Exchange of phospholipid classes between liver microsomes and plasma: comparison of rat, rabbit, and guinea pig

D. B. ZILVERSMIT*

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

ABSTRACT Rat and guinea pig liver microsomes labeled with phospholipid ^{32}P were incubated with rat, guinea pig, and rabbit plasma in a KCl–Tris–EDTA buffer. A net transfer of microsomal phosphatidylcholine and phosphatidylethanolamine to plasma was observed. In addition, an exchange of phospholipids between microsomes and plasma took place. During 20-min incubations at 37°C, the exchange of phosphatidylcholine was the most extensive. Microsomal sphingomyelin exchanged with plasma sphingomyelin only very slowly. A soluble protein factor in liver, which had previously been observed to stimulate the exchange of liver mitochondrial and microsomal phospholipids, also increased the exchange of phosphatidylcholine between liver microsomes and plasma. The pronounced differences in the relative percentages of phosphatidylethanolamine of guinea pig, rabbit, and rat plasmas did not appear to be related to differences in the relative exchange of this phospholipid compared to that of other phospholipids in these plasmas.

SUPPLEMENTARY KEY WORDS phosphatidylcholine · phosphatidylethanolamine · phosphatidylinositol · lysophosphatidylcholine · sphingomyelin

In previous work from our laboratory, and in that of others, an exchange of phospholipids between subcellular particles of liver was demonstrated (1–4). This exchange was stimulated by a soluble protein fraction of liver. In one experiment we demonstrated that a soluble protein of liver also accelerated the exchange of phospholipids between rat liver mitochondria and rat plasma lipoproteins (2). In earlier studies on the turnover of plasma phospholipids in intact animals, we found that intravenously injected lipoproteins labeled with phospholipids disappeared from the bloodstream primarily by means of a reversible transport between liver and plasma (5).

If plasma and liver phospholipids are subject to ready exchange, one might think that the relative concentrations of phospholipid classes in liver and plasma phospholipids should be equal. It is well known, however, that this is not the case: plasma is richer in choline-containing phospholipids than liver and poorer in phosphatidylethanolamine (6). Moreover, it has been shown that the percentage of phosphatidylethanolamine of plasma in different species varies over a wide range. Human, dog, and rat plasma contain relatively low amounts of phosphatidylethanolamine, whereas rabbit and guinea pig plasma contain much higher amounts of this phospholipid. According to Nelson (7), only 1.3% of rat plasma phospholipid is phosphatidylethanolamine, but in the rabbit and the guinea pig the percentages are 6.7 and 21.7, respectively. It is the purpose of the present study to determine whether or not such differences in the composition of plasma phospholipids could be the result of selective affinities of a given phospholipid for plasma lipoprotein or for liver organelles, or whether or not these differences result from the properties of the soluble protein factor of liver which accelerates phospholipid exchange.

Abbreviations: phospholipid P or PLP, phospholipid phosphorus.

*Career Investigator of the American Heart Association.

EXPERIMENTAL

Preparation of Subcellular Fractions

Rats (Holtzman strain, 200–300 g) or guinea pigs[1] (300–400 g) were fasted overnight. They were bled before removal of the livers, which were minced immediately. After rinsing the fragments with 0.25 M sucrose, 1 mM EDTA (pH 7.4), the livers were homogenized with 10 volumes of this solution. The homogenates were centrifuged at 0°C for 20 min at 15,000 *g* (avg) in the high-speed attachment of the International centrifuge (PR-2, International Equipment Co., Needham Heights, Mass.). The sediments of red blood cells, liver nuclei, Golgi apparatus, heavy and light mitochondria were discarded. The supernatant fractions of each liver were centrifuged at 0°C at 105,000 *g* (avg) for 60 min (angle rotor, Spinco No. 40) and the sediments, consisting primarily of microsomes, were washed first with 40 ml of 10 mM Tris–HCl (pH 8.6) and then with 40 ml of 1 mM Tris–HCl (pH 8.6). According to Wallach and Kamat (8) the washing procedure removes intravesicular soluble proteins. The microsomal suspensions were centrifuged both times at 105,000 *g*(avg) for 30 min and the microsomes were finally resuspended in 135 mM KCl, 50 mM Tris, 1 mM EDTA (pH 7.4).[2] In some experiments labeled microsomes were stored frozen in 0.25 M sucrose, 1 mM EDTA; before use they were sedimented and resuspended in KCl–Tris.

Labeled liver microsomes were derived from rats injected intraperitoneally with 100–200 μCi $^{32}P_i$ (Cambridge Nuclear Corp., Cambridge, Mass.) and with 100 μCi leucine-4,5-^{3}H at 16 hr and 1 hr, respectively, before they were killed. Guinea pigs received higher doses corresponding to their larger body weights.

The protein-containing fraction of liver, which was previously found to accelerate the exchange of phospholipids between mitochondria and microsomes (2), was prepared from the supernatant fractions after sedimentation of mitochondria by adjustment of the pH to 5.1 with 1 N HCl. The fraction, hereafter designated as pH 5.1 supernatant, was obtained from the supernatant after centrifugation at 15,000 *g*(avg) for 20 min, and 16–30 hr dialysis against KCl–Tris.

Incubations

Unless stated otherwise, incubations were performed[3] for 20 or 40 min at 37°C. Aliquots of washed, doubly labeled microsomes were added to 1–4 ml of plasma with or without 2 ml of pH 5.1 supernatant. Sufficient KCl–Tris was added to make the total volume equal to 6 ml. Blood samples were collected in EDTA (4 mM final concn) from animals fasted overnight. The blood was centrifuged and in all experiments, except one, the plasma was dialyzed at 4°C against KCl–Tris. Usually dialysis was carried out during the night following the preparation of plasma.

After incubation, the samples were centrifuged at 105,000 *g*(avg) for 60 min at 0°C. A known aliquot of the supernatant was removed for determination of total ^{3}H and of phospholipid P and ^{32}P. A layer of the supernatant just above the pellet was removed and discarded. One ml of water was added to the tube and taken off with a transfer pipette in order to remove practically all the supernatant. The microsomes were resuspended in 3 ml of H_2O, and phospholipids were extracted with chloroform–methanol 2:1.

In each experiment a number of control incubations were performed with washed microsomes in KCl–Tris with or without pH 5.1 supernatant, and in one experiment 6.5% bovine serum albumin (Pentex 2 × crystallized) was substituted for the equivalent amount of plasma. In each instance release of ^{3}H and of phospholipid P and ^{32}P into the medium was measured as an additional check on the adequacy of microsome sedimentation after incubation. Net release of phospholipid P and of ^{3}H into buffer, albumin-containing solution, or plasma was usually less than 1 or 2% of that present in the microsomes and thus sufficiently small so that no corrections for "contamination" of the supernatants were required.

Analysis of Phospholipids, Protein, and Radioactivity

Phospholipids from microsomes and from plasma were extracted with chloroform–methanol 2:1 and the extracts were purified according to Folch, Lees, and Sloane Stanley (9). Individual phospholipids were separated by thin-layer chromatography on activated Silica Gel H (E. Merck A.G., Darmstadt, Germany) plates (20 × 20 cm) with chloroform–methanol–acetic acid–water 25:15:4:2 (v/v/v/v) as the developing solvent. Areas of gel containing lipids, detected with I_2 vapor, were scraped into counting vials for liquid scintillation counting in the mixture of Gordon and Wolfe (10). ^{3}H in supernatant fractions and in microsomes was determined by delivering aliquots into the same counting medium. In the later experiments, self-absorption of ^{3}H emissions was minimized by solubilizing the microsomes in NCS reagent (Nuclear-Chicago Corp., Des Plaines, Ill.) before addition to the counting mixture. Total counts in the tritium channel of a Packard Tri-Carb Liquid Scintillation Spectrometer No. 3375 (Packard Instrument Co., Inc., Downers Grove, Ill.) were cor-

[1] From the colony of Dr. W. L. Nelson, Cornell University, who kindly donated the animals for this study.

[2] This solution will henceforth be designated as KCl–Tris.

[3] These relatively short time intervals were chosen because differences in exchange rates are seen more readily long before isotopic equilibrium is reached. At even shorter time intervals the amount of isotope transferred becomes so small that cross contamination and counting accuracy limit the overall accuracy of the procedure.

rected for ^{32}P overlap. Lipid phosphorus of the total lipid extract was determined by the method of Bartlett (11). For determination of specific activities of individual phospholipid fractions Silica Gel H, scraped from thin-layer plates, was eluted with 25 ml chloroform–methanol–water 80:35:5(v/v/v). Protein of plasma, microsomes, and pH 5.1 supernatant fractions was determined by the biuret method (12).

RESULTS

Incubations of microsomes with plasma must be performed in a medium of sufficiently high ionic strength to keep the serum globulins in solution. In all experiments except one, plasma samples were dialyzed overnight at 4°C against KCl–Tris, the same medium in which the microsomes were suspended. Table 1 shows the differences in phospholipid composition of fresh and dialyzed rat, rabbit, and guinea pig plasmas. In each instance, one plasma sample was extracted with chloroform–methanol both in the fresh state and after dialysis. These data were combined (in Table 1) with analyses obtained from one fresh and three dialyzed rat plasmas and from two dialyzed samples of guinea pig plasma. In all instances, dialysis resulted in loss of phosphatidylcholine and an increase in the corresponding lyso derivative, presumably the result of the phospholipid–cholesterol acyltransferase reaction (13). Slight decreases in total phospholipid concentrations after dialysis were due to 1–2% dilution of the plasma sample as determined by protein analyses. The low value for dialyzed total plasma phospholipid for the guinea pig is not the result of dialysis, but is due to the very low phospholipid values in the second plasma sample (8.6 μg phospholipid P per ml of plasma).

The data in Table 1 confirm the observation of Nelson (7) that guinea pig plasma is much richer in phosphatidylethanolamine, relative to that of other phospholipids, than the plasma of the rabbit and especially than that of the rat. However, Nelson reported that 21.7% of guinea pig plasma phospholipid is phosphatidylethanolamine while our analysis shows percentages between 10 and 11%.

The data in Tables 2 and 3 were obtained to determine the effect of pH 5.1 supernatant on the exchange of microsomal and plasma phospholipids. In one experiment, ^{32}P-labeled liver microsomes in KCl–Tris were incubated with fresh plasma (Table 2, top). In a second experiment, a different batch of microsomes was incubated with plasma dialyzed against KCl–Tris. Because the dialysis

TABLE 1 PHOSPHOLIPIDS OF FRESH AND DIALYZED PLASMA*

	Rat		Rabbit		Guinea Pig	
	Fresh (2)	Dialyzed (4)	Fresh (1)	Dialyzed (1)	Fresh (1)	Dialyzed (2)
	moles %		*moles %*		*moles %*	
Origin	0.1	0.8	0.1	0.4	0.0	0.0
Lyso PC	16.9	27.3	16.1	21.6	4.3	10.4
Sphing	8.5	10.4	7.9	8.1	4.2	6.3
PC	66.9	52.6	65.8	60.2	75.8	67.3
PI	4.6	5.6	3.4	3.5	4.0	4.0
PE	2.6	2.5	6.0	5.6	10.5	10.8
Front	0.4	0.8	0.7	0.6	1.2	1.2
Total	43.5	39.1	36.3	35.7	16.4	12.5

*All values are means of several analyses of plasma from one or more animals, and, except for the bottom line, are moles % as determined by elution from TLC scrapings. Total phospholipid P (μg/ml) was determined on separate aliquots of plasma; dilution of plasma during overnight dialysis in KCl–Tris–EDTA was 1–2% as determined by protein concentration. Total phospholipid P after dialysis was not corrected for dilution effects. Numbers in parentheses refer to the number of animals. Phospholipids are listed in the sequence in which they appear on the thin-layer plates: PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; Sphing, sphingomyelin. Small amounts of phosphorus were found at the origin and solvent fronts.

TABLE 2 PHOSPHOLIPID EXCHANGE BETWEEN LIVER MICROSOMES AND PLASMA. INCUBATIONS WITH FRESH (TOP) AND DIALYZED (BOTTOM) RAT PLASMA

	Microsomal Phospholipid (Initial)			Plasma Phospholipid P†		Plasma Phospholipid ^{32}P		Plasma Phospholipid S.A.*	
	P	^{32}P	S.A.*	Initial	Final	−pH 5.1 Spnt	+pH 5.1 Spnt	−pH 5.1 Spnt	+pH 5.1 Spnt
	%	%		%	%	%	%		
PC	58	63	1000	68	69	71	73	317	362
PE	23	28	1090	2.3	5.9	16	15	830	904
PI	13	5.8	400	3.0	8.9	5.0	5.7	189	205
Lyso PC	0.8	0.5	573	19.9	7.1	4.8	4.5	197	236
Sphing	3.9	2.3	536	6.5	7.8	1.0	1.3	44	55
Total PLP (μg)				49.8	58.0				
S.A.*			1020					328	368
PC	58	61	1000	58	65	63	70	347	472
PE	24	27	1085	1.3	6.1	15	14	935	1038
PI	13	7.4	520	5.9	8.2	11	8.0	498	429
Lyso PC	0.5	0.6	980	24	13	6.3	4.8	151	196
Sphing	4.0	3.0	700	10	7.0	2.7	1.8	125	144
Total PLP (μg)				45.8	54.9				
S.A.			955					330	403

In the experiment reported at the top, ^{32}P-labeled rat liver microsomes (118 μg phospholipid P) were incubated with fresh rat plasma (49.8 μg phospholipid P) with or without pH 5.1 supernatant. At the bottom are data from experiments in which microsomes (122 μg phospholipid P) from a different donor rat were incubated with rat plasma (45.8 μg phospholipid P) dialyzed against KCl–Tris for 16 hr at 4°C. Both sets of incubations were for 20 min in the presence or absence of pH 5.1 supernatant which contained 0.13 μg phospholipid P and 9.2 mg protein. For abbreviations see Table 1.

* The specific activities of individual and total phospholipid fractions in the two experiments are expressed relative to that of microsomal PC before incubation, which is set at 1000.

† "Initial" refers to plasma before incubation with microsomes; "final" refers to the average phospholipid P after incubation of the microsomal supernatants with and without added pH 5.1 supernatant. Total phospholipid is given as μg phospholipid P per incubation flask.

TABLE 3 EXCHANGE AND NET TRANSFER OF MICROSOMAL AND PLASMA PHOSPHOLIPIDS

	Fresh Plasma		Dialyzed Plasma	
	−pH 5.1 Spnt	+pH 5.1 Spnt	−pH 5.1 Spnt	+pH 5.1 Spnt
	%		%	
PC				
Net transfer	10.0	9.1	12.2	13.7
Exchange	10.6	13.7	6.8	11.1
PE				
Net transfer	8.8	7.7	8.8	10.1
Exchange	2.9	3.8	1.8	1.9

The conditions of incubation, the microsomes, and the plasma were the same as described in Table 2. Net transfer and exchange were calculated as a percentage of the given phospholipid fraction originally present in the microsomes. For abbreviations see Table 1.

did not appear to alter the results, the findings of these two experiments will be discussed together.

In both experiments the concentration of total phospholipids in plasma was increased by about 8–9 μg phospholipid P per incubation, i.e., per 1 ml of plasma. The largest percentage increase in plasma phospholipid composition occurred in the phosphatidylethanolamine and phosphatidylinositol fractions (Table 2). A marked decrease in the percentage of lysophosphatidylcholine was observed.[4]

The percentage of labeled phospholipid present in the plasma did not parallel the plasma phospholipid composition but showed a disproportionally high percentage of phosphatidylethanolamine. These proportions were not significantly altered by the presence of phospholipid exchange protein which is present in the pH 5.1 supernatant. From the specific activities of the individual phospholipid fractions, which are expressed relative to that of the value for microsomal phosphatidylcholine before incubation, it is evident that the major plasma phospholipid fraction, phosphatidylcholine, had not yet equilibrated in 20 min. However, compared to the other choline-containing phospholipid, i.e., sphingomyelin, the exchange of phosphatidylcholine was quite rapid.

Because both net transfer of phospholipid from microsomes to plasma and phospholipid exchange are taking place, some of the data in Table 2 have been further analyzed as shown in Table 3. Net transfer of phospholipid was calculated from the difference in plasma phospholipid concentrations before and after incubation with microsomes. It is seen that about 10% of microsomal phosphatidylcholine and of phosphatidylethanolamine are transferred from microsomes to plasma. When the amount of phospholipid ^{32}P in the plasma is corrected for this net transfer of phospholipid, one may conclude that the remaining phospholipid ^{32}P in plasma is derived from an exchange reaction. The data in Table 3 show that the exchange, expressed as a percentage of the phospholipid fraction present in the microsomes, is about three times greater for phosphatidylcholine than for phosphatidylethanolamine. Although such a difference parallels that observed in the exchange of phosphatidylcholine and phosphatidylethanolamine between mitochondria and microsomes (1, 2), some caution is required in the interpretation of the exchange data for phosphatidylethanolamine in Table 3. At the end of 20 min incubation, the specific activities of phosphatidylethanolamine in plasma and microsomes differ only slightly. This is due to the fact that the original amount of phosphatidylethanolamine present in plasma is small and that net transfer of this phospholipid from the microsomes doubles or triples that amount in a 20-min incubation. The calculation of phosphatidylethanolamine exchange is, therefore, based on the difference between total phosphatidylethanolamine ^{32}P in plasma and that present due to net transfer of this phospholipid, a difference which is relatively small.

For phosphatidylcholine the case is quite different. Net transfer and exchange are about equal. The conclusion that exchange of phosphatidylcholine takes place in addition to a net transfer is confirmed by a decrease in the specific activity of microsomal phosphatidylcholine which matches that calculated from the amount of phosphatidylcholine ^{32}P in the plasma after correction for net transfer of this phospholipid. For example, the decrease in phosphatidylcholine specific activity in the microsomes incubated with fresh plasma was 7.2% without pH 5.1 supernatant and 10.2% with pH 5.1 supernatant, which agrees reasonably well with an exchange of 10.6 and 13.7%, respectively, calculated from the phosphatidylcholine ^{32}P in plasma after correction for net transfer (Table 3).

One additional observation may be made about the data in Table 3. In both experiments the addition of the pH 5.1 supernatant fraction stimulated phosphatidylcholine exchange. The extent of stimulation was rather variable, about 30% in one experiment and 60% in the other. In an additional experiment, with different microsomal, plasma, and pH 5.1 preparations, the exchange of phosphatidylcholine was doubled by the addition of pH 5.1 supernatant. One can also see the effect of pH 5.1 supernatant on the exchange of the *total* microsomal phospholipid. When the total amount of phospholipid ^{32}P in plasma after incubation is corrected for net transfer, we find the average exchange in four experiments without added pH 5.1 supernatant to be 35.0 ± 0.7%, whereas, in the same experiments with

[4] In a parallel experiment, ^{32}P-labeled lysophosphatidylcholine isolated from rat plasma was added to unlabeled plasma and incubated for 20 and 40 min with or without unlabeled microsomes. In the presence of microsomes about one-half of the ^{32}P became water-soluble in 40 min while about 10% of the label was found in phosphatidylcholine.

pH 5.1 supernatant added, the average exchange was 49.7 ± 1.2%.

In the following experiments we have reduced the number of variables by using only dialyzed plasma samples and by performing all incubations in the presence of pH 5.1 supernatant fractions derived either from rat or guinea pig liver. In Table 4, the distribution of phospholipid ^{32}P in plasmas of a rat, a rabbit, and a guinea pig is shown after incubation with labeled rat liver microsomes. Net transfer of phospholipid and transfer of total phospholipid ^{32}P was most pronounced in rat plasma, while little release of phospholipid ^{32}P was observed in the blank consisting of KCl–Tris. The presence of phospholipid exchange was demonstrated by a 7–8% lower phospholipid specific activity of the microsomal pellets after incubation than before. Notwithstanding the much higher relative percentage of phosphatidylethanolamine in guinea pig plasma than in that of the rabbit or rat, the percentage of ^{32}P in the phosphatidylethanolamine fraction of guinea pig serum was, if anything, somewhat smaller than that in the rat and rabbit. In another experiment, performed in the same manner with rat and guinea pig plasmas, the phospholipid ^{32}P in the phosphatidylethanolamine fraction of rat plasma was 13.6% of that originally present in the microsomes and for guinea pig plasma it was 16.5%.

The next experiments were devised to test the hypothesis that the nature of the microsomes or of the pH 5.1 supernatant from rat and guinea pig liver might differ sufficiently to account for the differences in plasma phospholipid pattern in these two species. Doubly labeled rat and guinea pig microsomes were incubated with guinea pig plasma. In both instances either rat or guinea pig pH 5.1 supernatant from liver was added to the system. The results are shown in Table 5. The distribution of ^{32}P in phospholipid classes in the two microsomal fractions, measured before incubation, is substantially the same. About 30% of the label is present in phosphatidylethanolamine and about twice as much is in phosphatidylcholine. In the plasma fractions, after 40 min of incubation, the phosphatidylethanolamine ^{32}P represents about 15% of the total label irrespective of the source of the microsomes or of the pH 5.1 supernatant fraction. In the presence of rat microsomes somewhat more lysophosphatidylcholine was formed than with guinea pig microsomes, but the sum of phospholipid ^{32}P in phosphatidylcholine and its lyso compound was about 75% in all instances.

TABLE 4 TRANSFER OF MICROSOMAL PHOSPHOLIPID TO PLASMA-CONTAINING FRACTIONS

Medium	Phospholipid P*		Phospholipid ^{32}P				
				% Distribution			
	Before Incub	After Incub	Total*	PE	PI	PC	Lyso PC
	μg	μg	%				
KCl–Tris–EDTA	0	3.9	0.5				
Rat plasma	69.6	77.2	22.9	20	7.5	66	6.3
Rabbit plasma	71.3	76.1	17.1	17	6.9	65	10.5
Guinea pig plasma	62.0	64.3	14.8	14	6.3	67	11.2

^{3}H- and ^{32}P-labeled rat liver microsomes (170 μg phospholipid P, 11 mg protein) incubated at 37°C for 40 min with KCl–Tris–EDTA or plasma. After incubation microsomes were removed by centrifuging 1 hr at 105,000 *g* (avg). Corrections for incomplete removal of microsomes based on ^{3}H released into supernatant fraction (2%) were not made. Small amounts of phospholipid P and ^{32}P in supernatant after incubation with KCl–Tris–EDTA also show nearly complete removal of microsomal material. Incubation media with plasma also contained 2 ml of pH 5.1 supernatant from rat liver (about 0.6 μg phospholipid P). Rat and rabbit plasma 2 ml, guinea pig plasma 4 ml. Total incubation volume was 6.3 ml in all samples. For abbreviations see Table 1.

* Total phospholipid P expressed per incubation. Total phospholipid ^{32}P expressed as the percentage of that originally present in the microsomes.

TABLE 5 TRANSFER OF MICROSOMAL PHOSPHOLIPID ^{32}P TO GUINEA PIG PLASMA

Phospholipid Fraction	Incubation with Rat Microsomes			Incubation with Guinea Pig Microsomes		
		Plasma PL ^{32}P After Incubation			Plasma PL ^{32}P After Incubation	
	Microsomal PL ^{32}P	Rat pH 5.1 Spnt	Guinea Pig pH 5.1 Spnt	Microsomal PL ^{32}P	Rat pH 5.1 Spnt	Guinea Pig pH 5.1 Spnt
	%	%	%	%	%	%
Origin	0.4	0.3	—	0.1	0.3	—
Lyso PC	3.2	7.9	7.2	2.7	4.2	2.4
Sphing		0.6	0.8		0.9	1.1
PC	55	65	64	58	72	71
PI	9.8	11	12	5.4	6.9	7.1
PE	29	14	16	32	15	17
Front	2.5	0.9	0.9	2.0	0.9	1.0

^{3}H- and ^{32}P-labeled rat microsomes (121 μg phospholipid P) or guinea pig microsomes (168 μg phospholipid P) incubated with 3 ml guinea pig plasma (28 μg phospholipid P) and pH 5.1 supernatant from rat liver (1.1 μg phospholipid P, 17.4 mg protein) or from guinea pig liver (1.3 μg phospholipid P, 15.3 mg protein). Incubation for 40 min at 37°C in a total volume of 6.0 ml. Removal of microsomes as in Table 4. Phospholipid ^{32}P distribution in plasma–supernatant fraction after incubation did not require corrections for incomplete removal of microsomes (based on ^{3}H). For abbreviations see Table 1.

DISCUSSION

The experiments presented here demonstrate that phospholipids of microsomes incubated with plasma undergo exchange as well as a net transfer from the membrane to the plasma-containing medium. Less than 0.5% of microsomal phospholipid ^{32}P appeared in the soluble fraction after incubating the labeled microsomes with KCl–Tris. In the presence of the pH 5.1 supernatant fraction 1.0–1.6% of the phospholipid ^{32}P was released from the

microsomes, while in the presence of 1.4% bovine serum albumin the release of phospholipid ^{32}P was about 3%. When plasma was present in the incubating medium, at a total protein concentration of 1.4%, the release of phospholipid ^{32}P into the medium was of the order of 20% of the microsomal phospholipid ^{32}P. The release was caused in part by a net transfer of microsomal phospholipids to plasma and, in part, by an exchange of phospholipids of microsomes and of plasma lipoproteins.

Several investigators have demonstrated exchange of plasma phospholipids with isolated liver mitochondria (2, 14) or with liver slices (15). These investigators have not concerned themselves with the question of whether differences in exchange rates of specific phospholipids might be related to differences in plasma phospholipid composition.

In the present investigation we attempted to determine whether or not the phospholipid exchange reaction in rats and guinea pigs is related to the relatively high percentage of phosphatidylethanolamine in the plasma of guinea pigs as compared to that of the rat. For this reason we chose to use whole plasma rather than isolated lipoprotein fractions and also to avoid inhibitors of the phospholipid–cholesterol acyltransferase reaction. Apparently the moderate increase in plasma lysolecithin during dialysis did not alter the results, as demonstrated by comparing the results in fresh plasma and in plasma dialyzed against KCl–Tris.

Therefore, one may ask: Is the high percentage of phosphatidylethanolamine in guinea pig plasma compared to that of the rat related to (*a*) a special affinity of phosphatidylethanolamine to some component of guinea pig plasma, (*b*) a more extensive release of phosphatidylethanolamine from guinea pig microsomes, or (*c*) a difference in specificity of the phospholipid exchange protein in the soluble fraction of guinea pig and rat liver? The latter fraction of guinea pig liver might, for example, favor the transfer from liver to plasma of phosphatidylethanolamine relative to that of phosphatidylcholine. Under the conditions of our in vitro experiments, none of these differences could be demonstrated.

Other features of these experiments were, however, quite interesting. A net transfer from microsomes to rat plasma of both major microsomal phospholipids, phosphatidylcholine and phosphatidylethanolamine, was observed. The amount of phosphatidylcholine transferred to plasma was more than twice that of phosphatidylethanolamine. Yet, because of the very low initial concentration of phosphatidylethanolamine in rat plasma, the percentage of this phospholipid present in plasma at the end of the incubation was 2–4 times as high as before incubation. In a similar manner, the percentage of phosphatidylinositol in plasma was markedly increased during the incubation with microsomes. Lysophosphatidylcholine concentrations in plasma consistently decreased during incubation with microsomes, apparently the result of enzymatic degradation, while sphingomyelin remained about the same. The latter phospholipid appeared to exchange more slowly than the other phospholipid fractions.

In a previous publication (2) we demonstrated exchange of phospholipids between mitochondria and isolated plasma lipoproteins. This observation was confirmed by observations[5] that mitochondrial and whole plasma phospholipids exchange as well. In the studies reported here, the exchange of plasma phospholipids with microsomes has been demonstrated. It is of interest to recall that this exchange did not appear to be affected by the type of lipoprotein present in plasma, inasmuch as rat plasma contains mostly high density lipoproteins (16) whereas guinea pig plasma consists largely of low density lipoproteins (17, 18).

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

The technical assistance of Miss Michela Edelbroek is gratefully acknowledged.

Manuscript received 25 June 1970; accepted 9 October 1970.

References

1. Wirtz, K. W. A., and D. B. Zilversmit. 1968. Exchange of phospholipids between liver mitochondria and microsomes in vitro. *J. Biol. Chem.* **243:** 3596–3602.
2. Wirtz, K. W. A., and D. B. Zilversmit. 1969. Participation of soluble liver proteins in the exchange of membrane phospholipids. *Biochim. Biophys. Acta.* **193:** 105–116.
3. McMurray, W. C., and R. M. C. Dawson. 1969. Phospholipid exchange reactions within the liver cell. *Biochem. J.* **112:** 91–108.
4. Akiyama, M., and T. Sakagami. 1969. Exchange of mitochondrial lecithin and cephalin with those in rat liver microsomes. *Biochim. Biophys. Acta.* **187:** 105–112.
5. Entenman, C., I. L. Chaikoff, and D. B. Zilversmit. 1946. Removal of plasma phospholipides as a function of the liver: the effect of exclusion of the liver on the turnover rate of plasma phospholipides as measured with radioactive phosphorus. *J. Biol. Chem.* **166:** 15–23.
6. Ansell, G. B., and J. N. Hawthorne. 1964. Phospholipids: Chemistry, Metabolism and Function. Elsevier Publishing Co., Amsterdam. 210.
7. Nelson, G. J. 1967. The phospholipid composition of plasma in various mammalian species. *Lipids.* **2:** 323–328.
8. Wallach, D. F. H., and V. B. Kamat. 1966. Preparation of plasma-membrane fragments from mouse ascites tumor cells. *In* Methods in Enzymology. S. P. Colowick and N. O. Kaplan, editors. Academic Press Inc., New York. **8:** 164–172.
9. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A

[5] D. B. Zilversmit and M. Edelbroek, unpublished results.

simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
10. Gordon, C. F., and A. L. Wolfe. 1960. Liquid scintillation counting of aqueous samples. *Anal. Chem.* **32:** 574.
11. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
12. Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177:** 751–766.
13. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
14. Tarlov, A. 1968. Turnover of mitochondrial phospholipids by exchange with soluble lipoproteins in vitro. *Fed. Proc.* **27:** 458.
15. Kook, A. I., and D. Rubinstein. 1970. The role of serum lipoproteins in the release of phospholipids by rat liver slices. *Biochim. Biophys. Acta.* **202:** 396–398.
16. Windmueller, H. G., and R. I. Levy. 1967. Total inhibition of hepatic β-lipoprotein production in the rat by orotic acid. *J. Biol. Chem.* **242:** 2246–2254.
17. Burstein, M., P. Amouch, and J. M. Fine. 1968. Mobilité des lipoprotéines sériques en gel d'agarose. *Nouv. Rev. Fr. Hematol.* **8:** 809–820.
18. Puppione, D. L. 1969. Physical and chemical characterization of the serum lipoproteins of marine mammals. Univ. Calif. Radiat. Lab. Rep., UCRL-18821. 1–111.