PHOSPHOLIPIDS IN HEPATIC MICROSOMAL MEMBRANES DURING DEVELOPMENT

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As shown in a previous paper (Dallner et al., 1965a) an extensive synthesis of microsomal membranes occurs in the liver of the rat fetus at the end of gestation. The activities of a number of constitutive microsomal enzymes appear, however, mainly in the early postnatal period and subsequently increase at different rates. Inhibitors of protein synthesis like actinomycin D and puromycin prevent this increase in enzymic activities, indicating that in the normal development the increased activity is due to the synthesis of new proteins. In this paper we report changes in the lipid components of the microsomal membranes during the same period.

Experimental. Materials and methods used were described previously (Dallner et al., 1965a). Microsomal membranes were prepared from the livers of 5-day old sucklings whose mothers had been fed ad libitum from the 7th day of pregnancy with either: (a) basic diet, (b) same supplemented with 20% corn oil, (c) same supplemented with 20% lard. The phospholipids of the microsomal membranes were extracted with chloroform-ethanol (1:1) and washed with 0.1 N HCl to separate proteolipids. α-tocopherol was present in the chloroform as antioxidant during these extractions. The separation of phosphatides and the measurements of phosphorus content was carried out by paper chromatography according to Marinetti (1962). When incorporation of P^{32} was determined, the chromatographic spots were counted directly without elution. For the determination of fatty acid composition, phospholipids were separated from neutral lipids on silicic acid column (Borgström, 1952)

and, after methylation (Stoffel et al., 1959), the fatty acids were determined by gas-liquid chromatography (Farquhar et al., 1959). Extraction of microsomes with acetone-water (9:1) was performed essentially as done for mitochondria by Lester and Fleischer (1961). Asolectin (Assoc. Conc., Woodside, N.Y.) micelles were prepared by sonication; lipid micelles from 8-day old and adult microsomes, mitochondria and purified beef heart phosphatides were prepared as described by Fleischer and Klouwen (1961). In incorporation experiments, glycerol-1,3-C^{14} (New England Nuclear Corp.) was injected intraperitoneally (1.5 μC/10g), and the extracted, washed microsomal lipids were directly plated and counted in a Geiger-Müller gas flow counter.

Results. The phospholipid composition of rat liver microsomes at the age of 2 hrs, 2 days and 90 days (adult) is shown in Table I. Within the

Table I. Phospholipid composition of hepatic microsomal membranes as a function of age. The values are given as % of the total.

Age of rats	2 hours	2 days	adult
Total	100	100	100
Phosphatidyl ethanolamine	17.9	15.3	15.2
Phosphatidyl serine	8.9	12.7	7.8
Phosphatidyl choline	47.8	39.8	44.6
Sphingomyelin	8.7	12.0	9.8
Phosphatidyl inositol	6.1	8.6	8.0
Lysophosphatidyl inositol	2.6	3.8	3.8
Phosphatidic acid	1.8	1.2	1.6
Recovery	93.8	93.4	89.8

resolution of the method used, no major differences appear among the different phosphatides as a function of age, if the extensive lipid peroxidation, elicited by the presence of iron compounds and hemoglobin, is prevented. There are also no great differences in the rate of incorporation of P^{32} into individual phosphatides during the same period (not shown in Table). However, the fatty acid composition of the total phospholipids exhibits great differences when microsomes from rats at birth, 5 and 90 days of age are compared to each other (Fig. 1). The changes involve

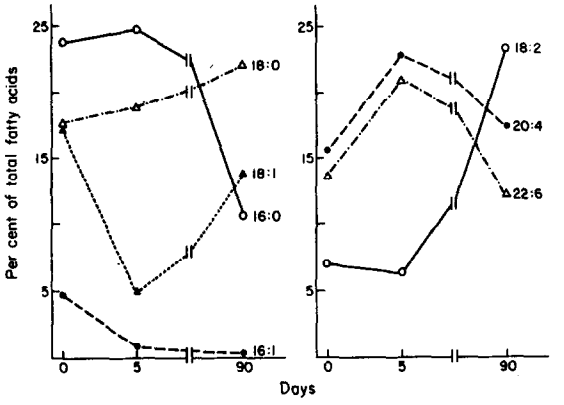

Fig. 1. Fatty acid composition of microsomal phospholipids as a function of age.

practically all fatty acids and show different patterns for each fatty acid species.

Because of these variations in fatty acid content, it is conceivable that the changing enzymic pattern of the microsomes observed earlier in the newborn (Dallner et al., 1965a) could be due to differences in the lipid composition of the membranes, rather than reflect real differences in the enzyme content of the cell. Thus, the changes in enzyme composition with age could be due to an inability of these enzymes to bind to, or otherwise became integrated into microsomal membranes. To test this possibility, female rats were fed diets supplemented with corn oil (rich in 18:2 fatty acid) or lard (rich in 18:1 fatty acid) throughout pregnancy and postpartum. This treatment was found to cause significant changes in the fatty acid composition of the microsomes of the 5-day old sucklings. However, despite large changes in the 18:1, 18:2 and 22:6 content produced by the corn oil- or lard-enriched diet as compared to the basic diet (Table II), no changes occurred in the enzymic pattern of the hepatic microsomal membranes (Table II) obtained from the 3 groups of sucklings. Thus, there is no apparent correlation between changes in fatty acid composition (Fig. 1) and changes

Table II. Effect of diet on phospholipid-fatty acids and enzymic composition of hepatic microsomes.

Diet	Corn oil	Lard	Basic	
Age of rats	5-day old	5-day old	5-day old	adult
Phospholipid/protein	0.24	0.23	0.24	0.31
Fatty acid composition (% of total fatty acids)				
16:0	34.37	29.94	24.80	10.64
18:0	18.43	22.55	18.91	22.12
18:1	6.60	10.84	4.99	13.86
18:2	12.55	5.72	6.35	23.54
20:4	18.72	21.01	22.89	17.39
22:6	9.33	9.93	21.10	12.12
Specific enzyme activities*				
TPNH-cyt.c reductase	0.023	0.017	0.019	0.023
DPNH-cyt.c reductase	0.099	0.101	0.111	1.11
Demethylation	0.84	0.89	0.98	4.37
IDPase	2.34	2.02	2.04	12.1
ATPase	0.91	1.04	1.17	1.17

*μmoles TPNH or DPNH ox./min./mg. protein; μmoles formaldehyde/min./mg. protein (demethylation); μmoles P_i/20 min./mg. protein.

in enzyme activity (Dallner et al., 1965a) observed during this time.

When microsomes from 8-day old rats were treated with acetone-water (9:1), 95% of the phospholipids were extracted and concurrently 80% of the DPNH-cytochrome c reductase activity was lost (Table III). Half of the original activity could be restored by incubation of the extracted micro-

Table III. Effect of various phospholipids on DPNH-cytochrome c reductase activity of lipid-extracted microsomes of 8-day old rats. Lipid was added to the microsomes from 1 g liver and preincubated for 10 min. at 4° before assay.

Microsomes	Preincubated with lipid (mg)	Mg lipid added in assay	μmoles DPNH ox./ min./mg protein
Non-extracted	none	none	0.200
"	adult lipid (10)	3	0.120
"	8 days lipid (7)	2.1	0.102
Extracted	none	none	0.042
"	adult lipid (25)	4	0.093
"	8 days lipid (18)	2.8	0.083
"	asolectin (100)	16	0.093
"	cardiolipin (24)	1.5	0.126
"	lecithin (24)	3	0.066
"	mitochondrial lipid (30)	10	0.084

somes with different lipid micelles. This reactivation was not dependent on the nature of the added phospholipids, indicating again that the enzyme activity pattern of the microsome membranes were independent of the nature of the phosphatides of the membrane.

Incorporation of glycerol-C^{14} into total lipids of 2-hour old rats was significantly higher in the rough membrane lipids than in the smooth membrane lipids shortly after the injection (Fig. 2), similar to the picture obtained with leucine-C^{14} incorporation into the proteins of these membranes (Dallner et al., 1965a).

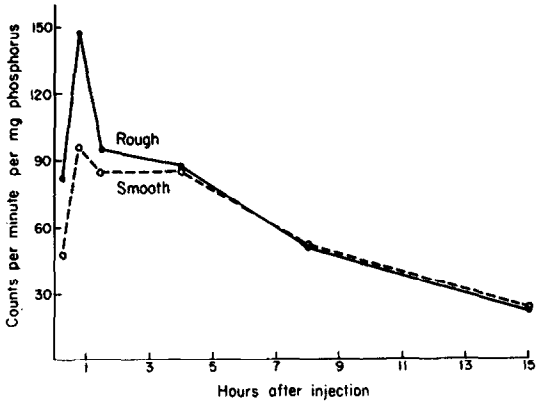

Fig. 2. Incorporation of glycerol-C^{14} into total lipids of hepatic microsomal membranes (2-hour old rats).

Discussion. Our data indicate that, during the period studied (0 to 8 days), the phospholipid composition of the microsomal membranes and the fatty acid composition of these phospholipids do not influence the developmental pattern of microsomal enzymes. The series of findings reported in this and previous papers (Dallner et al., 1964, 1965a) could be explained by the following sequence of events:

1) In the immediately prenatal period, newly formed microsomal membranes first appear as rough surfaced ER. These membranes are comparable to adult microsomal membranes since they have a similar phospholipid composition and phospholipid/protein ratio.

2) In the newborn, two different processes affect the ER: a) the production of a large amount of smooth ER membranes, and b) the appearance of membrane-bound enzyme activities in a characteristic temporal sequence.

a) Available data do not indicate the means by which the production of smooth membrane is achieved; it may be the result of large scale conversion of rough to smooth membranes by ribosome detachment; or it may represent outflow of smooth membrane from rough surfaced areas.

b) The enzymes are probably synthesized by attached ribosomes and subsequently incorporated into an ER membrane which could be an old, pre-existing structure, or alternatively, a new structure synthesized pari passu with the enzymes. Present data can not decide this alternative. The first possibility is compatible with the fact that rough ER exists already at birth, and that some enzymes are synthesized at a very rapid rate. This alternative could also explain the fact that some of the elements (X_1, X_2) of the coupled electron transport systems are added late in the developmental process to membranes which otherwise appear to be complete. The second possibility is compatible with the findings that membrane synthesis and enzyme synthesis are concomitant processes, that leucine incorporation into membrane protein and glycerol incorporation into membrane lipid both occur first in the rough ER, and that spacing among groups of attached ribosomes increases during the same period.

Finally the newly synthesized enzymes appear relatively rapidly in the smooth surfaced parts of the ER. The finding indicates that a highly efficient transfer mechanism is involved, and suggests that membrane is rapidly converted or moved from rough to smooth areas, or that ribosomes can attach to previously smooth membrane, implant the new enzyme and subsequently detach. In either case the implication is that the ER can be a dynamic, rapidly changing structure.

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