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TURNOVER OF RAT LIVER PLASMA MEMBRANE PHOSPHOLIPIDS

COMPARISON WITH MICROSOMAL MEMBRANES

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SUMMARY

The turnover rates of various phospholipids in the plasma membranes of normal rat liver, determined by a single-isotope administration method and double-labeling technique, have been compared with those of microsomal membranes. Individual phospholipid classes of plasma membranes had similar half-lives to those of microsomal membranes, except that the sphingomyelin of the plasma membranes turned over more slowly than that of microsomal membranes. Our data demonstrate that within a given membrane fraction, the various phospholipid classes are degraded at different rates.

INTRODUCTION

It is now recognized that lipid, together with protein, forms an essential part of cellular membranes. Several investigators[1,2], based mainly on the results from the turnover studies of different membrane proteins, have suggested that all membranes within the hepatocytes are in a dynamic state with a similar overall rate of renewal. In the present study, we have tested this concept by comparing the turnover rates of the phospholipid constituents of the plasma membranes and the microsomal membranes of normal rat liver.

EXPERIMENTAL PROCEDURE

Preparation of plasma membranes

Livers from female Charles River (CD strain) rats weighing 150–165 g and fasted 16 h before killing were used in all experiments. We isolated the plasma membranes using a combination of the methods of Berman *et al.*[3] and Neville[4]. The homogenates were prepared in buffered isotonic sucrose (0.25 M sucrose–0.5 mM $CaCl_2$–5 mM Tris–HCl, pH 7.4). After centrifugation at 2000 × *g*, the pellet formed was washed several times by recentrifuging at 2000 × *g* and finally suspended in buffered sucrose. Based on refractometer (Bausch and Lomb, Inc.) readings, the suspensions were then adjusted with 66% stock sucrose solution or water to 44.0 ± 0.1% sucrose; 20-ml portions of this suspension were then poured into three separate S-25 tubes. Next, 10 ml of 42.3 ± 0.1% sucrose was layered on top of the homogenate.

The samples were then centrifuged at 90 000 × *g* for 2 h in a Spinco SW 25.1 rotor. The float was removed with a spatula and homogenized in the original media. We adjusted the sucrose concentration of this sample to 48% (density = 1.22) and placed equal portions into three or four Spinco S-40 rotor tubes. On top of this suspension we layered a discontinuous gradient of 3 ml of 45% sucrose (density = 1.20), 4 ml 41% sucrose (density = 1.18), and 3 ml 37% sucrose (density = 1.16). The tubes were centrifuged at 90 000 × *g* for 2 h. The membrane fractions between densities 1.16 and 1.18 were collected and resuspended in distilled water and used immediately or stored at −20 °C until analyzed.

Preparation of microsomal membranes

After centrifuging the total homogenate at 2000 × *g* for 20 min, microsomal membrane fractions were prepared from the supernatants by first centrifuging at 15 000 × *g* for 10 min to sediment mitochondria and then at 105 000 × *g* for 60 min to sediment the microsomes. The resulting pellets were washed once with water and once with 0.15 M KCl–10 mM EDTA to remove most of the intracisternal contamination[5]. We used the total microsomal membrane fractions because both submicrosomal fractions are similar in their composition and turnover (see ref. 6 for references).

Enzyme assays

The following enzyme markers were used to check the purity of the membrane fractions. 5′-Nucleotidase (EC 3.1.3.5), characteristic of plasma membranes, was assayed at pH 7.5 in the presence of 10 mM AMP and 5 mM $MgCl_2$ (ref. 7). Cytochrome oxidase (EC 1.9.3.1), a mitochondrial marker, was determined by monitoring the oxidation of cytochrome *c* (ref. 8) in a Beckman Model DU spectrophotometer equipped with a Gilford Model 2000 recorder. Glucose-6-phosphatase (EC 3.1.3.9), a microsomal marker, was measured by the method of Swansen[9].

Administration of labeled compounds

When the turnover of membrane lipid components was determined by a single-isotope administration method, we injected each rat intraperitoneally with sodium [2-^{14}C]acetate (26 μmoles and 100 μCi per 100 g body weight) dissolved in 0.9% NaCl.

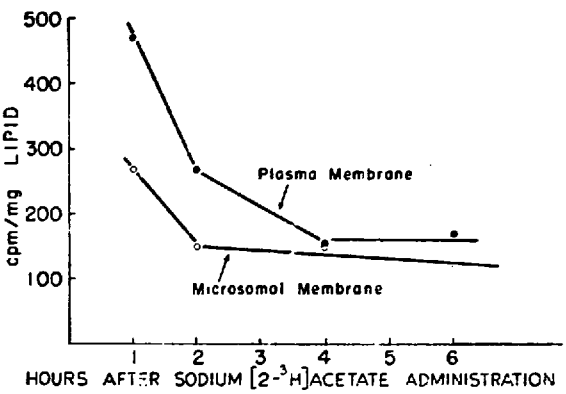

Fig. 1. Decay of radioactivity from total lipids of rat liver plasma membranes and microsomal membranes after a single administration of sodium [2-^{3}H]acetate. Rats weighing 150–165 g each were injected intraperitoneally with sodium [2-^{3}H]acetate (100 μCi per 100 g body weight; spec. act. 3.9 mCi/mmole). The rats were killed at the time indicated and the plasma membranes and microsomes were isolated and analyzed as described under Experimental Procedure.

Pairs of two animals were killed at 23, 47, 117, 191 and 239 h after the injection. In the double-label experiments, the rats were injected intraperitoneally with sodium [2-^{14}C]acetate (3.4 μmoles and 200 μCi) and then 60 h later each rat was injected with sodium [2-^{3}H]acetate (10 μmoles and 1 mC). The animals were killed 4 h after receiving the ^{3}H label. The scheme for the administration of labeled glycerol was similar to that described for acetate except that 18.4 μmoles and 250 μCi of [2-^{14}C]-glycerol and 0.6 μmole and 125 μCi of [2-^{3}H]glycerol were used. The rationale for killing the animals 4 h after administration of the second isotope was based on the results showing that the lipids associated with the membranes are in a state of isotope decay after this period (Fig. 1). The initial rapid loss of radioactivity between 1 and 4 h presumably does not represent the turnover of lipids associated with the membrane, but rather a secretion of lipoproteins (also see ref. 1).

Lipid analyses and radioassay

The methods used for the extraction of total lipids from both the plasma and microsomal membrane suspensions, the separation and the quantitative determination of individual phospholipid classes, and the measurement of radioactivity were the same as those reported elsewhere[6]. The neutral lipids were resolved on thin layers (250 μm) of Silica Gel G in a solvent system consisting of hexane–diethyl ether–acetic acid (70:30:1, by vol.). The mass of the components was measured by photodensitometric analysis[10].

RESULTS AND DISCUSSION

Enzymatic evaluation of the purity of the isolated cell fractions revealed that less than 10% of the specific activity of the 5′-nucleotidase was in the microsomal membranes and less than 20% of the specific activity of glucose-6-phosphatase was in the plasma membranes. We detected no activity of cytochrome oxidase in either membrane fraction.

The chemical compositions of plasma membranes and microsomal membranes are distinctly different. The molar ratio of cholesterol to total phospholipid phosphorus in the plasma membranes (0.53) was much higher than that in the microsomal membranes (0.15). In addition, the analysis of the distribution of lipids (Table I) indicates that there is a high content of sphingomyelin and cholesterol in the plasma membranes. Our data are consistent with the relationship noted by Patton[11], reflecting a positive correlation between the levels of cholesterol, sphingomyelin, and total lipid content in different membranes of rat hepatocytes. The amount of these lipid constituents tends to be low in mitochondrial membranes and high in plasma membranes, whereas the nuclear envelope, endoplasmic reticulum, and Golgi membranes have intermediate values. Our results agree with published data on the neutral lipid content, phospholipid composition, and the molar ratio of cholesterol to total phospholipid phosphorus of the plasma membranes[12–14].

Fig. 2 shows the decrease with time in specific radioactivities of total phospholipids derived from plasma membrane and microsomal membrane fractions. The appearance of more than one decay slope suggests a heterogeneity of turnover rates among the various phospholipid classes and is a finding that has also been reported by other investigators[15,16]. The half-life of the fractions exhibiting either the fast

TABLE I

LIPID COMPOSITION OF PLASMA AND MICROSOMAL MEMBRANES OF RAT LIVER

The means ± S.E. for five different membrane preparations are given. Individual phospholipids are expressed as percentage of total phospholipid phosphorus. Individual neutral lipids are expressed as percentage of the total neutral lipid fraction.

Lipid fraction	*Percent of total fraction*	
	Plasma membranes	*Microsomal membranes*
Total phospholipids	67.1 ± 1.6	80.0 ± 0.5
Sphingomyelin	18.2 ± 0.6	9.6 ± 1.1
Phosphatidylcholine	40.0 ± 1.1	56.9 ± 0.3
Phosphatidylinositol *plus* phosphatidylserine	13.7 ± 1.6	8.1 ± 0.7
Phosphatidylethanolamine	27.8 ± 1.0	25.5 ± 0.9
Total neutral lipids	32.9 ± 1.6	20.0 ± 0.5
Cholesterol*	66.8 ± 1.2	50.7 ± 1.2
Free fatty acid	12.2 ± 1.7	19.0 ± 0.8
Triglyceride	16.5 ± 1.8	26.3 ± 0.9
Cholesterol esters	4.5 ± 0.5	4.0 ± 0.6

* When a solvent system of hexane–diethyl ether–methanol–acetic acid (80:20:5:1, by vol.) was used to separate cholesterol and diglycerides, no diglycerices were found in the plasma membranes, and less than 8% of the cholesterol fraction in the microsomal membranes was contaminated with diglycerides.

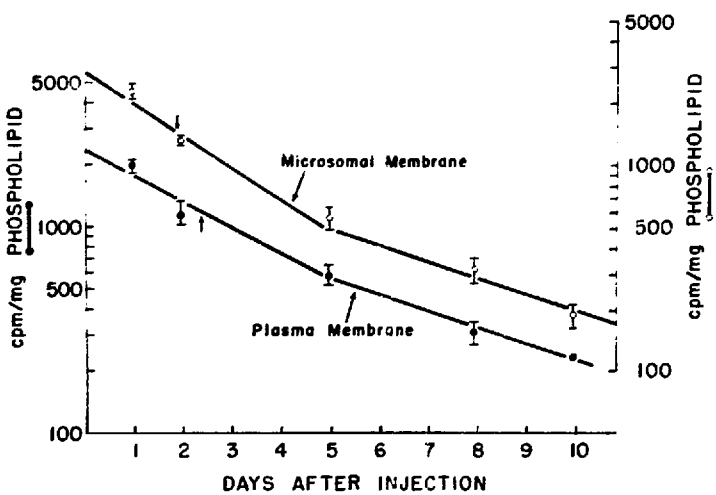

Fig. 2. Decay of specific radioactivity of total phospholipids in plasma membranes and microsomes after injecting sodium [2-^{14}C]acetate. Rats weighing 150–165 g each were injected intraperitoneally with sodium [2-^{14}C]acetate (26 μmoles and 100 μCi per 100 g body weight). The rats were killed at the time indicated and the plasma membranes and microsomes were isolated and analyzed as described under Experimental Procedure. Arrows denote half-life times based on graphical extrapolation

or slow turnover was quite similar for the phospholipids of the two membranes studied. More detailed analyses of the turnover of individual phospholipid classes of the membranes in the fraction exhibiting a fast turnover (Fig. 3) indicate that phosphatidylcholine, phosphatidylinositol *plus* phosphatidylserine, and phosphatidylethanolamine have similar half-lives and the turnover rates for these phospholipids

are the same in both plasma and microsomal membranes. In contrast, the half-life of sphingomyelin is slightly longer than that of the other phospholipids and the turnover rates of sphingomyelin in the plasma membrane are different from those in the microsomal membranes. We confirmed and extended these data in the experiment done with the double-labeled precursors.

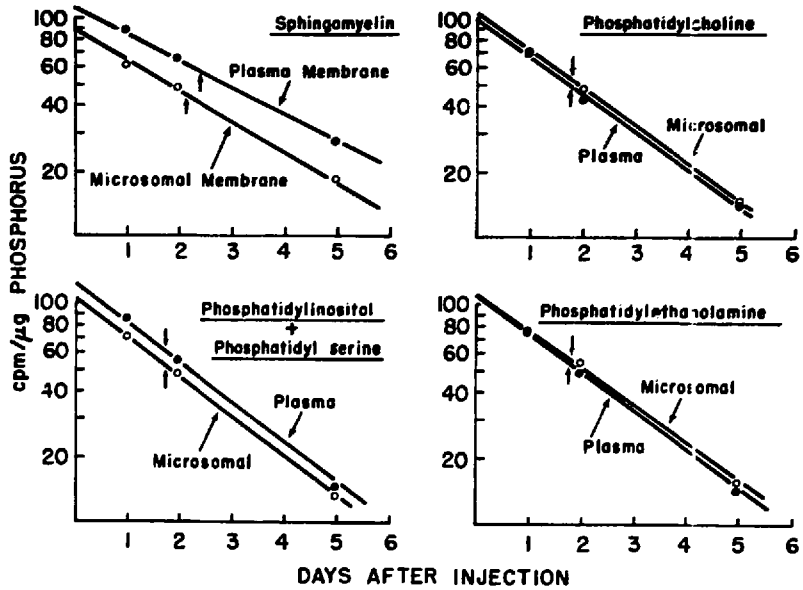

Fig. 3. Decay of specific radioactivity of individual phospholipids in plasma membranes and microsomes after injecting sodium [2-^{14}C]acetate. The experimental conditions were the same as those described in the legend of Fig. 2. Arrows denote half-life times based on graphical extrapolation.

Tables II and III list the relative turnover rates of individual phospholipid classes of plasma and microsomal membranes, determined by using acetate or glycerol labeled with ^{14}C and ^{3}H as the lipid precursors. In general, the conclusions were similar to those we reached by single-isotope administration. However, we noted that phosphatidylcholine and phosphatidylethanolamine turn over much more rapidly than the phosphatidylinositol *plus* phosphatidylserine fraction. This probably can best be explained by the fact that the double-isotope technique is a more sensitive method for detecting differences in turnover rates[1,17].

TABLE II

RELATIVE TURNOVER OF PHOSPHOLIPID CLASSES IN PLASMA AND MICROSOMAL MEMBRANES OF RAT LIVERS AFTER THE ADMINISTRATION OF ^{14}C AND ^{3}H-LABELED ACETATE

Experimental conditions are described under Experimental Procedure. Each value is the mean of duplicate samples.

Phospholipid	*Ratio $^{3}H/^{14}C$*	
	Plasma membrane	*Microsomal membrane*
Sphingomyelin	0.4	0.7
Phosphatidylcholine	2.2	2.3
Phosphatidylinositol	1.2	1.1
Phosphatidylserine	1.6	1.5
Phosphatidylethanolamine	1.9	2.1

TABLE III

RELATIVE TURNOVER OF PHOSPHOLIPID CLASSES IN PLASMA AND MICROSOMAL MEMBRANES OF RAT LIVERS AFTER THE ADMINISTRATION OF ^{14}C AND ^{3}H-LABELED GLYCEROL

Experimental conditions are described under Experimental Procedure. Each value is the mean of duplicate samples.

Phospholipid	*Ratio $^{3}H/^{14}C$*	
	Plasma membrane	*Microsomal membrane*
Sphingomyelin	0.5	1.1
Phosphatidylcholine	5.7	5.9
Phosphatidylinositol *plus* phosphatidylserine	2.5	2.3
Phosphatidylethanolamine	5.8	6.9

Patton[11] has proposed that increases in levels of cholesterol and sphingolipids render membranes more lipophilic and therefore less permeable and less easy to degrade. It would appear that the specific nature of a membrane is partly directed by the degradative enzymes responsible for its turnover. Our finding that sphingomyelin of plasma membranes turns over more slowly than the sphingomyelin of microsomal membranes, and the fact that the content of cholesterol and sphingomyelin of plasma membranes is higher than microsomal membranes certainly deserve further investigation.

The exact mechanism(s) for the degradation of membrane phospholipids is still unclear, but it would appear that one or more of the phospholipases that have been shown to be present in microsomes[18,19], plasma membranes[20,21], and lysosomes[22–24] is involved. Since our data show that the half-lives of certain plasma membrane phospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol *plus* phosphatidylserine) approach that of microsomal phospholipids, it would seem there is a dynamic relationship between these two membrane systems even though marked differences in their biosynthetic enzymes exist. For example, plasma membranes do not possess the enzyme(s) required for the *de novo* synthesis of nitrogen-containing phospholipids such as phosphatidylcholine[20]; the *de novo* synthesis of these phospholipids apparently occurs exclusively in the endoplasmic reticulum[25]. Thus, the supply of phospholipid components of the plasma membrane would seem to be totally dependent on the endoplasmic reticulum. The exchange of intact phospholipid molecules between membranes[26,27] might play an important role in such processes.

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