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Biosynthetic pathways in the formation of individual molecular species of rat liver phospholipids

Recent investigations on the metabolism of the individual molecular species of rat liver phospholipids suggested that the arachidonic acid present in phosphatidylcholine and phosphatidylethanolamine is introduced mainly through acylation of the corresponding 1-acyl lysoderivatives[1-5]. A *de novo* synthesis as established by KENNEDY[6] would be more responsible for the formation of molecules containing linoleic and oleic acid. In order to investigate the relative contributions of both pathways to the synthesis of the various molecular species of phospholipids, rat liver microsomes and slices were incubated in the presence of both [2-^{3}H]glycerophosphate or [2-^{3}H]glycerol and [1-^{14}C]fatty acids[7].

Rat liver microsomes were isolated by differential centrifugation as described elsewhere[7]. About 5 mg of protein, as determined by the method of LOWRY *et al.*[8], were incubated with 50 nmoles of [^{3}H]glycerophosphate and 20 nmoles of ^{14}C-labelled fatty acids in the presence of 25 μmoles ATP, 0.3 μmole CoA and 0.125 M KCl–0.02 M Tris (pH 7.4). The total volume was 2 ml. Rat liver slices (200 mg) were incubated under O_2–CO_2 (95:5, v/v) atmosphere in 3 ml of Krebs–Ringer solution (Ca^{2+} omitted) in the presence of 200 nmoles [2-^{3}H]glycerol and potassium salts of [1-^{14}C]fatty acids, which were complexed with albumin[9]. After the indicated periods of incubation the slices were extracted according to the procedure of BLIGH AND DYER[10]. Known aliquots of the extracted lipids were applied on thin-layer plates for the isolation of phosphatidylcholine, phosphatidylethanolamine, diglycerides, and phosphatidic acid as described before[2,5].

Phosphatidylcholine and phosphatidylethanolamine were separated into their different molecular classes by conversion with phospholipase C from *Bacillus cereus* and subsequent fractionation of the diglycerides formed, on silver nitrate-impregnated silica plates[2]. The various species of phosphatidic acid could be resolved by argentation chromatography of their dimethyl derivatives[5,11]. After the elution of the isolated products, radioactivity incorporated was measured by means of a Packard-Tricarb instrument using an external standard for quenching corrections.

Table IA shows the ratios of [^{3}H]glycerophosphate and [^{14}C]fatty acids incorporated by rat liver microsomes which had been incubated in both simultaneously. The very low ratios observed for phosphatidylcholine and phosphatidylethanolamine compared with the high ratios of phosphatidic acid and diglycerides demonstrate that in isolated microsomes, uptake of fatty acids in phosphatidylcholine and phosphatidylethanolamine proceeds mainly *via* acylation of endogenous lysophospholipids. Similar results were obtained when rat liver homogenates were used as enzyme source. Homogenisation and isolation of cell particles may induce an impairment of the *de novo* synthesis or an increase of endogenous monoacylphosphoglycerides.

To investigate whether rat liver slices are still capable of the *de novo* synthesis, slices were incubated for the indicated periods with [^{3}H]glycerol and [^{14}C]palmitate. As shown in Table IB a relatively high uptake of glycerol into phosphatidic acid could already be seen after 2.5 min, whereas phosphatidylcholine, phosphatidyl-

TABLE I

THE SIMULTANEOUS INCORPORATION OF [1-^{14}C]FATTY ACIDS AND [2-^{3}H]GLYCEROPHOSPHATE OR [2-^{3}H]GLYCEROL INTO RAT LIVER PHOSPHOLIPIDS AFTER INCUBATION WITH MICROSOMES (A) AND SLICES (B)

A. Microsomes. 20 nmoles of the [^{14}C]fatty acid indicated and 50 nmoles of [^{3}H]glycerophosphate were incubated with 5 mg of microsomal protein for 30 min. The ratios of incorporated glycerophosphate (^{3}H) and fatty acids (^{14}C) are presented.

Fatty acid	*Phosphatidylcholine*	*Phosphatidylethanolamine*	*Diglycerides*	*Phosphatidic acid*
16:0	0.07	0.17	1.60	1.76
18:0	0.22	0.09	—*	13.8
18:1	0.16	0.27	6.0	7.8
18:2	0.11	0.20	2.6	2.6

B. Slices. 200 nmoles of [^{14}C]palmitate and 200 nmoles of [^{3}H]glycerol were incubated with 200 mg of rat liver slices for the period of time indicated. The ratios of incorporated glycerol (^{3}H) and fatty acids (^{14}C) are presented.

Incubation time (min)	*Phosphatidylcholine*	*Phosphatidylethanolamine*	*Diglycerides*	*Phosphatidic acid*
2.5	—	—	—	12.1
5	1.28	1.42	—*	10.9
10	1.65	1.59	3.3	6.2
15	1.82	3.54	4.5	4.8

* Not determined.

ethanolamine and diglycerides were not yet labelled. After prolonged incubation, however, the ratio of incorporated glycerol to fatty acid decreases for phosphatidic acid and increases for phosphatidylcholine and phosphatidylethanolamine. When the isotopic ratios of phosphatidylcholine and phosphatidylethanolamine after 15 min incubation are compared with that of phosphatidic acid, it is evident that rat liver slices, in sharp contrast to isolated particles, are capable of considerable *de novo* synthesis of phosphatidylcholine and phosphatidylethanolamine. In agreement with the previous work of SCHERPHOF[12] and POSSMAYER *et al.*[5] on the positional distribution of the incorporated fatty acids upon the addition of glycerophosphate to rat liver microsomes and the studies of HILL *et al.*[3] on rat liver slices, it was found that

TABLE II

THE INCORPORATION OF [^{3}H]GLYCEROL AND [^{14}C]STEARATE INTO THE MAJOR MOLECULAR SPECIES OF RAT LIVER PHOSPHATIDIC ACID, PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE AFTER SIMULTANEOUS INCUBATION WITH RAT LIVER SLICES FOR 45 min

The detailed composition of the incubation mixture is described in the text. Results are expressed as nmoles.

		Total species	*Tetraenoic species*	*Dienoic species*	*Monoenoic species*	*Disaturated Species*
Phosphatidic acid	[^{3}H]Glycerol	3.12	0.28	1.21	0.89	0.26
	[^{14}C]Stearate	0.94	0.10	0.21	0.20	0.27
	Ratio ^{3}H/^{14}C	3.32	2.80	5.76	4.45	0.95
Phosphatidylcholine	[^{3}H]Glycerol	18.61	3.30	8.62	2.86	0.80
	[^{14}C]Stearate	11.39	2.88	2.20	0.79	0.62
	Ratio ^{3}H/^{14}C	1.63	1.15	3.92	3.62	1.29
Phosphatidylethanolamine	[^{3}H]Glycerol	6.30	1.35	2.69	1.01	0.18
	[^{14}C]Stearate	3.54	1.53	0.84	0.27	0.31
	Ratio ^{3}H/^{14}C	1.78	0.89	3.20	3.73	0.58

glycerol, after conversion into glycerophosphate, is esterified in a nonrandom fashion. Table II presents the amounts of [^{3}H]glycerol and [^{14}C]stearate incorporated into the individual molecular species of phosphatidic acid, phosphatidylcholine and phosphatidylethanolamine. It is clear from the data presented that the arachidonic acid-containing species of phosphatidylcholine and phosphatidylethanolamine have a much lower ^{3}H/^{14}C ratio than the mono- and dienoic molecules. This implies that at least in rat liver slices, a *de novo* synthesis *via* phosphatidic acid is highly operative for the formation of mono- and dienoic molecular species of phosphatidylcholine and phosphatidylethanolamine. However, as may be concluded from the relatively low incorporation of glycerol, the polyunsaturated molecules are synthesized mainly by acylation of endogenous lysophospholipids. Further work on the contributions of both pathways to phospholipid synthesis in other tissues and other animal species is in progress.

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Aldosterone biosynthesis by human fetal adrenal *in vitro*

Though adrenal tissue from a 32-week-old human fetus was capable of converting labelled progesterone to labelled aldosterone, incubations of adrenals of younger fetuses were not able to make this transformation[1]. More recently PASQUALINI *et al.*[2] reported the presence of [^{3}H]aldosterone in the adrenals of a 20-week-old fetus perfused with [1,2-$^3H_2$]corticosterone. We have investigated the conversion of [4-^{14}C]-