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TRANSFER OF PHOSPHOLIPIDS BETWEEN THE ENDOPLASMIC RETICULUM AND MITOCHONDRIA IN RAT HEPATOCYTES *IN VIVO*

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SUMMARY

The transfer of phospholipids from the endoplasmic reticulum to the inner mitochondrial membrane was investigated by pulse labeling *in vivo*. With [^{3}H]glycerol microsomal phosphatidylethanolamine and phosphatidylcholine were rapidly labeled during the first 30 min; while maximum incorporation into the inner mitochondrial membrane occurred only after about 5 hours. It appears that the *in vivo* transfer of these phospholipids between the two membrane compartments is a relatively slow process.

INTRODUCTION

The site of phospholipid synthesis in the liver cell has been extensively investigated and there is a general agreement that many of the major phospholipids are synthesized mainly or exclusively in the endoplasmic reticulum (1-3). Phosphatidylethanolamine and phosphatidylcholine are among those lipids which are made exclusively in this organelle. Consequently, a very active intracellular mechanism for the redistribution of phospholipids must exist.

Phospholipid exchange proteins are found in the cytoplasm and some of them have been isolated and well characterized (4,5). Under *in vitro* conditions in the presence of large amounts of exchange proteins the transfer of lipids between microsomes and mitochondria or between microsomes and liposomes is a very rapid process (6,7); in the latter case equilibrium is reached within a few minutes. The present paper describes experiments in which rats were treated with [^{3}H]glycerol and the distribution of labeled lipid in isolated subcellular fractions was followed.

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MATERIALS AND METHODS

Male rats weighing 180 g were starved for 20 h before the experiments. [1(3)-^{3}H]-Glycerol (2.5 Ci/mmol), [1-^{3}H]ethanolamine (19.5 Ci/mmol) and [^{3}H]choline (32 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. NaCl solution was added to these radioisotopes to give a final concentration of 0.9 % and injections were made into the portal vein under pentobarbital anesthesia.

Total and rough microsomes and outer and inner mitochondrial membranes were prepared as described previously (8,9). Marker enzymes were measured as reported earlier (10,11). The fractions were precipitated with 6 % TCA, washed with water and extracted with chloroform-methanol (2:1) at 4°C overnight in the presence of 0.15 % butylated hydroxytoluene. Partition was performed according to Folch et al. (12). Chromatography was performed on silica gel G plates (Merck, Darmstadt) in a two-dimensional system: in the first direction chloroform-methanol-7 M ammonia (65:22.5:4) was used as eluent and in the second direction chloroform-acetone-methanol-acetic acid-water (50:20:10:10:5). The individual spots were visualized with iodine vapor, scraped off, and used for determination of lipid or radioactivity. For measurement of lipid the gel was supplemented with 2 ml perchloric acid, the lipids were hydrolyzed by heating, and phosphorus was measured according to Marinetti (13). For determination of radioactivity the gel was extracted with 1 ml methanol at 40°C for 60 min and radioactivity was measured after the addition of 10 ml scintillator. Protein was determined using the Biuret reaction (14).

RESULTS AND DISCUSSION

The chemical and enzymic properties of the isolated microsomes and the inner mitochondrial membrane are shown in Table 1. According to previous findings (15), the inner mitochondrial membrane contains only one third as much phospholipid per mg protein as do total microsomes or microsomal subfractions. Contamination of microsomes with inner mitochondrial membrane is negligible, as is apparent from the distribution of cytochrome *c* oxidase activity. Conversely, there are no significant amounts of microsomal enzymes present in the inner mitochondrial membrane fraction. Our outer mitochondrial membrane fraction is probably also reasonably free from cross contamination, but for the sake of clarity this fraction was not analyzed for phospholipid turnover.

Phosphatidylcholine is the major lipid component in both microsomes and inner mitochondrial membranes, representing 50 % and 40 %, respectively, of the total phospholipid content (Table 2). Phosphatidylethanolamine is the next largest lipid component, constituting 20 % of the lipid content of both membranes.

Table 1. ENZYMIC CHARACTERIZATION OF ISOLATED MICROSOMAL AND INNER MITOCHONDRIAL MEMBRANE FRACTIONS

	Total microsomes	Rough	Inner mitochondrial membranes	Outer
Protein[1]	22.6	11.5	6.58	0.70
Phospholipid[1]	6.8	3.3	0.79	0.19
$\frac{\text{Phospholipid}}{\text{Protein}}$	0.30	0.29	0.12	0.27
Cytochrome c oxidase[2]	0.017	0.022	1.42	
Monoamine oxidase[3]	21.3	6.5	11.2	198
NADPH-cytochrome c reductase[4]	0.041	0.043	0.002	
Glucose-6-phosphatase[5]	0.40	0.46	0.01	

[1]mg/g liver; [2]μmoles cytochrome c ox./min/mg protein; [3]nmoles benzaldehyde produced/min/mg protein; [4]μmoles NADPH ox./min/mg protein; [5]μmoles P_i/min/mg protein.

When [^{3}H]glycerol is injected directly into the liver through the portal vein, a rapid increase followed by a rapid decrease of the cytoplasmic pool of this radioisotope occurs, resulting in pulse labeling. This fact is reflected by the labeling pattern of the microsomal phospholipids, which represent newly synthesized components (Fig. 1). Both lipids investigated are heavily labeled as little as half an hour after the portal injection. Subsequently, their specific radioactivity decreased to a relatively low value. In contrast, there is no labeling initially in the two lipids in the inner mitochondrial membrane, and peak incorporation is reached only about five hours after the injection. As expected, the maximum specific radioactivity is much lower than in microsomes.

Table 2. PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE CONTENTS OF SUBCELLULAR FRACTIONS

Fraction	Total phospholipid	Phosphatidyl-ethanolamine[1]	Phosphatidyl-choline[1]
Total microsomes	6.8	1.4	3.6
Rough microsomes	3.3	1.3	1.7
Inner mitochondrial membrane	0.79	0.19	0.34

[1]mg/g liver

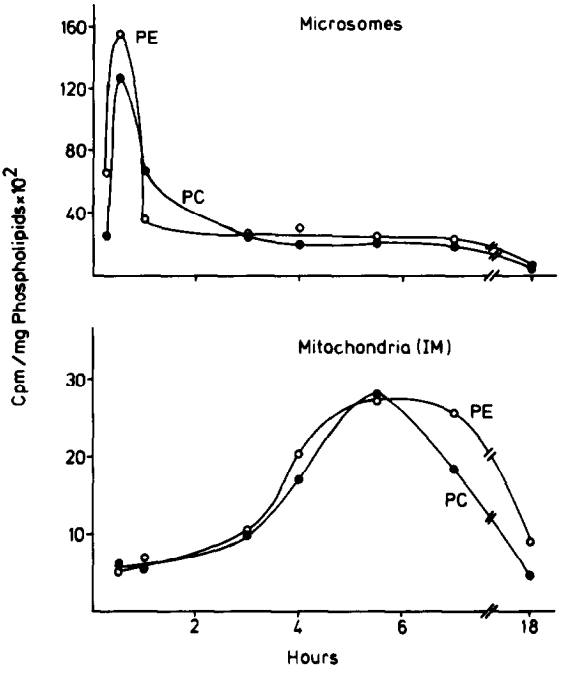

Fig. 1. In vivo incorporation of [^{3}H]glycerol into phosphatidylethanolamine (PE) and phosphatidylcholine (PC) of microsomes and inner mitochondrial membrane (IM).
Rats were injected in the portal vein with 300 µCi [^{3}H]glycerol at various times before decapitation. The amount of lipid phosphorus and radioactivity in the individual lipids were determined after thin-layer chromatographic separation. Each value represents the mean of 5-7 experiments.

Incorporation experiments were also performed in vivo with labeled bases (Fig. 2). Injections of both [^{3}H]ethanolamine and [^{3}H]choline result in heavy labeling of the corresponding lipid fractions of microsomes in the initial phase, in contrast to the findings with mitochondria. The increase in mitochondrial labeling, both in the case of phosphatidylcholine and phosphatidylethanolamine, takes several hours, a situation resembling that seen with [^{3}H]glycerol. However, the overlap of these labels is considerable, which may be attributed to the fact that base exchange is occurring to a relatively large extent.

From the point of view of membrane biogenesis it is important to know to what extent and at what rate newly synthesized phospholipids of the endoplasmic reticulum are transferred to the preexisting framework of other

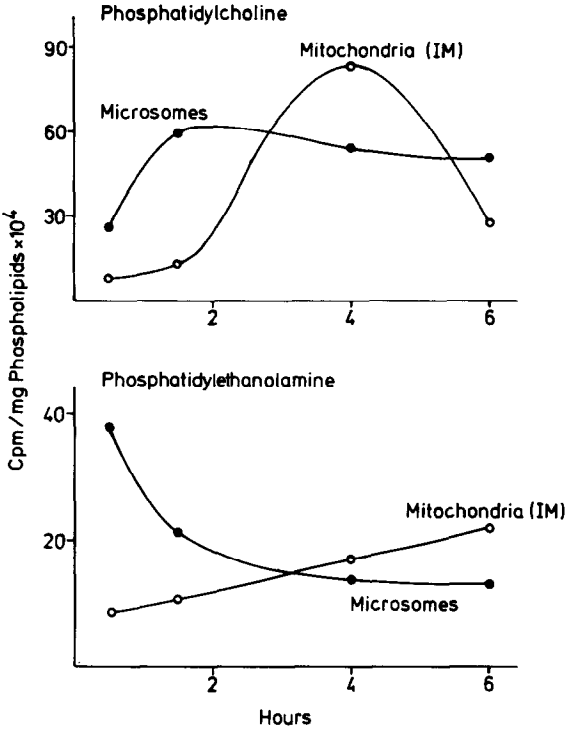

Fig. 2. In vivo incorporation of [^{3}H]choline and [^{3}H]ethanolamine into phosphatidylcholine and phosphatidylethanolamine of microsomes and inner mitochondrial membrane (IM).
For labeling 300 µCi [^{3}H]choline and [^{3}H]ethanolamine were injected into the portal vein. The values show specific activities and each value is the mean of 4-6 experiments.

intracellular membranes such as mitochondria. Experiments described in this paper indicate that *in vivo* transfer of the labeled lipids from the endoplasmic reticulum to the inner mitochondrial membrane is a slow process requiring several hours. These results are consistent with *in vivo* experiments using [^{32}P]phosphate (15,16). A number of investigations on *in vitro* systems have yielded variable results (6,7,17,18). The exchange process in such experiments has been reported to require minutes or hours, and to be either limited to certain lipids or to include most of them. Obviously, the experimental conditions used *in vitro* strongly influence the results obtained. Our findings indicating a relatively slow relocation of newly synthesized phospholipid molecules is of interest in conjunction with studies of mem-

brane renewal and membrane synthesis in which the biosynthesis, completion, processing and transfer of protein components to existing membranes was found also to be a relatively slow process (19,20).

Phospholipid synthesis occurs on the cytoplasmic surface of the endoplasmic reticulum, and newly synthesized molecules can thus be transferred to other membranes. However, a rapid and complete exchange of all phospholipids in the endoplasmic reticulum would require a free "flip-flop", which is not yet supported by experimental observations.

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