Exchange of Phospholipids between Liver Mitochondria and Microsomes in Vitro*

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SUMMARY

A 10% rat liver homogenate in 0.25 M sucrose-10^{-3} M ethylenediaminetetraacetate was fractionated into mitochondria, microsomes, and a supernatant fraction. Liver mitochondria derived from rats injected with leucine-3H and with phosphate-32P or with glycerol-14C were incubated with nonlabeled microsomes in the presence of supernatant or of sucrose-EDTA. Labeled microsomes were similarly exposed to nonlabeled mitochondria. 32P or glycerol-14C was used to measure exchange of phospholipid; 3H was used to correct for contamination of mitochondria by microsomes. After 90 min at 37° 30 to 40% of the phospholipid in the particulate fractions had exchanged while at 0° 10% exchange took place. Phosphatidyl choline appeared to exchange to a greater extent than phosphatidyl ethanolamine, whereas cardiolipin of the mitochondrial fraction did not exchange. The extent of exchange was reduced 50 to 60% when sucrose-EDTA was substituted for the supernatant fraction as incubation medium.

Radioactive phosphorus is rapidly incorporated into liver phospholipids of the intact rat. The question whether the endoplasmic reticulum is solely responsible for their synthesis or whether other subcellular elements synthesize phospholipids has not been clearly established. Gurr, Prottey, and Hawthorne (1) argue on the basis of isotopic studies on intact rats that, even though liver lecithin synthesis is most active in the microsomal fraction, there must be substantial synthesis of this phospholipid in both nuclear and mitochondrial fractions. This conclusion is at variance with that derived from experiments in vitro which show that only minor phospholipid fractions of mitochondria became labeled (2–4). Wilgram and Kennedy (5) showed the presence of the appropriate enzymes for phospholipid synthesis in liver microsomes, but failed to show the same in mitochondria. These observations would be compatible if microsomal synthesis of phospholipid were followed by rapid exchange of the newly synthesized compounds with mitochondria. Exchange in vitro of phospholipids involving high and low density serum lipoproteins (6, 7), chylomicrons (8), or red cells (9, 10) has been observed repeatedly. It was the purpose of the present investigation to examine the potentialities for exchange of phospholipids between microsomes and mitochondria.

EXPERIMENTAL PROCEDURE

Preparation of Subcellular Fractions—Female 250- to 300-g rats of the Holtzman strain were fasted overnight and anesthetized with ether before removal of the liver. Livers were excised and immersed in ice-cold 0.25 M sucrose containing 10^{-3} M EDTA (pH 7.0). Homogenization and isolation of subcellular particles were carried out with the same medium and all operations were performed at 0–4°. Livers were minced and the pieces were rinsed thoroughly with the sucrose-EDTA to remove blood and homogenized with two strokes of a Teflon pestle in a glass homogenizer containing 9 ml of sucrose-EDTA and 1 g of liver.

The 10% homogenate was centrifuged at 600 \times g_{\text{max}} for 15 min to sediment nuclei, red cells, whole cells, and cell debris. The supernatant was centrifuged for 20 min at 5500 \times g_{av} (rotor SW 41, Spinco) to sediment the mitochondria. The mitochondria were washed twice by resuspending the pellets in a homogenizer with half of the initial volume of sucrose-EDTA and centrifuging as before.

The 6,500 \times g_{av} supernatant was centrifuged at 15,000 \times g_{av} for 15 min (angle rotor No. 40, Spinco) to sediment the microsomes. The mitochondria were washed twice by resuspending the pellets in a homogenizer with half of the initial volume of sucrose-EDTA and centrifuging as before.

The resulting supernatant was centrifuged at 105,000 \times g_{av} for 60 min (angle rotor No. 40, Spinco) to isolate the microsomes. The surface of the microsomal pellet was rinsed several times with sucrose-EDTA.

Radioactive subcellular fractions were prepared by injecting rats intraperitoneally with inorganic phosphate-32P (Cambridge Nuclear Corporation) and with leucine-4,5-3H (New England...
Lipid Glycerol-1*C—Mitochondria and microsomes were extracted by the procedure of Folch et al. (11) as before. Phospholipids were separated from the total lipids by chromatography on a small silicic acid-Supercel (1:1) column (8). Purity of the fractions was checked by thin layer chromatography. 1*C was determined as described for 32P.

Leucine-3**H and Protein Determination—Small aliquots (100 µl) of subcellular fractions containing 32P and with leucine-3**H were incubated with an unlabeled 15,000 × g supernatant. In other experiments doubly labeled microsomes were incubated with unlabeled mitochondria suspended in unlabeled 105,000 × g supernatant. In two experiments glycerol,14C was used instead of 32P. The incubations were performed in 25-ml Erlenmeyer flasks containing 6 to 11 ml of particulate suspension, with gentle agitation at 37°. One Erlenmeyer flask containing the complete incubation mixture was kept in ice from the moment at which the mitochondria, microsomes, and 105,000 × g supernatant were mixed until the time at which the particles were separated.

After incubation the suspensions were centrifuged at 15,000 × g for 15 min (rotor No. 40, Spinco) to sediment the mitochondria. The mitochondria were washed twice as described before, and after each resuspension sedimented at 6,500 × g for 20 min (rotor SW 41, Spinco). Microsomes and 105,000 × g supernatant were isolated as before. Finally, the mitochondrial and microsomal pellets were suspended in a small volume of water for chemical analysis.

Lipid Phosphorus and 32P—Lipids from the subcellular fractions and the 105,000 × g supernatant were extracted with 20 volumes of chloroform-methanol (2:1, v/v) overnight at 4°. The extract was washed by the procedure of Folch, Lees, and Sloane Stanley (11). Lipid phosphorus was determined by Bartlett’s method (12).

Individual phospholipids were separated by thin layer chromatography. Thin layer plates were prepared with Silica Gel H (E. Merck, Darmstadt, Germany). Plates were activated at least 1 hour at 110° before chromatography. Chloroform-methanol-acetic acid-water (25:15:4:2, v/v) was used as developing solvent. Lipids were detected by iodine vapor. Areas containing lipid were marked and scraped off after the iodine stain had disappeared. The specific activities of the individual phospholipids were determined after eluting the lipids from the silica gel scrapings with methanol. Phosphatidyl-EA fractions contained small amounts of phosphatidyl serine; cardiolipin included all 32P at the solvent front; phosphatidyl inositol, sphingomyelin, and lyssolecithin were clearly separated but are not included in Tables V and VI.

The lipid phosphorus, 32P, was determined by liquid scintillation counting in 20 ml of toluene containing 0.4% 2,5-diphenyloxazole (POPOP) and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP). The 32P in the phospholipids, separated by thin layer chromatography, was also determined by transferring the silica gel directly to counting vials containing 20 ml of counting medium described by Gordon and Wolfe (13). In the latter instance losses of phospholipids due to incomplete elution were avoided.

1 The abbreviations used are: phosphatidyl-EA, phosphatidyl ethanolamine; phosphatidyl-C, phosphatidyl choline; PLP, phospholipid phosphorus.

2 The measured specific activity is x/a. Since w is the amount of 3H due to microsomal contamination and u is the PLP/3H in the microsomes we represents PLP/3H in the microsomes. u = PLP/3H in the microsomes. Similarly, w/u/a is the PLP due to microsomal contamination. The corrected specific activity, 2 (x - wa)/(a - uw/a), in which x is the phospholipid, 32P; a is the chemical phospholipid P; and u is the 3H recovered in the mitochondrial pellet; w is the ratio of phospholipid, 32P to 3H in the microsomes while s is the microsomal phospholipid specific activity, both measured at the end of incubation.

3 The reason for this difference is not that the extent of microsomal contamination differs but that the specific activities of mitochondria after incubation at 0° are much lower than after incubation at 37°.

4 The loss of microsomal 3H to the 105,000 × g supernatant was, as determined in separate experiments, about 10%. If from microsomes initially attached to mitochondria a similar amount of 3H leaks out, the correction factor would be underestimated by 10%.
The specific activities of the microsomal phospholipid were corrected on the basis of leucine-3H (see text).

The subcellular fractions were isolated as described under "Experimental Procedure." The incubation mixture contained 230 µg of mitochondrial PLP, 312 µg of microsomal PLP, and 43 µg of 105,000 X g supernatant PLP in a total volume of 9 ml. The mitochondria were isolated 14 hours after injection of inorganic phosphate-32P (75 µC). Total radioactivity in the mitochondrial phospholipid before incubation was 97,300 cpm. After incubation at 37°C for 10 and 30 min, the samples were kept in ice for 80 min each, respectively, before centrifugation.

Specific activity of mitochondrial phospholipid before incubation. When labeled mitochondria were incubated with unlabeled microsomes, the transfer of 3H to the microsomes that contaminate the mitochondria before incubation. Evidently, in 1h at 37°C 43% of the mitochondrial phospholipid-32P has exchanged (Experiment I). The extent of exchange is time- and temperature-dependent. In Experiment I, we calculated from [3H]leucine that, in the mitochondria before incubation, 2% of the phospholipid was due to microsomal contamination.

The specific activities of the phospholipids (s) and the ratio (ω) of phospholipid-32P to 3H in the microsomes that contaminate the mitochondria from liver-L1 are the same as that measured in the microsomal pellet of liver-L1; x, a, and s are defined as before, except that they apply to the microsomal fraction obtained after incubating microsomes from liver-L0 with mitochondria from liver-L0.

It is of interest that when 3H-labeled mitochondria are incubated with nonlabeled microsomes, the transfer of 3H to the microsomal fraction is a measure of the microsomal contamination in the mitochondria before incubation. When mitochondrial pellets were isolated as described under "Experimental Procedure," about 2% of their phospholipids were due to microsomal contamination.

RESULTS

When labeled mitochondria were incubated with unlabeled microsomes, the fall in specific activity of phospholipid in the mitochondria and rise in specific activity of phospholipid in the microsomes and supernatant indicate that exchange of phospholipids between subcellular particles occurred (Table I). Quantitative aspects of the exchange can be seen more readily in the graph. However, there are significant differences in meaning of the symbols. In the present experiment mitochondria and microsomes were isolated from the liver (L1) of a rat injected with phosphate-32P and leucine-3H and, in addition, microsomes were obtained from a second nonlabeled liver (L2). We have assumed that the specific activities of the phospholipids (ω) and the ratio (ω) of phospholipid-32P to 3H in the microsomes that contaminate the mitochondria from liver-L1 are the same as that measured in the microsomal pellet of liver-L1; x, a, and s are defined as before, except that they apply to the microsomal fraction obtained after incubating microsomes from liver-L0 with mitochondria from liver-L0.

The large exchange of phospholipid-32P observed in Experiments II and III might reflect a metabolically active phospholipid exchange. In Experiment III we therefore labeled the mitochondria by injecting phosphate-32P 228 hours before isolating the particles. Under these conditions, the mitochondrial phospholipids should be uniformly labeled. The data in Table I indicate an exchange of 30% compared with 43% before. This difference can be accounted for by the amount of 32P present in the nonexchangeable cardiolipin fraction (see Table V).
In Experiment IV the mitochondria were isolated from the same homogenate as that used in Experiment III, but at lower centrifugal forces. According to measurements with leucine-3H the contamination with microsomes was decreased to one-half. Again, the extent of exchange was independent of the degree of contamination.

In the next set of experiments 32P- and 3H-labeled microsomes were added to unlabeled mitochondria suspended in unlabeled 105,000 g supernatant. After 90 min at 37°, 34% of the microsomal phospholipid had exchanged with nonmicrosomal phospholipid (Experiment I, Table II). Again, the extent of exchange appears to depend on time of incubation and temperature. To purify the unlabeled mitochondria as completely as possible, we repeated the experiment with microsomes isolated at 15,000 g for 5 min and washed six times. The results in Experiment II show that 29% of the microsomal phospholipid had exchanged in 90 min at 37°.

Although the amounts of microsomal and mitochondrial phospholipid in the incubation mixtures of Experiment II in Tables I and II are not the same, one can obtain a rough idea about the movement of phospholipids between mitochondria and microsomes in both directions. In Experiment II, Table I, 35% of the mitochondrial 32P is present in the microsomes at the end of 90 min. If this represents a transfer of 35% of the mitochondrial phospholipid one finds that 0.35 × 168 = 59 µg of PLP has been transferred to the microsomal fraction. In the opposite direction (Experiment II, Table II) 0.20 = 300 = 60 µg of PLP has been transferred. This calculation is valid even though it is applied to a mixture of phospholipids because, 66 hours after 32P injection, the specific activities of the different phospholipid fractions are nearly equal (see Experiment I, Tables V and VI).

The previous experiments have shown only the exchange of the phosphate portion of the phospholipid molecules. In the next two experiments (Table III) we used mitochondria and microsomes with phospholipids labeled in their glycerol backbone. At 37° after 90-min incubation 40% of the phospholipid-4C had disappeared from the mitochondria. This corresponds to the figures of 40 to 45% reported in Table I (Experiments I and II). When 4C-labeled microsomes were incubated with unlabeled mitochondria, 33% of the microsomal phospholipid-4C disappeared which corresponds to a value of 34% for the similar experiment with 32P reported in Table II (Experiment I).

From the previous experiments, it is evident that, when the microsomes were labeled, the specific activities of the 105,000 g supernatant at the end of incubation fell between those of the mitochondria and microsomes (Table II). When labeled mitochondria were used, the specific activities of the 105,000 g supernatant were decreased to one-half. Again, the extent of exchange was independent of the degree of contamination.

### Table II

**Labeled microsomes incubated with unlabeled mitochondria**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time of incubation</th>
<th>Specific activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>37° min</td>
<td>Mitochondria</td>
<td>Microsomes</td>
</tr>
<tr>
<td>1</td>
<td>90 min</td>
<td>63</td>
<td>894</td>
</tr>
<tr>
<td>10</td>
<td>80 min</td>
<td>124</td>
<td>802</td>
</tr>
<tr>
<td>30</td>
<td>60 min</td>
<td>197</td>
<td>749</td>
</tr>
<tr>
<td>90</td>
<td>303</td>
<td>672</td>
<td>477</td>
</tr>
<tr>
<td>00</td>
<td>90 min</td>
<td>81</td>
<td>863</td>
</tr>
</tbody>
</table>

* The specific activities of the mitochondrial phospholipid were corrected on the basis of leucine-3H (see text).

* The subcellular fractions were isolated as described under "Experimental Procedure." The incubation mixture contained 220 µg of mitochondrial PLP, 255 µg of microsomal PLP, and 29 µg of 105,000 g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 16 hours after injection of glycerol-1,3-14C (45 µC). Total radioactivity in the mitochondrial phospholipid before incubation was 12,800 cpm.

* Specific activities of the phospholipids before incubation.

* The subcellular fractions were isolated as described above.

The incubation mixture contained 283 µg of mitochondrial PLP, 325 µg of microsomal PLP, and 41 µg of 105,000 g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 16 hours after injection of glycerol-1,3-14C (45 µC). Total radioactivity in the microsomal phospholipid before incubation was 8,600 cpm.

* Specific activities of the mitochondrial and microsomal phospholipid were corrected on the basis of leucine-3H (see text).

* The subcellular fractions were isolated as described under "Experimental Procedure." The incubation mixture contained 222 µg of mitochondrial PLP, 392 µg of microsomal PLP, and 41 µg of 105,000 g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 16 hours after injection of glycerol-1,3-14C (45 µC). Total radioactivity in the mitochondrial phospholipid before incubation was 8,600 cpm.

* The subcellular fractions were isolated as described above.

The incubation mixture contained 253 µg of mitochondrial PLP, 325 µg of microsomal PLP, and 41 µg of 105,000 g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 16 hours after injection of glycerol-1,3-14C (45 µC). Total radioactivity in the microsomal phospholipid before incubation was 12,800 cpm.

### Table III

**Mitochondria incubated with microsomes (glycerol-1,3-14C)**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time of incubation</th>
<th>Specific activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>37° min</td>
<td>Mitochondria</td>
<td>Microsomes</td>
</tr>
<tr>
<td>1</td>
<td>90 min</td>
<td>1009</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>90 min</td>
<td>720</td>
<td>269</td>
</tr>
<tr>
<td>II°</td>
<td>90 min</td>
<td>1026</td>
<td>94</td>
</tr>
</tbody>
</table>

* a The specific activities of the mitochondrial and microsomal phospholipid were corrected on the basis of leucine-3H (see text).

* b The subcellular fractions were isolated as described under "Experimental Procedure," except that the mitochondria were isolated and washed in the high speed attachment of the International centrifuge (PR-2) by centrifuging the nuclear supernatant for 5 min at 15,000 x gmax. The incubation mixture contained 222 µg of mitochondrial PLP, 392 µg of microsomal PLP, and 41 µg of 105,000 g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 16 hours after injection of glycerol-1,3-14C (45 µC). Total radioactivity in the mitochondrial phospholipid before incubation was 8,600 cpm.

* c Specific activities of the phospholipids before incubation.

* d The subcellular fractions were isolated as described above.
Table IV

Mitochondria incubated with microsomes: sucrose-EDTA versus 105,000 X g supernatant fraction as incubation medium

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation conditions</th>
<th>Incubation medium</th>
<th>Specific activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°</td>
<td>0°</td>
<td>Mitochondria³</td>
<td>Microsomes³</td>
</tr>
<tr>
<td>I</td>
<td>min</td>
<td>min</td>
<td>cpm/µe PLP</td>
<td>% nonincubated microsomes³</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>Sucrose</td>
<td>17</td>
<td>7.95³</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>Supernatant²</td>
<td>48</td>
<td>7.00³</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Sucrose</td>
<td>94</td>
<td>6.91³</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>Supernatant²</td>
<td>221</td>
<td>5.35³</td>
</tr>
<tr>
<td>II</td>
<td>30</td>
<td>Sucrose</td>
<td>448</td>
<td>3.7³</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Supernatant²</td>
<td>374</td>
<td>8.3³</td>
</tr>
</tbody>
</table>

³ The specific activities of the mitochondrial and microsomal phospholipid were corrected on the basis of leucine-3H (see text).
² The subcellular fractions were isolated as described under “Experimental Procedure.” The incubation mixture consisted of 225 µg of mitochondrial PLP and 295 µg of microsomal PLP in either 58 µg of 105,000 X g supernatant PLP or 0.25 M sucrose-10 mM EDTA in a total volume of 11 ml. The microsomes were isolated 11 hours after injection of inorganic phosphate-<sup>32</sup>P (75 µC). Total radioactivity in the microsomal phospholipid before incubation was 210,700 cpm.
³ Specific activities of the phospholipids before incubation.
² Supernatant fraction, 105,000 X g.

Table V

Labeled mitochondria incubated with unlabeled microsomes: exchange of individual phospholipids

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Individual phospholipids</th>
<th>Specific activity of mitochondria before incubation</th>
<th>Distribution of individual &lt;sup&gt;32&lt;/sup&gt;P-phospholipids⁶</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Phosphatidyl-C</td>
<td>1049</td>
<td>Mitochondria before incubation</td>
<td>Mitochondria after incubation</td>
</tr>
<tr>
<td></td>
<td>Phosphatidyl-EA</td>
<td>992</td>
<td>40.9 (63,543)</td>
<td>26.8 (24,777)</td>
</tr>
<tr>
<td></td>
<td>Cardiolipin</td>
<td>761</td>
<td>38.9 (60,435)</td>
<td>45.5 (42,066)</td>
</tr>
<tr>
<td>II</td>
<td>Phosphatidyl-C</td>
<td>1049</td>
<td>13.3 (20,663)</td>
<td>23.7 (21,911)</td>
</tr>
<tr>
<td></td>
<td>Phosphatidyl-EA</td>
<td>992</td>
<td>38.9 (60,435)</td>
<td>45.5 (42,066)</td>
</tr>
<tr>
<td></td>
<td>Cardiolipin</td>
<td>761</td>
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</tr>
</tbody>
</table>

⁶ Phospholipids-<sup>32</sup>P are expressed as a percentage of the total phospholipid-<sup>32</sup>P in a given subcellular fraction. These are measured directly on thin layer chromatography scrapings (see “Experimental Procedure”). The figures in parentheses indicate the total number of counts per min of the individual phospholipids in a given subcellular fraction. These values were obtained by multiplying the percentage figures with the total phospholipid-<sup>32</sup>P in a given subcellular fraction. For the mitochondria before incubation, the total PL-<sup>32</sup>P is measured directly. For the subcellular fractions after incubation, the total PL-<sup>32</sup>P is calculated by multiplying the measured specific activity with the amount of phospholipid phosphorus present in that fraction before incubation (see “Results,” Paragraph 3).
⁰ The amount of individual phospholipid-<sup>32</sup>P at the end of incubation expressed as a percentage of that phospholipid-<sup>32</sup>P present in the nondenuded mitochondria.
⁴ See Footnote a, Table I.
⁵ See Footnote c, Table I.

supernatant were nearly equal to those of the microsomes (Table I). When incubations were performed in the presence of sucrose-EDTA instead of 105,000 X g supernatant (Table IV), a 60% decrease in the transfer of phospholipid-<sup>32</sup>P between the particulate fractions was observed.

In Tables I to IV, the exchange of the composite phospholipid fraction has been considered, but it is possible that significant differences in exchange of individual phospholipids exist. Table V lists the distribution of phospholipid-<sup>32</sup>P in mitochondria and microsomes before and after 90-min incubations at 37°. In the mitochondria of Experiment I, 61% of the phosphatidyl-C and 30% of the phosphatidyl-EA were replaced. In Experiment II,
the exchange was somewhat less, which might have been caused by the smaller ratio of microsomal to mitochondrial phospholipids present in the incubation mixture. In both experiments, the cardiolipin-32P of the mitochondria had not exchanged. Experiments with labeled microsomes are reported in Table VI. Again the percentage of radioactive microsomal phosphatidyl-EA replaced appears to be less than for phosphatidyl-C.

One can roughly calculate the minimum number of micromoles of phosphatidyl-C or phosphatidyl-EA transferred in both directions in 90 min by dividing the total 32P transferred by the specific activity (counts per min per μmole) of the phospholipid in the labeled particle before incubation. The transfer of phosphatidyl-C from microsomes to mitochondria is 1.4 micromoles, and 1.1 micromoles are transferred in the opposite direction. For phosphatidyl-EA the two figures are 0.3 and 0.4 micromole, respectively.

** DISCUSSION **

The interpretation of phospholipid exchange experiments described in the previous section depends on the availability of homogeneous subcellular fractions. To avoid contamination of the initially labeled microsomal fraction with "light" mitochondria and lysosomes, we discarded from the original homogenate all particles of the mitochondrial supernatant sedimenting after centrifuging at 15,000 × g for 15 min. The purity of the mitochondrial fraction could not be assured so readily and we, therefore, used leucine-3H to calculate contamination with microsomes (see "Experimental Procedure"). The glucose 6-phosphatase assay (17) has been observed, it has been proposed that the exchange of phospholipids is mediated in part by an exchange of lysophospholipid coupled with reacylation (19). The role, if any, of lysosphospholipids in our studies is not clear. Although exchange of phospholipids between serum lipoproteins and erythrocytes has been observed, it has been proposed that the exchange of phospholipids is mediated in part by an exchange of lysophospholipid coupled with reacylation (19). The role, if any, of lysosphospholipids in our studies is not clear.

The synthesis of phospholipids from other precursors such as phospholyl choline is also unlikely since Schneider and Belki (21) have shown that without added cofactors, synthesis is negligible. Moreover, exchange of phospholipid choline could not explain the exchange of phospholipid-32P observed in our experiment since virtually identical exchange of glycerol-labeled phospholipids took place.

Under the conditions of our experiments, phosphatidyl-C of microsomes and mitochondria exchanges readily so that, if one starts out with 32P-labeled mitochondria, only 40% of the radioactivity is left in that fraction 90 min later. Exchange of phosphatidyl-EA appears to be less extensive so that under similar conditions 70% of the mitochondrial phosphatidyl-EA is left after 90 min at 37°. This may indicate that phosphatidyl-EA is more tightly bound to the membrane structure than the phosphatidyl-C. Strong binding might also explain the persistence of cardiolipin-32P in mitochondria exposed to nonlabeled incubation media. Alternately one could explain the lack of exchange of this lipid by the fact that there is virtually no cardiolipin in the microsomal or supernatant fractions.

From experiments with phosphate-32P and with acetate-14C (22) administered to intact rats, it has been shown that the
isotopic disappearance curve for mitochondrial phospholipids is biphasic. The half-time for the rapid phase was 1.6 days for the slow phase 10 days. If there are two metabolic pools of phospholipids, as was suggested by Fletcher and Sanadi (23) and period might be more exchangeable than the more “structural” that mitochondrial phospholipids labeled with 32P for a short 

The observed value was 30%.

The experiments reported here do not prove that exchange of particulate phospholipids takes place in vivo. On the basis of electron microscopic observations, it has been suggested that the endoplasmic reticulum and the outer membrane of mitochondria are contiguous structures (18). Such an arrangement would facilitate exchange. The observation that the half-life of mitochondrial phospholipids (22) approaches that of mitochondrial phospholipid in vivo is lower than that of the microsomes (1, 29).

Our experiments show that the exchange of phospholipids is enhanced by the presence of 105,000 × g supernatant. Although enzymes may play a role in the exchange reaction, it is also possible that lipoproteins in the supernatant serve as intermediaries in the exchange process. The latter is consistent with the finding that the specific activity of the supernatant phospholipid is intermediate between that of the two particulate fractions. The failure of Fleischer (26) to observe exchange between micellar and mitochondrial phospholipids might be ascribable to the absence of a lipoprotein carrier in their incubating medium.

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