rough (having attached ribosomes) microsomes was

performed with a two-layered sucrose gradient, containing CsCl (Dallner, 1963). To separate membrane components, both rough and smooth microsomes were treated with 0.26% deoxycholate (DOC) according to Ernster et al. (1962), except that centrifugation at 40,000 rpm was prolonged to 4 hrs. The pellets, containing both the ribosomes and the membranes, were resuspended in 0.25 M sucrose; DOC was added to a final 0.5% concentration and the suspension then centrifuged at 40,000 rpm for 2 hrs. The pellets contain only the ribosomal particles, while the supernates contain the membranes. Enzyme activities and the amounts of cytochrome b_5 and CO-binding pigment were measured as described earlier (Ernster et al., 1962; Dallner, 1963; Orrenius et al., 1964a). In incorporation experiments, DL-leucine-1-C^{14} (New England Nuclear Corp.) was injected intraperitoneally (1.5 μC/10 g). The liver was excised and fractionated at selected time points. The proteins of the various fractions were precipitated with 12% TCA and extracted by the procedure of Siekevitz (1952). The final pellets were dissolved in 88% formic acid, and were plated and counted in a Geiger-Müller gas flow counter.

Results. Hepatocytes at 3 days before birth contain a large number of mitochondria, and a moderately developed ER, primarily of rough surfaced type characterized by tight packing of ribosomes (ribosome spacing ~ 80 Å) on the cytoplasmic surface of its limiting membrane. The cytoplasmic matrix contains many free ribosomes and relatively large aggregates of glycogen particles. At the time of birth, the glycogen deposits appear greatly increased, and contain very few, if any smooth ER elements. At 3 days after birth the appearance approaches that seen in the adult. The ER is highly developed and is comprised of both smooth and rough elements. The groups of attached ribosomes of the latter are separated, however, by large distances (~ 500 Å). The characteristic relationship between smooth ER and glycogen deposits is already established.

Before birth, both electron transport enzymes and phosphatases exhibit low activity (Figs. 1 and 2). After birth the enzymes can be divided into

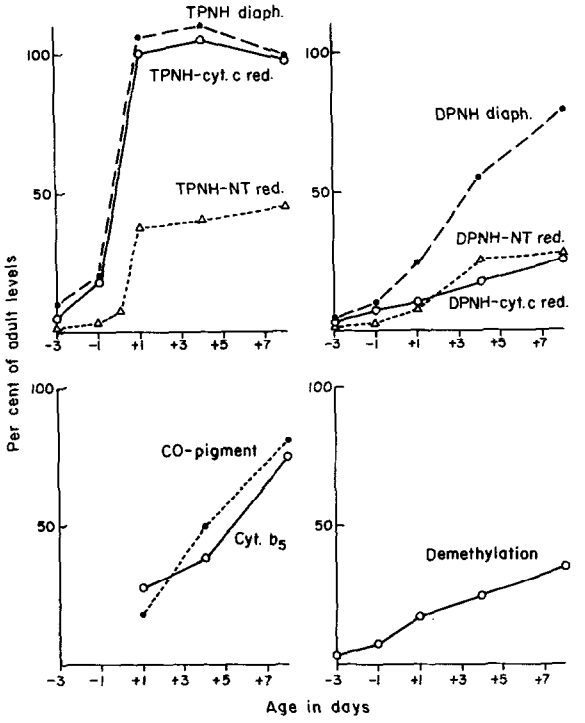


Fig. 1. Electron transport enzymes in hepatic microsomes as a function of age. Specific enzyme activities (on protein basis), expressed as per cent of adult level, are plotted on the ordinate.

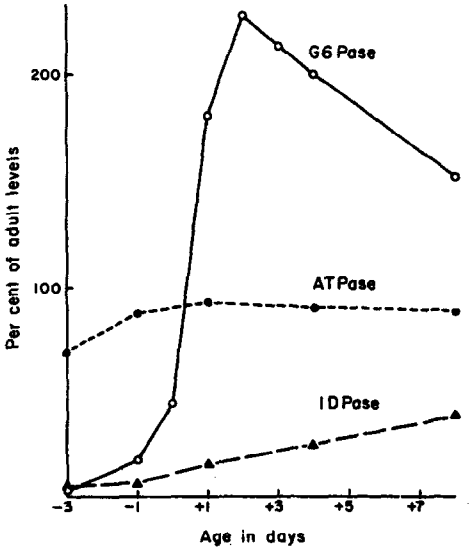


Fig. 2. Phosphatases in hepatic microsomes as a function of age.

several groups depending on the rates of increase in their activity. 1) Nucleoside triphosphatase (using ATP as substrate) displays the same specific activity at all stages of embryonic and neonatal development. 2) Nucleoside diphosphatase (using IDP as substrate), TPNH-neotetrazolium (NT) reductase, DPNH-cytochrome c reductase, DPNH-NT reductase and oxidative demethylation activity (using aminopyrine as substrate) increase slowly and at 8 days after birth are still considerably lower than the adult value. 3) DPNH diaphorase activity (assayed with ferricyanide as electron acceptor), the amounts of CO-binding pigment and cytochrome b_5, increase more rapidly than the enzymes in group 2. 4) TPNH diaphorase (measured with ferricyanide) and TPNH-cytochrome c reductase activity quickly reach the adult level at the first day after birth. 5) Finally, glucose-6-phosphatase (G6Pase) activity rises immediately following birth, greatly exceeding the activity of the adult liver, as previously described (Nemeth, 1954; Weber and Cantero, 1957). Increases in G6Pase, TPNH-cyt. c and TPNH-NT reductase activities were inhibited by actinomycin D (2x0.8 mg/kg) and puromycin (2x10 mg/kg), indicating that the assays measured increase in enzyme amount.

In the developing liver the newly appearing enzymes, such as G6Pase and TPNH-cyt.c reductase initially have higher specific activities in the rough microsomes, and are presumably synthesized there (Fig. 3). On the other hand, ATPase, which does not change during development, is found in both subfractions, but is concentrated mainly in smooth microsomes. In the adult rat liver these enzymes are distributed equally between rough and smooth microsomes (Dallner, 1963).

Fig. 4 shows the incorporation of leucine-C^{14} into the membrane proteins of rough and smooth microsomes. The labeled amino acid was injected into 2-hour old rats and the membranes were isolated from 0.5 to 32 hours later. As can be seen, the specific activity of the membrane protein of the rough microsomes is higher than that of the smooth microsomes at an early time point, but is lower later on.

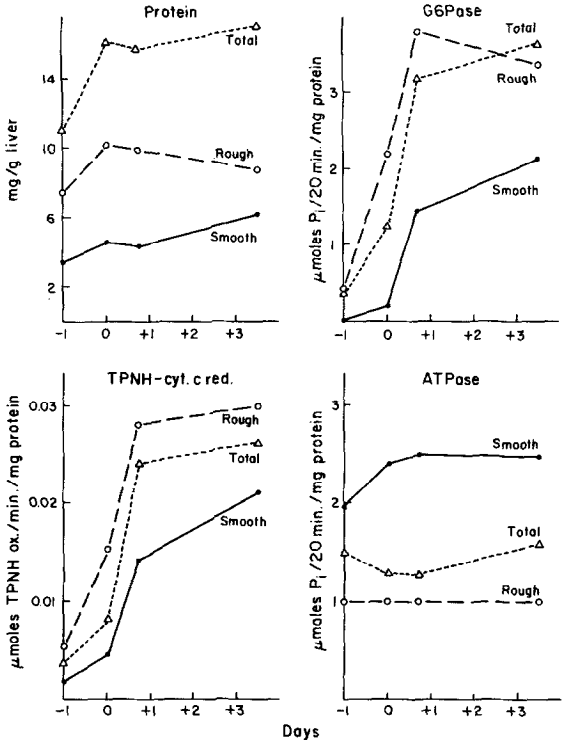


Fig. 3. Distribution of some enzymes in rough and smooth microsomes of developing rat liver.

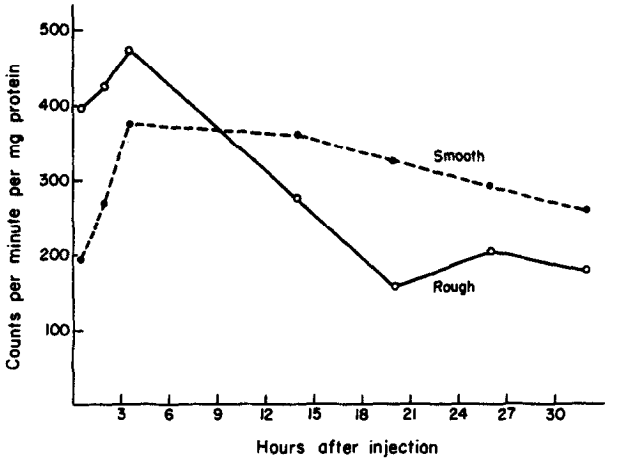
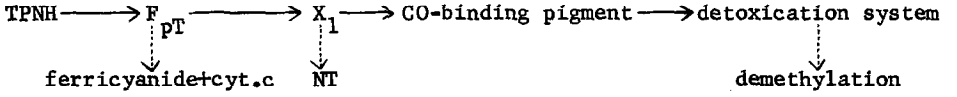


Fig. 4. Incorporation of leucine-C^{14} into microsomal membranes of 2-hour old rats.

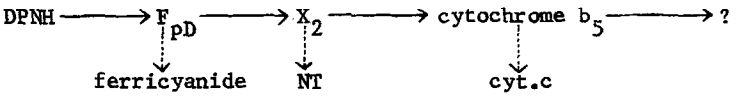
Discussion. Electron microscopic evidence (Dallner et al., 1965b) indicates that at birth, rat hepatocytes already have an ER system, including some smooth membranes. However, as the data of this paper show, only after birth do many of the constitutive membrane enzymes appear. The question arises whether these new enzymes are integrated into already existing membranes, or whether a small amount of new membrane (containing lipids and non-enzymic protein) is synthesized concomitantly with the synthesis of specific enzyme protein. To this intent an inquiring into lipid synthesis during the same developmental period was carried through and will be reported in a subsequent paper (Dallner et al., 1965a).

The above data also throw some light on the components of the two microsomal electron transport chains. The available evidence (Orrenius et al., 1964a, 1964b; Sato et al., 1964) indicates the following electron flow diagram for the microsomal TPNH oxidase:



In the microsomes of newborn rats the low detoxication activity appears to be due to a deficiency of the component X_1, here measured as TPNH-NT reductase, for it is this latter activity which seems to be rate limiting in the neonatal microsomal TPNH chain.

Cytochrome b_5 (Strittmatter and Ball, 1952), the DPNH oxidizing flavoprotein (Strittmatter and Velick, 1956) and the PCMB sensitive DPNH-NT reductase (Dallner, 1963) appear to be integrated in the following sequence for the microsomal DPNH oxidase:



Again, the rate limiting factor of the chain appears to be a component (X_2), which reacts with NT, for the DPNH-NT reductase is very low in the neonatal microsomal membranes.

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