Exchange of Phospholipids between Liver Mitochondria and Microsomes in Vitro*

K. W. A. WIRTZ AND D. B. ZILVERSMIT†

From the Graduate School of Nutrition and Section of Biochemistry and Molecular Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14850

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-^{3}H and with phosphate-^{32}P or with glycerol-^{14}C were incubated with nonlabeled microsomes in the presence of supernatant or of sucrose-EDTA. Labeled microsomes were similarly exposed to nonlabeled mitochondria. ^{32}P or glycerol-^{14}C was used to measure exchange of phospholipid; ^{3}H was used to correct for contamination of mitochondria by microsomes. After 90 min at 37°C 30 to 40% of the phospholipid in the particulate fractions had exchanged while at 0°C 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 become 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°C. 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 90 min at 6,500 \times g_{\text{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_{\text{av}} supernatant was centrifuged at 15,000 \times g_{\text{av}} for 15 min (angle rotor No. 40, Spinco) to sediment a composite fraction, consisting of light mitochondria, lysosomes, and heavy microsomes. This fraction was discarded.

The resulting supernatant was centrifuged at 105,000 \times g_{\text{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-^{32}P (Cambridge Nuclear Corporation) and with leucine-4,5-^{3}H (New England Nuclear Corporation) 16–18 hr before sacrifice.

* This research was supported in part by funds provided through the State University of New York and in part by Public Health Service Research Grant HE-10940 from the National Heart Institute, United States Public Health Service.

† Career Investigator of the American Heart Association.
Nuclear). Leucine-3H (25 μCi) was always given 1 hour before killing the rats; phosphate-32P was administered in doses varying between 100 and 500 μCi and at intervals between 12 hours and 9 days before anesthesia and excision of the livers. Glycerol-1,3-14C (45 μCi, New England Nuclear) was administered 16 hours before termination of the experiment.

Incubation—The exchange of phospholipids between mitochondria and microsomes was investigated in two kinds of experiments. In some experiments mitochondria labeled with phospholipid-32P and with protein-bound 3H were incubated with an unlabeled 15,000 × g supernatant. In other experiments doubly labeled microsomes were incubated with unlabeled microsomes suspended in unlabeled 105,000 × g supernatant. In two experiments glycerol-14C was used instead of 32P. The incubations were performed in 25-mil 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 or more 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 II (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. Phosphatidylcholine-labeled 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.

Lipid Glycerol-14C—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. 14C was determined as described for 32P.

Leucine-3H and Protein Determination—Small aliquots (100 μl) of subcellular fractions containing 3H and 32P, as well as fractions containing 3H only, were pipetted directly into counting vials containing the scintillator of Gordon and Wolfe (13). Total counts in the tritium channel of the Packard Tri-Carb model 3075 were corrected for 32P present in the same channel. The overlap of 32P in the 3H channel was determined in each experiment and amounted to about 15%.

Microsomal Contamination of Mitochondrial Pellets—Although the mitochondrial pellets were washed twice the extent of microsomal contamination had to be investigated. For this purpose we used, in each experiment, subcellular fractions labeled with phosphate-32P and with leucine-3H. As has been shown by Kadenbach (14), proteins from leucine-3H-labeled microsomes are not transferred to mitochondria under the incubation conditions used in our experiments. Thus, when 32P- and 3H-labeled microsomes were incubated with nonlabeled mitochondria, the amount of 3H recovered in the latter at the end of incubation reflects the proportion of mitochondrial phospholipid-32P due to microsomal contamination. The corrected specific activity 2 equals (x - w)/a - w/a/x in which x is the phospholipid-32P, a is the chemical phospholipid P, and w is the 3H recovered in the microsomes while 3H is the microsomal phospholipid specific activity, both measured at the end of incubation.

Specific activities reported under "Results" have been corrected for microsomal contamination. These corrections, expressed as a percentage of the measured specific activity, were small for long incubations at 37° (2% for Experiment II, Table II) and greater for shorter incubations or those at 0° (20% for Experiment II, Table II). 3

When doubly labeled mitochondria (3H and 32P) are incubated with nonlabeled microsomes, labeled microsomes that contaminated the mitochondria might be transferred in toto to the bulk microsomal fraction. The total shift in phospholipid-32P must, therefore, be corrected to yield a correct measure of phospholipid exchange. According to the results of O'Brien and Kaif (15), microsomes present in the mitochondrial fraction rapidly equilibrate with microsomes in the supernatant. If this is so, the amount of 3H in the microsomal pellet isolated at the end of incubation reflects the release of intact labeled microsomes. 4 The formula for correction is the same as the one in the previous para-

The measured specific activity is x/a. Since w is the amount of 3H due to microsomal contamination and w is the PLP/3H in the microsomes we represents PLP/3H due to microsomal contamination. Similarly, w/a/x is the PLP due to microsomal contamination.

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 leaves out, the correction factor would be underestimated by 10%.

The abbreviations used are: phosphatidyl-EA, phosphatidyl ethanolamine; phosphatidyl-C, phosphatidyl choline; PLP, phospholipid phosphorus.
The mitochondria were isolated 14 hours after injection of inorganic phosphate-32P (500 cP). Total radioactivity in the mitochondrial phospholipid before incubation was 97,300 cpm. After incubation at 37° for 10 and 30 min, the samples were kept in ice for 80 min. The subcellular fractions were isolated as described under “Experimental Procedure.” The incubation mixture contained 230 pg of mitochondrial PLP, 312 pg of microsomal PLP, and 43 pg of 105,000 X g supernatant PLP in a total volume of 6 ml.

In Experiment II, we calculated from leucine-3H that, in the initially labeled mitochondrial pellet, 2% of the phospholipid appeared to be nonmitochondrial. Therefore, in Experiment II the mitochondria were isolated at 15,000 g for 5 min compared to 6,500 g for 20 min and were washed six times instead of twice. By this procedure, the nonmitochondrial phospholipid calculated on the basis of leucine-3H was reduced to one-half (1%). The data for exchange in Experiment II (Table I) agree closely with those observed for Experiment I.

The large exchange of phospholipid-32P observed in Experiments I and II might reflect a metabolically active phospholipid fraction and does not prove that “structural” phospholipid exchanges. 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-$^3$H 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 $^{32}$P- and $^3$H-labeled microsomes were added to unlabeled mitochondria suspended in unlabeled 105,000 $\times$ 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 mitochondria 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 $\times$ gmax. The incubation mixture contained 222 $\mu$g of mitochondrial PLP, 332 $\mu$g of microsomal PLP, and 41 $\mu$g of 105,000 $\times$ g supernatant PLP in a total volume of 9 ml. The mitochondria were isolated 6 hours after injection of glycerol-1,3-$^3$C (45 $\mu$C). Total radioactivity in the mitochondrial phospholipid before incubation was 8,000 cpm.

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 $^{32}$P is present in the microsomes at the end of 90 min at 37°.

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

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 $\times$ gmax. The incubation mixture contained 222 $\mu$g of mitochondrial PLP, 332 $\mu$g of microsomal PLP, and 41 $\mu$g of 105,000 $\times$ g supernatant PLP in a total volume of 9 ml. The mitochondria were isolated 16 hours after injection of glycerol-1,3-$^3$C (45 $\mu$C). Total radioactivity in the mitochondrial phospholipid before incubation was 12,800 cpm.

The previous experiments have shown only the exchange of the phosphatic portion of the phospholipid molecules. In the next two experiments (Table III) we used mitochondria and microsomes with phospholipids labeled in their glycerol backbone.

### Table II

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time of incubation</th>
<th>Specific activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I $^a$</td>
<td>min</td>
<td>cpm/µg PLP</td>
<td>% nonincubated</td>
</tr>
<tr>
<td>90</td>
<td>63</td>
<td>501</td>
<td>5.5</td>
</tr>
<tr>
<td>100</td>
<td>80</td>
<td>303</td>
<td>10.9</td>
</tr>
<tr>
<td>60</td>
<td>90</td>
<td>1036</td>
<td>3.4</td>
</tr>
<tr>
<td>60</td>
<td>90</td>
<td>81</td>
<td>8.3</td>
</tr>
</tbody>
</table>

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

The subcellular fractions were isolated as described under "Experimental Procedure." The incubation mixture contained 220 $\mu$g of mitochondrial PLP, 305 $\mu$g of microsomal PLP, and 29 $\mu$g of 105,000 $\times$ g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 15 hours after injection of inorganic phosphate-$^{32}$P (100 $\mu$C). Total radioactivity in the microsomal phospholipid before incubation was 256,300 cpm.

### Table III

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>Specific activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I $^a$</td>
<td>0°</td>
<td>917</td>
</tr>
<tr>
<td>90</td>
<td>720</td>
<td>59.9</td>
</tr>
<tr>
<td>90</td>
<td>94</td>
<td>82.1</td>
</tr>
<tr>
<td>90</td>
<td>346</td>
<td>66.9</td>
</tr>
</tbody>
</table>

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

* 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 $\times$ gmax. The incubation mixture contained 222 $\mu$g of mitochondrial PLP, 332 $\mu$g of microsomal PLP, and 41 $\mu$g of 105,000 $\times$ g supernatant PLP in a total volume of 9 ml. The mitochondria were isolated 16 hours after injection of glycerol-1,3-$^3$C (45 $\mu$C). Total radioactivity in the microsomal phospholipid before incubation was 8,000 cpm.

* The specific activities of the phospholipids before incubation.

* The subcellular fractions were isolated as described above. The incubation mixture contained 223 $\mu$g of mitochondrial PLP, 325 $\mu$g of microsomal PLP, and 41 $\mu$g of 105,000 $\times$ g supernatant PLP in a total volume of 9 ml. The microsomes were isolated 16 hours after injection of glycerol-1,3-$^3$C (45 $\mu$C). Total radioactivity in the microsomal phospholipid before incubation was 12,800 cpm.

* The specific activities of the phospholipid fractions are nearly equal (see Experiment I, Tables V and VI).

The previous experiments have shown only the exchange of the phosphatic 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-$^{32}$P had disappeared from the mitochondria. This corresponds to the figures of 40 to 43% reported in Table I (Experiments I and II). When $^{14}$C-labeled microsomes were incubated with unlabeled mitochondria, 33% of the microsomal phospholipid-$^{14}$C disappeared which corresponds to a value of 34% for the similar experiment with $^{32}$P 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 $\times$ 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 $\times$ g supernatant at the end of incubation fell between those of the mitochondria and microsomes (Table II).
Exchange of Phospholipids

**TABLE IV**

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

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation conditions</th>
<th>Specific activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
<td>Microchondria</td>
<td>% nonincubated microsomes</td>
</tr>
<tr>
<td></td>
<td>0°C</td>
<td>Microsomes</td>
<td>105,000 × g supernatant</td>
</tr>
<tr>
<td>I</td>
<td>90 min</td>
<td>795±25</td>
<td>1.8 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>742±38</td>
<td>1.8 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>451±16</td>
<td>2.0 ± 0.10</td>
</tr>
<tr>
<td>II</td>
<td>90 min</td>
<td>673±35</td>
<td>6.0 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>304±16</td>
<td>9.4 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>212±14</td>
<td>78.9 ± 2.1</td>
</tr>
</tbody>
</table>

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 × g supernatant (Table IV), a 60% decrease in the transfer of phospholipid-32P 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-32P in mitochondria and microsomes before and after 90-min incubations at 37°C. 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 micromole) 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" mitochondrial and lysosomal cardiolipin, we discarded from the original homogenate all particles of the mitochondrial supernatant sedimenting after centrifuging at 16,000 × 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, a marker enzyme for microsomal contamination, could not be used because of the loss of enzyme activity at 37°C (16). In nonincubated mitochondria prepared by the standard procedure the leucine-3H method showed that 2% of the phospholipid might be due to microsomal contamination. On the basis of glucose 6-phosphatase assays (17), the calculated contamination varied from 5% to as high as 20% in an unpredictable manner. Parsons et al. (18) also report nonreproducible results with glucose 6-phosphatase and believe that some of this enzyme is present in the outer mitochondrial membrane. We, therefore, chose the leucine-3H method as the more reliable index of microsomal contamination.

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 lysophospholipids in our studies is not clear, since at 10^{-3} M EDTA some phospholipases may still be active (20). It is important to note that under our conditions no synthesis de novo of phospholipid from phosphatidyl-choline-32P takes place. The synthesis of phospholipids from other precursors such as phosphoryl choline is also unlikely since Schaefer and Belki (21) have shown that without added cofactors synthesis is negligible. Moreover, exchange of phosphoryl 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°C. 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 and for the slow phase 10 days. If there are two metabolic pools of phospholipids, as was suggested by Fletcher and Sanadi (23) and supported by Bailey, Taylor, and Bartley (22), one could imagine that mitochondrial phospholipids labeled with $^32$P for a short period might be more exchangeable than the more “structural” phospholipids. This is disproved, however, by our experiments with mitochondria isolated 9.5 days after $^32$P injection. At first sight the exchange of 30% of mitochondrial phospholipid (Experiment III, Table I) seems significantly less than the 43% observed for mitochondria isolated 14 hours after $^32$P injection (Experiment I, Table I). However, in the mitochondria of Experiment III, 29% of the $^32$P was present in the nonexchangeable cardiolipin as compared to 3% in the mitochondria of Experiment I. If we assume that the other phospholipids, which represent 71% of the total $^32$P, exchange to the same extent as the total phospholipid in Experiment I, i.e. 43%, the calculated exchange would be 0.71 x 43%, which equals 31%. 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 microsomal phospholipid (24) suggests a dynamic relationship between these subcellular particles. The failure to find synthesis of phosphatidyl-C and phosphatidyl-EA in liver mitochondria in vitro suggests that the conditions of incubation were inappropriate or that the most important mitochondrial phospholipids are generated elsewhere. Such a precursor-product relationship is also suggested by the observation that, after administration of an isotope, the specific activity of the 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.

Acknowledgment—The technical assistance of Mrs. A. Sherman is gratefully acknowledged.

REFERENCES
Exchange of Phospholipids between Liver Mitochondria and Microsomes in Vitro
K. W. A. Wirtz and D. B. Zilversmit


Access the most updated version of this article at http://www.jbc.org/content/243/13/3596

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at 
http://www.jbc.org/content/243/13/3596.full.html#ref-list-1