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PARTICIPATION OF SOLUBLE LIVER PROTEINS IN THE EXCHANGE OF MEMBRANE PHOSPHOLIPIDS

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SUMMARY

1. When rat liver mitochondria and microsomes were incubated separately with a 105000 × *g* supernatant fraction which contained some phospholipid, a rapid exchange of phospholipids between the particulate and soluble fractions was observed. The exchange was not accompanied by a net transfer of phospholipids. The exchange was temperature dependent. Phosphatidyl choline was exchanged to a greater extent than was phosphatidyl ethanolamine.

2. The 105000 × *g* supernatant fraction was adjusted to pH 5.1, and the precipitate was removed by centrifugation. This yielded a virtually phospholipid-free supernatant fraction. This "pH 5.1 supernatant fraction" stimulated the exchange of phospholipids between mitochondria and microsomes at least as much as the original 105000 × *g* supernatant fraction. This exchange of phospholipids between cell membrane fractions was also temperature dependent. Phosphatidyl choline of the particulate fractions exchanged more rapidly than phosphatidyl ethanolamine. The extent of exchange depended on the amount of pH 5.1 supernatant protein present in the medium.

3. The factor in the pH 5.1 supernatant fraction, stimulating the exchange of phospholipids, is probably a protein because it was found to be nondialyzable, precipitable with $(NH_4)_2SO_4$, sensitive to temperature and digestible by trypsin. It appears likely that the stimulatory effect of the pH 5.1 supernatant fraction does not depend on the presence of a lipoprotein which carries phospholipids from one organelle to another.

4. Phospholipids also exchanged between mitochondria and plasma lipoproteins. The presence of pH 5.1 supernatant fraction greatly stimulated the exchange.

INTRODUCTION

The exchange of phospholipids between liver mitochondria and microsomes *in vitro* was demonstrated in a previous paper[1] and by McMURRAY AND DAWSON[2]. This exchange was greater when 105000 × *g* supernatant fraction was present. One interpretation was that lipoproteins in the supernatant fraction acted as intermediates in

the exchange of phospholipids between mitochondria and microsomes. This interpretation is compatible with the observations that phospholipids in plasma lipoproteins exchange with phospholipids in chylomicrons[3] and in red blood cells[4,5]. The purpose of this paper is to clarify the role of the supernatant lipoproteins in the exchange process. In addition some general characteristics of the soluble factor, active in the exchange process, were determined.

MATERIALS AND METHODS

Preparation of subcellular fractions

Female rats (200–300 g) of the Holtzman strain were fasted overnight and decapitated before removal of the liver. Livers were minced and the pieces were rinsed thoroughly with ice-cold 0.25 M sucrose containing 1 mM EDTA (pH 7.4) to remove blood. A 10% homogenate was made in the same medium with two strokes of a Teflon pestle in a glass homogenizer.

The homogenate was centrifuged at 600 × *g* max. for 15 min to sediment nuclei, red blood cells, whole cells and cell debris. The supernatant fraction was centrifuged for 5 min at 15000 × *g* av. in the high-speed attachment of the International centrifuge (PR-2) to sediment mitochondria. This subcellular fraction was washed 2–4 times with half of the initial volume of sucrose–EDTA and was centrifuged each time as before. The 15000 × *g* av. supernatant fraction was centrifuged at 15000 × *g* av. for 20 min in the same centrifuge to sediment light mitochondria, lysosomes and heavy microsomes. The sedimented material was discarded.

The resulting supernatant fraction was centrifuged at 105000 × *g* for 60 min (angle rotor No. 40, Spinco) to isolate the microsomes. The microsomes were washed twice by resuspending the pellets of one liver first in 40 ml of 10 mM Tris–HCl (pH 8.6) and then in 40 ml of 1 mM Tris–HCl (pH 8.6) essentially following the procedure of WALLACH AND KAMAT[6]. The microsomal suspensions were centrifuged both times at 105000 × *g* av. for 30 min. The washed microsomes were resuspended in sucrose–EDTA (20 mg of protein per ml) and stored in the freezer.

In some instances the 105000 × *g* supernatant fraction was isolated after perfusion of the liver *via* the portal vein with 25 ml of ice-cold 0.9% NaCl and 25 ml of ice-cold sucrose–EDTA solutions.

Radioactive subcellular fractions were obtained from rats injected intraperitoneally with $^{32}P_i$ (Cambridge Nuclear Corp.) or with [4,5-$^3H_2$]leucine (New England Nuclear). [4,5-$^3H_2$]Leucine (100 μC) was injected 45 min before the rats were killed; $^{32}P_i$ (100–200 μC) was given 12–16 h before excision of the livers to achieve uniform specific activities of the various phospholipid pools.

Preparation of plasma lipoproteins

Blood was collected from the abdominal aorta of a nonstarved rat. Clotting of the blood was prevented with EDTA present in the syringe. Blood was centrifuged at 1000 × *g* av. for 20 min to sediment red and white blood cells.

Plasma was centrifuged at 105000 × *g* av. for 60 min (angle rotor No. 40, Spinco) to float the chylomicrons which were carefully pipetted off and discarded.

The density of the remaining fluid was adjusted to 1.21 (ref. 7). Centrifugation at 105000 × *g* av. for 16–20 h (angle rotor No. 40, Spinco) floated a crude lipoprotein

mixture $d < 1.21$. The lipoprotein containing layer was carefully pipetted off and dialyzed at 4° overnight against 1000 vol. of a solution containing 135 mM KCl, 50 mM Tris and 1 mM EDTA (pH 7.4).

Incubation

Mitochondria labeled with phospholipid-^{32}P and unlabeled microsomes were incubated with an unlabeled 105000 × *g* supernatant fraction. The incubations were performed in unstoppered Pyrex tubes containing 4 ml of particulate suspension with gentle agitation in a water bath maintained at 37°.

Microsomes were not present in experiments in which we measured the exchange of phospholipids between mitochondria and 105000 × *g* supernatant fraction or between mitochondria and plasma lipoproteins. After incubation the suspensions were centrifuged at 15000 × *g* av. for 15 min in the International centrifuge (PR-2) to sediment the mitochondria. When microsomes were present, the mitochondria were washed 2–4 times and sedimented at 15000 × *g* for 5 min. Microsomes were isolated as before. When no microsomes were present in the incubation medium, the mitochondrial pellet was washed once.

Lipid phosphorus and ^{32}P

Lipids were extracted from the subcellular fractions and the plasma lipoproteins with 20 vol. of chloroform–methanol (2:1, v/v) overnight. The extract was washed by the procedure of FOLCH *et al.*[8]. Lipid phosphorus was determined by the method of BARTLETT[9]. Individual phospholipids were separated by thin-layer chromatography. Thin-layer plates were prepared with Silicagel H (E. Merck, Darmstadt, Germany). Plates were activated at least 1 h at 110° before chromatography. Chloroform–methanol–acetic acid–water (25:15:4:2, by vol.) was used as developing solvent. Lipids were detected by iodine vapor. Areas containing lipid were marked and scraped off in counting vials after the iodine stain had disappeared. The ^{32}P in the phospholipids, separated by thin-layer chromatography, was determined by liquid scintillation counting in the medium described by GORDON AND WOLFE[10].

The ^{32}P in the total phospholipid mixture was determined in 20 ml of toluene containing 0.4 % 2,5-diphenyloxazole and 0.01 % 1,4-bis-(5-phenyloxazolyl-2)benzene.

Microsomal contamination of mitochondrial pellets

Contamination of mitochondrial pellets with microsomes was measured with [^{3}H]leucine-labeled microsomes as described in the previous paper[1]. When ^{3}H-labeled microsomes were incubated with ^{32}P-labeled mitochondria, the amount of ^{3}H recovered in the latter at the end of incubation showed that only about 1 % of the mitochondrial phospholipid-P was actually of microsomal origin. Because the corrections were small they were not determined in each experiment.

[^{3}H]Leucine and protein determination

Small aliquots (100 μl) of mitochondrial fractions containing ^{32}P, with or without ^{3}H, and of microsomal fractions with ^{3}H, were pipetted directly into the scintillation mixture of GORDON AND WOLFE[10]. Total counts in the tritium channel of the Packard Tri-Carb model 3075 were corrected for ^{32}P counts present in the same channel. The overlap of ^{32}P counts in the ^{3}H channel was about 5 %.

Protein was determined by the biuret method[11] or by the method of LOWRY *et al.*[12].

RESULTS AND DISCUSSION

Exchange of phospholipids between subcellular particles and 105000 × g supernatant fraction

In the 105000 × *g* supernatant fraction, isolated from a perfused rat liver, 2 μg of phospholipid-P were present per 10 mg of supernatant protein. Phospholipids in this soluble fraction exchanged rapidly with phospholipids in mitochondria and microsomes as shown by the fall in phospholipid specific activity in the particulate fractions and the rise in phospholipid specific activity in the soluble fraction (Fig. 1). The rise in phospholipid specific activity cannot be accounted for by a release of membrane material into the soluble fraction because the phospholipid content of the particle-free supernatant fraction remained constant during incubation. These observations are in accord with an exchange mechanism. The rapid rise in the specific activities of supernatant phospholipids in these experiments may be related to the fact that the amount of phospholipid in the supernatant fraction was small compared to that in the particulate fractions. Under these conditions the exchange of only that portion of the membranous phospholipids present on the surface of the particles might account for the observed results.

In the next experiments we studied the temperature dependence of the exchange reaction. Fig. 2 shows clearly the relationship between temperature of incubation and extent of exchange. One can calculate from the decrease in mitochondrial specific activity that after 40 min of incubation 5 times as much phospholipid had exchanged at 37 as at 0°.

Analyses of individual mitochondrial phospholipids showed that this temperature dependence held for each individual phospholipid; *e.g.* 16% of mitochondrial phosphatidyl choline had exchanged at 12° compared to 22% at 37°. It is interesting that at each temperature more phosphatidyl choline had exchanged than phospha-

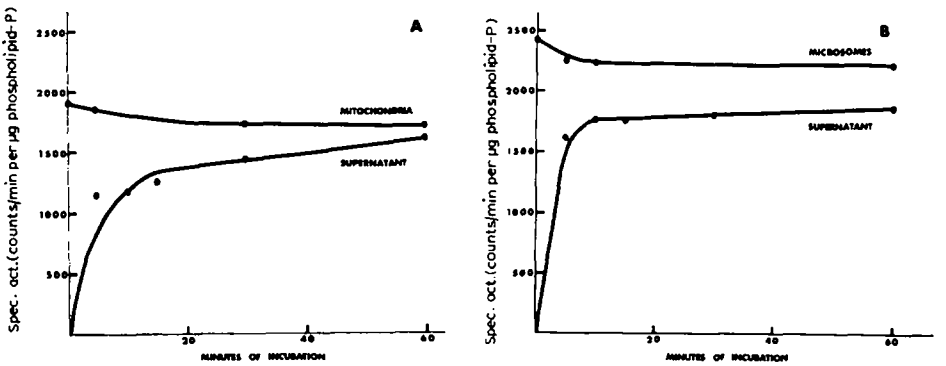

Fig. 1. Exchange of phospholipid of the 105000 × *g* supernatant fraction with mitochondrial and microsomal phospholipid as followed by the rise in the specific activities of the 105000 × *g* supernatant phospholipid-P and the drop in the specific activities of the mitochondrial and microsomal phospholipid-P. 9 μg 105000 × *g* supernatant phospholipid-P were incubated with labeled mitochondria containing 66 μg phospholipid-P (A) and with labeled microsomes containing 106 μg phospholipid-P (B) at 37°.

tidyl ethanolamine; *e.g.* at 12° 16% phosphatidyl choline had exchanged compared to 9% phosphatidyl ethanolamine (Table I). Cardiolipin, present only in the mitochondria, did not exchange.

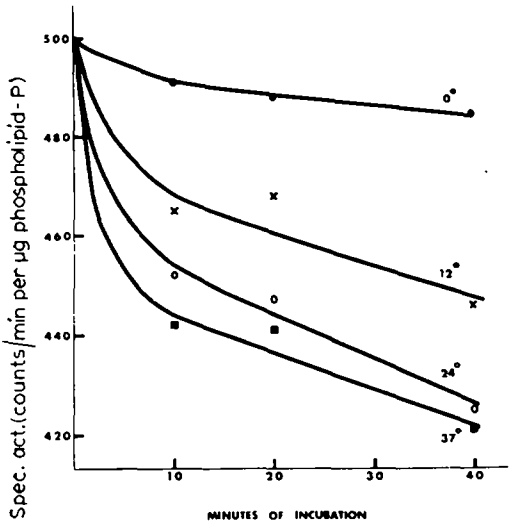

Fig. 2. The effect of time and temperature of incubation on the exchange of phospholipids between labeled mitochondria and unlabeled 105000 × *g* supernatant fraction as reflected in the specific activity of the mitochondrial phospholipid-P. The incubation mixture contained 105 µg mitochondrial phospholipid-P and 30 µg 105000 × *g* supernatant phospholipid-P in a total volume of 8 ml 0.25 M sucrose–1 mM EDTA (pH 7.4).

TABLE I

THE EXCHANGE OF PHOSPHOLIPIDS BETWEEN LABELED MITOCHONDRIA AND UNLABELED 105000 × *g* SUPERNATANT FRACTION

The incubation mixture contained 105 µg mitochondrial phospholipid-P and 30 µg 105000 × *g* supernatant phospholipid-P in a volume of 8 ml of 0.25 M sucrose–1 mM EDTA (pH 7.4). Incubations were performed at 37° for 40 min. The total ^{32}P in the individual phospholipids are measured directly on thin-layer chromatography scrapings (see MATERIALS AND METHODS). The figures in parentheses indicate the percentage of ^{32}P left in each individual phospholipid after incubation.

Individual phospholipids	*Distribution of total ^{32}P in individual mitochondrial phospholipids (counts/min)*		
	Before incubation	*After incubation for 40 min at 12°*	*After incubation for 40 min at 37°*
Phosphatidyl choline	23 862	20 062 (84.1%)	18 619 (78.0%)
Phosphatidyl ethanolamine	20 453	18 638 (91.1%)	17 616 (86.1%)
Cardiolipin	5 308	5 535 (104.3%)	5 442 (102.5%)

Exchange of phospholipids between mitochondria and microsomes

When unlabeled microsomes were added to the system containing labeled mitochondria and unlabeled 105000 × *g* supernatant fraction, differences between the rate of exchange of phosphatidyl choline and phosphatidyl ethanolamine became even more striking (Fig. 3). After 40 min of incubation at 37°, 57% of the mitochon-

drial phosphatidyl choline had exchanged compared to 20% phosphatidyl ethanolamine. The exchange of both phospholipids increased markedly with the temperature of incubation similar to that observed when mitochondria were incubated with supernatant alone.

The exchange of phospholipids between mitochondria and microsomes did not depend only on time and temperature of incubation but was also greatly influenced by the amount of pH 5.1 supernatant fraction added to the incubation medium (Fig. 4). Addition of increasing amounts of supernatant protein increased the phospholipid exchange reaction but did not increase the small (1%) release of membrane phospholipid into the medium. A little exchange took place in the absence of added supernatant protein as may be seen from the decrease in specific activity of mitochondrial phospholipid from 1300 to 1180. This might indicate that some exchange of phospholipids between mitochondria and microsomes occurs without intervention of the supernatant factor. Alternatively, some supernatant proteins might still be present in the subcellular particles even though in the present study microsomes and mitochondria were washed more thoroughly than in the previous study[1]. The washing procedure described in MATERIALS AND METHODS removed about 40% of the microsomal protein but no phospholipid.

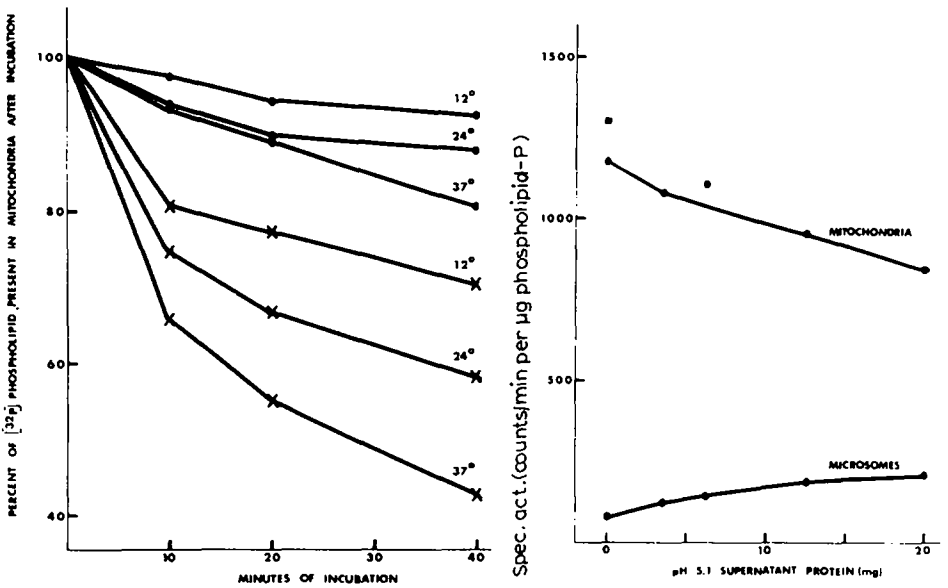

Fig. 3. The effect of time and temperature of incubation on the loss of phospholipid-^{32}P from labeled mitochondria incubated with unlabeled microsomes. The incubation mixture contained 133 μg mitochondrial phospholipid-P, 267 μg microsomal phospholipid-P and 17 μg 105000 $\times$ g supernatant phospholipid-P in the total volume of 8 ml 0.25 M sucrose–1 mM EDTA (pH 7.4). The percentages were calculated by comparing the counts present in each individual phospholipid after incubation with the counts present in the same phospholipid before incubation. ●—●, phosphatidyl ethanolamine; ×—×, phosphatidyl choline.

Fig. 4. The effect of various amounts of pH 5.1 supernatant protein on the exchange of phospholipids between labeled mitochondria and unlabeled microsomes as reflected by the specific activities of mitochondrial and microsomal phospholipid-P. 52 μg mitochondrial phospholipid-P were incubated with 96 μg microsomal phospholipid-P in the presence of pH 5.1 supernatant protein for 40 min at 37°. ■, specific activity of mitochondrial phospholipid-P before incubation.

Role of lipoproteins

Because the phospholipids in the 105000 × *g* supernatant fraction exchange with the phospholipids of mitochondria and microsomes (Fig. 1) it is likely that these phospholipids contribute to the exchange of phospholipids between subcellular particles. However, the presence of these phospholipids in an incubation medium containing mitochondria and microsomes is not crucial to the overall exchange process between subcellular particles as is seen in the following experiments. By precipitation of phospholipids and proteins from the 105000 × *g* supernatant fraction at a pH of 5.1 the phospholipid-P content of the supernatant decreased from 1.5 μg/ml to 0.05 μg/ml, a decrease of 97 %. A similar decrease was observed when the phospholipid-^{32}P was measured before and after adjustment of the pH. The protein content of the supernatant fraction fell from 7.8 mg/ml to 4.8 mg/ml. The resulting fraction was as active as the original supernatant fraction in the exchange of phospholipids between mitochondria and microsomes (Table II). On a mg protein basis the activity actually increased. The effect of this fraction cannot be duplicated by protein *per se*. Bovine serum albumin, for example, had no effect on the exchange (Table II). A number of protein fractions, representing the bulk of the 105000 × *g* supernatant protein, obtained during purification of the active factor also did not affect the exchange reaction.

Phospholipase C incubated with 105000 × *g* supernatant fraction broke down 85% of the phospholipid in this fraction. When the resulting fraction was tested phospholipid exchange was stimulated to the same extent as by the untreated supernatant fraction (Table III).

These two kinds of experiments show that lipoproteins of the 105000 × *g* supernatant are not important intermediates in the exchange of phospholipids between

TABLE II

THE EFFECT OF A PHOSPHOLIPID-DEPLETED pH 5.1 SUPERNATANT FRACTION ON THE EXCHANGE OF MITOCHONDRIAL AND MICROSOMAL PHOSPHOLIPID

The phospholipids in the 105000 × *g* supernatant fraction were removed by adjusting the pH of the supernatant fraction to 5.1 with 0.1 M HCl and centrifuging at 15000 × *g* for 15 min in the International centrifuge (PR-2). The clear supernatant fraction was dialyzed at 4° overnight against sucrose–EDTA (pH 7.4). 65 μg mitochondrial phospholipid-P were incubated with 105 μg microsomal phospholipid-P for 40 min. In Expt. I the microsomes were labeled, in Expt. II the mitochondria.

Expt. No.	*Protein added (mg)*	*Specific activity (counts/min per μg phospholipid-P)*	
		Mitochondria	*Microsomes*
I	0	106	2206
	15.6*	341	2027
	9.5**	368	2042
II	0	821	—
	10.0*	716	—
	10.0**	697	—
	10.0 albumin	817	—

* Original 105000 × *g* supernatant protein.

** pH 5.1 supernatant protein. In Expt. I, 2 ml of supernatant fraction contained 15.6 mg protein before and 9.5 mg after precipitation at pH 5.1.

mitochondria and microsomes. Apparently some other soluble factor is involved in the exchange reaction. This factor is probably not identical with the protein described by ROHEIM *et al.*[18]. In their experiments a protein, isolated from plasma, reacted with liver lipids and formed stable lipid–protein complexes resembling plasma lipoproteins. In our experiments the active factor, present in the particle-free supernatant fraction, did not increase the phospholipid concentration in the supernatant fraction during incubation with mitochondria and microsomes. It is possible, of course, that a labile lipid–protein complex might serve as an intermediate in the exchange process.

TABLE III

THE EFFECT OF A PHOSPHOLIPASE-C-TREATED SUPERNATANT FRACTION ON THE EXCHANGE OF MITOCHONDRIAL AND MICROSOMAL PHOSPHOLIPIDS

Preincubation: 105000 × *g* supernatant fraction (19 μg phospholipid-P) was incubated for 2 h at 37° in 25 mM $CaCl_2$ and 0.1 ml of a phospholipase C (EC 3.1.4.3) solution (1 mg protein per 0.1 ml, Worthington Biochemical Corp.). After incubation the mixture was dialyzed overnight against 4 l of sucrose–EDTA to inactivate the enzyme by removing the $CaCl_2$. 85% of the phospholipid was hydrolyzed. 102 μg labeled mitochondrial phospholipid-P (specific activity 580) were incubated with 130 μg microsomal phospholipid-P and 105000 × *g* supernatant fraction (44 mg protein) for 60 min in a total volume of 8 ml sucrose–EDTA.

105000 × g supernatant fraction	*Specific activity (counts/min per μg phospholipid-P)*	
	Mitochondria	*Microsomes*
Untreated (19 μg phospholipid-P)	437	111
Preincubated with 1 mg phospholipase C (3 μg phospholipid-P)	451	114
1 mg phospholipase C*	441	111

* 1 mg phospholipase C was added to untreated 105000 × *g* supernatant fraction (19 μg phospholipid-P) at the start of the exchange process. The phospholipase C concentration matched the enzyme content of the preincubated supernatant fraction.

Properties of the active factor

The active factor in the 105000 × *g* supernatant fraction is probably a protein because it is nondialyzable, precipitable with $(NH_4)_2SO_4$, temperature sensitive and digestible by trypsin. Table IV shows that preincubation of the 105000 × *g* supernatant fraction at increasing temperatures decreased its effect on exchange progressively.

Table V shows that digestion of the protein in the pH 5.1 supernatant fraction with trypsin markedly reduced the ability of the supernatant fraction to promote exchange. During the digestion with trypsin 60% of the protein remained inside the dialysis bag. This fraction showed only about 40% of the original exchange stimulating activity. The trypsin and trypsin inhibitor mixture had no effect on phospholipid exchange.

The molecular weight of the active protein appears to be somewhat less than that of hemoglobin because the active material was eluted from a Sephadex G-100 column after hemoglobin.

TABLE IV

THE EFFECT OF TEMPERATURE ON THE ACTIVITY OF THE 105000 × *g* SUPERNATANT FRACTION

The 105000 × *g* supernatant fraction (Expt. I) or its pH 5.1 supernatant fraction (Expt. II) were preincubated for 10 min at the indicated temperatures. In Expt. I, 70 μg labeled mitochondrial phospholipid-P were incubated with 121 μg microsomal phospholipid-P and with heated 105000 × *g* supernatant fraction (10 mg protein) for 40 min. In Expt. II, 105 μg labeled microsomal phospholipid-P were incubated with 65 μg mitochondrial phospholipid-P and with heated pH 5.1 supernatant fraction (10 mg protein) for 40 min. Incubation volume, 4 ml.

Expt. No.	*Heating of supernatant*	*Specific activity**		*Δ Specific activity**	*Decrease in exchange*** (%)
		Mitochondria	*Microsomes*		
I		630***	—		
	None	501	—	129	
	50°	516	—	114	12
	60°	566	—	64	50
	70°	590	—	40	69
	90°	610	—	20	85
II		—	2206***		
	None	—	2042	164	
	45°	—	2052	154	6
	60°	—	2128	78	52
	75°	—	2177	29	82
	100°	—	2194	12	93

* Counts/min per μg phospholipid-P.

** The decrease in exchange was calculated by setting the specific activity in the case of untreated supernatant at 100%.

*** Specific activities after incubation of mitochondria and microsomes without supernatant.

TABLE V

THE EFFECT OF TRYPSIN DIGESTION ON THE ACTIVITY OF THE pH 5.1 SUPERNATANT PROTEIN

pH 5.1 supernatant protein (15 mg) was digested with 2 mg trypsin (EC 3.4.4.4; Sigma Chemical Co.) in 0.135 M KCl–50 mM Tris buffer (pH 8.0). Digestion was carried out at 5° overnight while the mixture was dialyzed against 2 l of the same buffer. Samples without enzyme or with enzyme and twice the amount of trypsin inhibitor (Sigma Chemical Co.) were dialyzed simultaneously. All samples were subsequently dialyzed for 24 h against sucrose–EDTA (pH 7.4). Labeled mitochondria (12.5 mg protein, specific activity of phospholipid-P = 1127) were incubated with microsomes (5.0 mg protein) in the presence of pH 5.1 supernatant fraction at 37° for 40 min in 4 ml of sucrose–EDTA.

pH 5.1 supernatant	*Mitochondria*		*Decrease in exchange* (%)
	*Specific activity**	*Δ Specific activity**	
None	1067	—	—
Untreated (12.5 mg protein)	900	167	0
Trypsin digested**	1003	64	62
Trypsin nondigested***	932	135	19

* Counts/min per μg phospholipid-P.

** After trypsin digestion of 12.5 mg protein, trypsin inhibitor was added before dialysis against sucrose–EDTA.

*** Trypsin and trypsin inhibitor were added simultaneously to 12.5 mg of supernatant protein.

TABLE VI

EXCHANGE OF PHOSPHOLIPIDS BETWEEN MITOCHONDRIA AND PLASMA LIPOPROTEINS

Expt. No.	*Time of incubation (min)*	*Incubation mixture**			*After incubation*			
		Mitochondria (μg phospholipid-P)	*Lipoprotein (μg phospholipid-P)*	*pH 5.1 supernatant (mg protein)*	*Supernatant*		*Mitochondria spec. act.** (counts/min per μg phospholipid-P)*	*Mitochondrial phospholipid-P exchanged*** (%)*
					Phospholipid-P (μg)	*Phospholipid-^{32}P (counts/min)*		
I	20	54	—	—	3.4	2 806	1000	—
		54	16.8	—	21.5	6 525	965	3.5
		54	16.8	17.2	20.7	9 003	913	8.7
II	20	63	—		1.4	1 017	1000	—
		63	—	17.3	1.6	1 124	1017	—
		63	20.7	—	22.1	4 702	965	3.5
		63	20.7	17.3	23.5	6 820	921	7.9
III	40	68	—		1.9	1 631	1000	—
		68	—	10.1	2.2	1 659	990	—
		68	39.5	—	48.4	11 491	974	2.6
		68	39.5	10.1	46.9	13 583	888	11.2

* The components of the incubation mixture were suspended in 4 ml of 135 mM KCl–50 mM Tris–1 mM EDTA (pH 7.4).

** The mitochondrial specific activities and the phospholipid-^{32}P in the supernatant were calculated relative to a specific activity of 1000 for the original mitochondria.

*** % mitochondrial phospholipid-P exchanged is $\frac{\Delta \text{ specific activity}}{1000} \times 100$.

Exchange between mitochondria and plasma lipoproteins

TARLOV[14] demonstrated the exchange of phospholipids between mitochondria and plasma lipoproteins. It was, therefore, of interest to determine whether the active factor in the 105000 × *g* supernatant fraction would stimulate this exchange process. Table VI shows the results of several such experiments. Exchange of phospholipid is demonstrated by the decrease in mitochondrial phospholipid specific activity after incubation of mitochondria with plasma lipoproteins. In the presence of pH 5.1 supernatant fraction the extent of exchange increased 2–3-fold after 20 min and 4–5-fold after 40 min of incubation.

The phospholipid-^{32}P recovered in the 15000 × *g* supernatant fraction after incubation is the sum of the phospholipid-^{32}P transferred from the mitochondria in the exchange reaction and the net release of phospholipid-^{32}P from the mitochondria. A net release of phospholipid from mitochondria into the supernatant fraction is apparent, especially after 40 min of incubation in which the supernatant phospholipid increased from 39.5 to 48.4 μg. Additional experiments showed, however, that after centrifugation at higher speed (60 min at 105000 × *g*) the phospholipid-^{32}P in the mitochondrial supernatant after incubation was almost exclusively due to the exchange reaction as no increase in the phospholipid-P in this supernatant was detected.

CONCLUSIONS

The experiments show that phospholipid exchange is greatly increased by a protein-like material in the 105000 × *g* supernatant fraction. Apparently the protein is not a lipoprotein which shuttles phospholipid back and forth between subcellular particles. This is shown by the observation that removal of 97% of the phospholipid from the 105000 × *g* supernatant fraction by precipitation at pH 5.1 or the removal of 85% by phospholipase C did not diminish the effectiveness of the supernatant fraction on phospholipid exchange. Although these observations do not exclude the possibility that the protein in the 105000 × *g* supernatant fraction acts as a molecular carrier for phospholipid, other mechanisms should be considered. In principle one possibility is that the pH 5.1 supernatant fraction might release a "carrier" phospholipid from the mitochondria or microsomes by a proteolytic or detergent action. No such release was observed, however. The protein in the pH 5.1 supernatant fraction might weaken the lipid–lipid or lipid–protein interaction in the membrane or it might serve to facilitate the formation of a collision complex between mitochondria and microsomes, or between subcellular particles and soluble lipoproteins.

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