Phospholipid Synthesis in Rat Liver Endoplasmic Reticulum after the Administration of Phenobarbitone and 20-Methylcholanthrene

By SUSAN C. DAVISON and ERIC D. WILLS

Department of Biochemistry and Chemistry, The Medical College of St. Bartholomew's Hospital,
Charterhouse Square, London EC1M 6BQ, U.K.

(Received 27 December 1973)

1. Phenobarbitone injection did not affect the concentration of phospholipids in the liver endoplasmic reticulum, but it increased the rate of incorporation of [32 P]orthophosphate into the phospholipids. 20-Methylcholanthrene caused a transient increase in total phospholipid but a decrease in the turnover rate of the phospholipids. 2. Incorporation of [32 P]orthophosphate into phosphatidylcholine, compared with that into phosphatidylethanolamine, was increased by phenobarbitone injection but decreased by 20-methylcholanthrene injection. 3. The activity of *S*-adenosylmethionine–phosphatidylethanolamine methyltransferase increased 12 h after phenobarbitone injection, when incorporation of [32 P]orthophosphate into phosphatidylcholine was a maximum, but at other times, and after 20-methylcholanthrene injection, the activity of the enzyme did not correlate with the rate of phosphatidylcholine synthesis. 4. [14 C]Glycerol was incorporated more rapidly into phosphatidylcholine than into phosphatidylethanolamine, whereas [32 P]orthophosphate and [14 C]ethanolamine were incorporated more rapidly into phosphatidylethanolamine than into phosphatidylcholine. 5. Incorporation of [32 P]orthophosphate into phosphatidylethanolamine of liver slices incubated *in vitro* was much more rapid than into phosphatidylcholine, and incorporation into phosphatidylcholine was markedly stimulated by addition of methionine to the medium. Changes in the incorporation of [32 P]orthophosphate into phospholipids observed *in vivo* after injection of phenobarbitone or methylcholanthrene could not be reproduced in slices incubated *in vitro*. 6. It is concluded that phenobarbitone injection causes an increased rate of turnover of total phospholipids in the endoplasmic reticulum and an increased conversion of phosphatidylethanolamine into phosphatidylcholine, whereas 20-methylcholanthrene injection depresses both the turnover rate of total phospholipids and the formation of phosphatidylcholine.

Induction of drug-metabolizing enzymes in the liver by injection of phenobarbitone is accompanied by proliferation of membranes of the endoplasmic reticulum (Remmer & Merker, 1963) and increased synthesis of phospholipids of the membrane, which can be demonstrated by the rapid incorporation of [32 P]orthophosphate (Orrenius *et al.*, 1965). Phenobarbitone may also cause a redistribution of phospholipid species in the membrane by stimulating the methylation of phosphatidylethanolamine to form phosphatidylcholine (Young *et al.*, 1971).

In view of the marked differences in phospholipid species and fatty acid composition that occur after injection of phenobarbitone and 20-methylcholanthrene (Davison & Wills, 1974) we have now made a study of the biosynthesis of phospholipids in the endoplasmic reticulum after injection of these two drugs.

Materials and Methods

Materials

Sources of most chemicals used have been described (Davison & Wills, 1974). Adenosyl-L-[Me - 14 C]-methionine (150 μ Ci/mmol), [2- 14 C]ethanolamine (30 mCi/mmol), [1- 14 C]glycerol (26 mCi/mmol) and [32 P]orthophosphate (high specific radioactivity) were supplied by The Radiochemical Centre, Amersham, Bucks., U.K.

[32 P]Orthophosphate (1 mCi/120 g of rat), [14 C]ethanolamine (50 μ Ci/kg) and [14 C]glycerol (100 μ Ci/kg) were injected intraperitoneally in 0.9% NaCl solution.

Animals

Male Wistar rats, each weighing 120–140 g, were injected as described by Davison & Wills (1974).

Animals were matched for weight to within 3g for experiments involving the injection of radioactive precursors.

Methods for preparation of the microsomal fraction, separation of lipids and protein determinations

These have been described (Davison & Wills, 1974).

Measurement of radioactivity

Phospholipids were separated by t.l.c. as described (Davison & Wills, 1974) and the radioactive spots were scanned with a Panax radiochromatogram scanner linked to an integrator. The radioactivity attributed to each phospholipid was summed and expressed as a percentage of the total phospholipids after correction for background radioactivity.

After scanning, phospholipid samples were scraped off into vials containing 0.4% BBOT [2,5-bis-(5-*t*-butylbenzoxazol-2-yl)thiophen] in toluene and were counted for radioactivity in a Packard liquid-scintillation spectrophotometer. Counts were corrected for quenching.

Phospholipid phosphorus

This was measured by the method of Rouser *et al.* (1966) and neutral lipids were determined by the method of Malins & Mangold (1960).

Assay of S-adenosylmethionine-phosphatidylcholine methyltransferase

This enzyme was assayed by the method of Young *et al.* (1971).

Liver tissue slices

Livers were perfused *in situ* with modified Locks solution (Hill *et al.*, 1968) via the hepatic portal vein. Slices (0.25 mm thick) were cut with a McIlwain tissue chopper and transferred to Petri dishes containing freshly oxygenated Krebs-Ringer bicarbonate buffer with the CaCl_2 concentration lowered to 55mM (Laser, 1961).

Liver slices (250mg) were incubated in 5.0ml of the Krebs-Ringer bicarbonate solution under $\text{O}_2 + \text{CO}_2$ (95:5) in a shaking water bath at 37°C.

Results

Effect of phenobarbitone and 20-methylcholanthrene on [^{32}P]orthophosphate incorporation into total phospholipids and on the distribution of [^{32}P]orthophosphate among the phospholipid classes

[^{32}P]Orthophosphate (1mCi) was injected into rats that had received either phenobarbitone or 20-methylcholanthrene (100mg/kg) 3, 6, 12 or 24h previously. At 1h after injection of [^{32}P]orthophos-

phate the microsomal fraction was prepared, the lipids were extracted, separated by t.l.c. and the radioactivity was measured by quantitative thin-layer radiochromatogram scanning. Phospholipids were determined by measuring the phosphate produced by digesting the phospholipid with 70% (w/v) HClO_4 (Rouser *et al.*, 1966).

At 6–12h after phenobarbitone injection the total phospholipid phosphorus was unchanged but the specific radioactivity of the phospholipids increased. After 24h, however, the quantity of phospholipid recovered from the microsomal fraction/g of liver had increased, but the specific radioactivity of the phospholipids had reverted to a value close to the control (Fig. 1*a*). The increased specific radioactivity of the phospholipids was mainly due to increase in incorporation into the phosphatidylcholine fraction, which was a maximum 12h after phenobarbitone injection (Fig. 1*a*).

At 6h after 20-methylcholanthrene injection the quantity of phospholipid recovered from the endoplasmic reticulum was increased but the incorporation of [^{32}P]orthophosphate into the phospholipid decreased. Both [^{32}P]orthophosphate incorporated and total phospholipid returned to control values after 12h, but this recovery was accompanied by a marked redistribution of [^{32}P]orthophosphate. Incorporation into phosphatidylethanolamine was greatly increased and incorporation into phosphatidylcholine decreased (Fig. 1*b*).

S - Adenosylmethionine - phosphatidylethanolamine methyltransferase activity in liver endoplasmic reticulum prepared from rats injected with phenobarbitone or 20-methylcholanthrene

The increased incorporation of labelled phosphate into phosphatidylcholine that occurred 12h after phenobarbitone injection could be the result of increased activity of the enzymes transmethyating phosphatidylethanolamine to phosphatidylcholine, and the activity of this enzyme was measured in the microsomal fraction after injection of phenobarbitone and methylcholanthrene.

The specific activity of S-adenosylmethionine-phosphatidylethanolamine methyltransferase increased to 130% of the control value 12h after the first injection of phenobarbitone (Table 1) at the same time as incorporation of [^{32}P]orthophosphate into phosphatidylcholine was a maximum (Fig. 1*a*). By 24h after the injection, the activity of the enzyme was no different from that of the controls and after 3 days it was decreased. Both 24 and 72h after injection of methylcholanthrene the enzyme activity was decreased (Table 1). Incorporation of [^{32}P]orthophosphate into phosphatidylcholine was decreased after 12h but unchanged after 24h (Fig. 1*b*).

Fig. 1. Effect of phenobarbitone or 20-methylcholanthrene on the incorporation of $[^{32}\text{P}]$ orthophosphate into the phospholipids of the liver endoplasmic reticulum

Phenobarbitone (a) or 20-methylcholanthrene (b) (100 mg/kg) was injected and the animals were killed for the preparation of microsomal phospholipids at the times specified. ●, Total phospholipid P (% of control); ○, specific radioactivity of phospholipids (% of control); □, $[^{32}\text{P}]$ orthophosphate in phosphatidylethanolamine (% of total incorporated into phospholipids); ■, $[^{32}\text{P}]$ orthophosphate in phosphatidylcholine (% of total incorporated into phospholipids).

Table 1. S-Adenosylmethionine-phosphatidylethanolamine methyltransferase activity in liver microsomal fractions from rats injected with either phenobarbitone or 20-methylcholanthrene

Phenobarbitone (100 mg/kg per day) was injected daily for 3 days, or single injections of 20-methylcholanthrene (either 100 mg/kg or 20 mg/kg) were given. The values are averages $\pm$ S.E.M. of quadruplicate measurements from at least four animals. P values greater than 0.05 are shown as N.S. (not significant).

Time after first injection (h)	Activity (d.p.m./mg of protein)			
	Control	+20-Methylcholanthrene		+Phenobarbitone (100 mg/kg)
		(100 mg/kg)	(20 mg/kg)	
12	4528 $\pm$ 123 (100 $\pm$ 2.7%)	4399 $\pm$ 136 (97.0 $\pm$ 3.0%) N.S.	—	5921 $\pm$ 201 (130.8 $\pm$ 4.4%) P < 0.001
24	6955 $\pm$ 218 (100 $\pm$ 3.1%)	5857 $\pm$ 105 (84.2 $\pm$ 3.0%) P < 0.001	6393 $\pm$ 112 (91.9 $\pm$ 1.6%) P < 0.05	7033 $\pm$ 187 (101.1 $\pm$ 2.7%) N.S.
72	6564 $\pm$ 76 (100 $\pm$ 1.1%)	5881 $\pm$ 158 (89.6 $\pm$ 2.4%) P < 0.001	5915 $\pm$ 195 (90.1 $\pm$ 3.0%) P < 0.01	5677 $\pm$ 262 (85.9 $\pm$ 4.0%) P < 0.01

Incorporation of $[^{14}\text{C}]$ ethanolamine into the phospholipids of liver and the microsomal fraction after injection of phenobarbitone and 20-methylcholanthrene

At 12, 24 or 72 h before being killed, rats were injected with either 100 mg of phenobarbitone/kg

per day or a single injection of 100 mg of 20-methylcholanthrene/kg, and 1 h before being killed they were injected with 50 μCi of $[2\text{-}^{14}\text{C}]$ ethanolamine/kg. Lipids were extracted from both whole livers and the liver microsomal fraction. They were

Table 2. Incorporation of [^{14}C]ethanolamine into phosphatidylcholine in the endoplasmic reticulum and whole liver of rats treated with either phenobarbitone or 20-methylcholanthrene

Phenobarbitone (100 mg/kg) was injected daily for 3 days or a single injection (100 mg/kg) of 20-methylcholanthrene was given. [^{14}C]ethanolamine was injected 1 h before the animals were killed. Incorporation of [^{14}C]ethanolamine into phosphatidylcholine is expressed as:

$$\frac{\text{counts in phosphatidylcholine} \times 100\%}{\text{counts in phosphatidylcholine} + \text{phosphatidylethanolamine}}$$

The values are averages $\pm$ S.E.M. of duplicate measurements from at least four animals.

Injection	Time after injection (h)	Percentage of phospholipid [^{14}C]ethanolamine incorporated into phosphatidylcholine	
		Whole liver	Endoplasmic reticulum
Control		9.53 $\pm$ 0.33 (100.0 $\pm$ 3.4%)	9.36 $\pm$ 0.67 (100.0 $\pm$ 7.2%)
Phenobarbitone	12	9.69 $\pm$ 0.25 (101.6 $\pm$ 2.6%)	8.7 $\pm$ 0.26 (92.9 $\pm$ 2.8%)
	72	5.41 $\pm$ 0.14 (56.7 $\pm$ 1.5%)	4.7 $\pm$ 0.14 (50.2 $\pm$ 1.5%)
20-Methylcholanthrene	12	11.84 $\pm$ 0.38 (124.2 $\pm$ 4.0%)	10.16 $\pm$ 0.23 (108.5 $\pm$ 2.5%)
	72	6.76 $\pm$ 0.09 (70.9 $\pm$ 0.9%)	6.65 $\pm$ 0.37 (71.0 $\pm$ 4.0%)

separated by t.l.c. and the radioactivity of the phospholipid spots was measured. As we have already established (Davison & Wills, 1974), the ratio of phosphatidylcholine/phosphatidylethanolamine was little changed after injection of phenobarbitone or 20-methylcholanthrene; the results of the experiment were expressed as a percentage of the [^{14}C]ethanolamine incorporation into phosphatidylcholine (Table 2). When measured by this method the rate of synthesis of phosphatidylcholine from phosphatidylethanolamine was unaffected 12 h after injection of phenobarbitone, but after 3 days the percentage of phospholipid [^{14}C]ethanolamine incorporated into phosphatidylcholine was decreased to 50% of control values (Table 2). In contrast, 12 h after injection of 20-methylcholanthrene the synthesis of phosphatidylcholine from phosphatidylethanolamine was increased by 25% in whole liver and by 8% in the endoplasmic reticulum, but it too was much less than the control after 3 days (Table 2).

The ratio of radioactivity (d.p.m.) of phosphatidylcholine/radioactivity (d.p.m.) of phosphatidylethanolamine was always greater in the whole liver phospholipid than in the endoplasmic reticulum (Table 2). Phosphatidylcholine is transported out of the endoplasmic reticulum more rapidly than phosphatidylethanolamine (McMurray & Dawson,

1969) and this transport may account for some of the differences observed.

Comparison between [^{14}C]glycerol and [^{32}P]orthophosphate as precursors for the study of glycerolipid synthesis in rat liver

To study the incorporation of [^{14}C]glycerol into phospholipids, rats were injected intraperitoneally with 100 μCi of [^{14}C]glycerol/kg 30 or 60 min before they were killed. Lipids were extracted from the microsomal fraction, separated by t.l.c., the individual phospholipid spots scraped off and the radioactivity was measured.

The ratio of radioactivity (d.p.m.) in phosphatidylcholine to that in phosphatidylethanolamine was 2.26 after 30 min and 2.24 after 60 min (Table 3), and this may be compared with the molar ratio of 2.6 for these species in the endoplasmic reticulum. Thus the specific radioactivities of phosphatidylcholine and phosphatidylethanolamine are almost equal both 30 and 60 min after injection of [^{14}C]glycerol (Table 3). In contrast, after injection of [^{32}P]orthophosphate the radioactivity ratio of phosphatidylcholine/phosphatidylethanolamine was 0.73 (Fig. 1) and it was 0.103 after injection of ethanolamine (Table 2).

Phospholipid biosynthesis studied *in vitro* by using liver slices prepared from untreated animals

For comparison with studies *in vivo*, phospholipid synthesis was studied in liver slices *in vitro*. Slices (500 mg) were incubated in Krebs–Ringer bicarbonate medium at 37°C under an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5). [^{32}P]Orthophosphate (0.2 mCi) was added and slices were withdrawn at intervals for 60 min.

Table 3. [^{14}C]Glycerol incorporation into the glycerolipids of rat liver endoplasmic reticulum *in vivo*

Animals were killed 30 min or 60 min after injection of [^{14}C]glycerol. The values are averages $\pm$ S.E.M. of duplicate measurements from at least four animals.

Lipids	Incorporation (d.p.m./ μg of lipid)	
	30 min	60 min
	Percentage of label in total lipids at times after injecting labelled precursor	
Phosphatidic acid + neutral lipid	25.2 $\pm$ 0.2	14.0 $\pm$ 1.7
Phosphatidylethanolamine	22.1 $\pm$ 0.2	25.3 $\pm$ 0.5
Phosphatidylinositol + phosphatidylserine	2.9 $\pm$ 0.1	4.0 $\pm$ 0.1
Phosphatidylcholine	49.9 $\pm$ 0.5	56.7 $\pm$ 2.0

They were homogenized in 5ml of chloroform-methanol (1:1, v/v) and lipids were extracted by the method of Bligh & Dyer (1959). Total phospholipid phosphorus and radioactivity of the phospholipids were measured and the phospholipids were separated by t.l.c., scanned and scraped off the plates for radioactivity counting.

[³²P]Orthophosphate incorporation into phosphatidylcholine was much slower than into phosphatidylethanolamine during the first 30 min incubation (Fig. 2a), but after a 40 min preincubation period the rates were similar (Fig. 2b).
Incorporation of [¹⁴C]glycerol into phospholipids was studied by a procedure similar to that described

Fig. 2. Incorporation of [³²P]orthophosphate into phospholipids extracted from liver slices incubated *in vitro*

After incubation of the liver slices phospholipids were extracted and separated as described in the text. □, Total phospholipid; ▲, phosphatidylinositol; ○, phosphatidylcholine; ●, phosphatidylethanolamine; △, phosphatidic acid. (a) no preincubation; (b) after 40 min preincubation.

Table 4. Progress of incorporation of [¹⁴C]glycerol into the glycerolipids of rat liver slices *in vitro*

Slices were preincubated for 40 min before addition of [¹⁴C]glycerol. The values are averages ± s.e.m. of duplicate measurements from at least four animals

Lipids	Incorporation (d.p.m./μg of lipid)			
	15 min	30 min	45 min	60 min
	1759 ± 29	2122 ± 174	2610 ± 247	2235
Percentage of total radioactivity in glycerolipids				
Lipids	15 min	30 min	45 min	60 min
Neutral lipid	57 ± 3.4	53.2 ± 2.4	64.2 ± 2.8	63.9
Phosphatidic acid	12.6 ± 1.5	6.0	2.6	0.8
Phosphatidylethanolamine	12.1 ± 0.3	12.2 ± 0.3	12.7 ± 0.3	12.1
Phosphatidylinositol + phosphatidylserine	2.3	2.1	2.2	2.2
Phosphatidylcholine	16.1 ± 1.6	16.8 ± 1.5	18.4 ± 1.5	21.9
Phospholipids (Total)	42.2 ± 4.1	37.1 ± 2.3	35.8 ± 0.4	36.3

for [^{32}P]orthophosphate, but, as glycerol is also incorporated into neutral glycerol-lipids, the neutral lipids were also separated by the method of Malins & Mangold (1960).

Fig. 3. Effect of methionine on the incorporation of [^{14}C]-ethanolamine into phosphatidylcholine extracted from liver slices incubated *in vitro*

Slices were preincubated for 40 min before addition of [^{14}C]ethanolamine. After a further 60 min incubation the slices were removed, phospholipids were extracted and phosphatidylcholine was isolated by t.l.c.

The ratio of synthesis of phosphatidylcholine/phosphatidylethanolamine measured by the incorporation of [^{14}C]glycerol into the phospholipids after 60 min incubation was less in rat liver slices than *in vivo*. The ratio of radioactivity in phosphatidylcholine/phosphatidylethanolamine was 2.24 *in vivo* (Table 3) and 1.81 *in vitro* (Table 4) at 60 min.

In view of the fact that the synthesis of phosphatidylethanolamine involves transmethylation from *S*-adenosylmethionine the importance of the presence of adequate methionine was studied. Addition of methionine caused a greatly increased incorporation of [^{14}C]ethanolamine into phosphatidylcholine and 1 mM was an optimum concentration (Fig. 3).

Phospholipid biosynthesis in vitro after treatment with phenobarbitone or 20-methylcholanthrene

Groups of rats were injected with either phenobarbitone (100 mg/kg) or 20-methylcholanthrene (100 mg/kg) and 12 h later the animals were killed and liver slices prepared.

The incubation conditions used were those found to be optimal in control experiments. Methionine (1 mM) was added to the medium and slices were subjected to a preincubation period of 40 min after which [^{32}P]orthophosphate was incorporated into the medium and radioactive phospholipids were extracted and separated as described.

After 30 or 60 min incubation both phenobarbitone and 20-methylcholanthrene caused decreases in the incorporation of [^{32}P]orthophosphate into total phospholipids. After 60 min incubation both phenobarbitone and 20-methylcholanthrene caused significant increases in the [^{32}P]orthophosphate incorporation into phosphatidylethanolamine but smaller decreases in incorporation into phosphatidylcholine (Table 5).

Table 5. Incorporation of [^{32}P]orthophosphate into the phospholipids of liver slices *in vitro*, from rats that had been injected with either phenobarbitone or 20-methylcholanthrene

Phenobarbitone or 20-methylcholanthrene (100 mg/kg) was injected 12 h before killing. The values are averages $\pm$ S.E.M. of duplicate measurements from at least four animals.

Injection	Incubation time (min)	Incorporation (d.p.m./ μg of total phospholipid P)	Percentage of total radioactivity of phospholipids			
			Phosphatidic acid	Phosphatidylethanolamine	Phosphatidyl-inositol+phosphatidyl-serine	Phosphatidylcholine
None (control)	30	3065 $\pm$ 252	2.1 $\pm$ 0.5	52.5 $\pm$ 1.2	8.1 $\pm$ 0.2	37.4 $\pm$ 1.2
	60	8880 $\pm$ 741	1.5 $\pm$ 0.2	45.0 $\pm$ 1.3	6.8 $\pm$ 1.3	46.6 $\pm$ 1.1
Phenobarbitone	30	2549 $\pm$ 55	2.5 $\pm$ 0.7	52.0 $\pm$ 2.2	7.7 $\pm$ 0.3	36.7 $\pm$ 1.7
	60	8259 $\pm$ 538	1.0 $\pm$ 0.3	49.9 $\pm$ 2.0	6.1 $\pm$ 0.3	42.4 $\pm$ 1.8
20-Methylcholanthrene	30	2787 $\pm$ 186	2.5 $\pm$ 0.5	51.6 $\pm$ 0.6	7.7 $\pm$ 0.2	37.5 $\pm$ 0.7
	60	7447 $\pm$ 409	1.2 $\pm$ 0.3	48.6 $\pm$ 0.7	6.3 $\pm$ 0.1	43.8 $\pm$ 0.5

Discussion

The concentration of total phospholipids in the endoplasmic reticulum was not significantly altered 6–12h after administration of phenobarbitone but the rate of incorporation of [32 P]orthophosphate into the phospholipids was markedly stimulated, thus clearly demonstrating a rapid increase in turnover rate (Fig. 1a). 20-Methylcholanthrene, on the other hand, caused a transient increase in the total phospholipids but decreased the turnover rate (Fig. 1b). In addition to these effects on turnover rates both phenobarbitone and methylcholanthrene caused the ratio of incorporation of [32 P]orthophosphate into phosphatidylcholine/phosphatidylethanolamine to change.

The increased incorporation of [32 P]orthophosphate into phosphatidylcholine compared with that into phosphatidylethanolamine after phenobarbitone injection could have been the result of an increased rate of transmethylation of phosphatidylethanolamine.

At 12h after phenobarbitone injection the rate of synthesis of phosphatidylcholine in the liver endoplasmic reticulum, determined by measuring incorporation of [14 C]ethanolamine, was slightly decreased (Table 2), whereas simultaneously incorporation of [32 P]orthophosphate into phosphatidylcholine was at a maximum (Fig. 1a). At this same time after 20-methylcholanthrene injection the incorporation of [14 C]ethanolamine into phosphatidylcholine was increased (Table 2), but here it was accompanied by a decreased incorporation of [32 P]orthophosphate into phosphatidylcholine (Fig. 1b).

The activity of *S*-adenosylmethionine–phosphatidylethanolamine methyltransferase *in vitro* 12h after phenobarbitone injection increased (Table 1), simultaneously with the increased incorporation of [32 P]orthophosphate into phosphatidylcholine (Fig. 1a). However, after 24h the activity of the enzyme returned to control values and it decreased to 86% of control after 72h (Table 1), but at this time the incorporation of [14 C]ethanolamine into phosphatidylcholine was decreased to 50% of the control value (Table 2). Young *et al.* (1971) reported that the activity of *S*-adenosylmethionine–phosphatidylethanolamine methyltransferase increased after administration of phenobarbitone for 3 days, but as they killed their animals 12h after the final injection, it would appear that an activation 12h after the injection was observed, which does not conflict with our findings (Table 1).

At 12h after 20-methylcholanthrene injection the activity of *S*-adenosylmethionine–phosphatidylethanolamine methyltransferase was little affected (Table 1) when the incorporation of [32 P]orthophosphate into phosphatidylcholine was much decreased (Fig. 1b).

These experiments in general therefore do not

support the concept that transmethylation of phosphatidylethanolamine to phosphatidylcholine is an important rate-controlling step in the redistribution of phospholipid classes after injection of phenobarbitone or 20-methylcholanthrene. However, they do not invalidate the hypothesis if, *in vivo*, *S*-adenosylmethionine–phosphatidylethanolamine methyltransferase is not a rate-limiting enzyme or is subject to allosteric control.

Interpretations of phospholipid metabolism based on measurements of incorporation of [32 P]orthophosphate must be treated with some caution, however, because [14 C]glycerol, [32 P]orthophosphate and [14 C]ethanolamine were incorporated into liver phospholipids at very different rates.

In untreated animals glycerol was incorporated more rapidly into phosphatidylcholine than into phosphatidylethanolamine (Table 3), whereas [32 P]orthophosphate and [14 C]ethanolamine were more rapidly incorporated into phosphatidylethanolamine than into phosphatidylcholine (Fig. 1) and Table 2.

The ratio of phosphatidylcholine/phosphatidylethanolamine in the phospholipids of liver endoplasmic reticulum is approx. 2.65:1 (Davison & Wills, 1974) and the ratio of incorporation of glycerol into these phospholipids is 2.25:1. These values for glycerol incorporation into phosphatidylcholine and phosphatidylethanolamine are thus, to a reasonable approximation, close to the ratio of the normal phospholipid composition of the membranes and indicate that the two phospholipids are turning over at approximately the same rate. The ratio of incorporation of [32 P]orthophosphate into phosphatidylcholine/phosphatidylethanolamine is, however, 0.73 (Fig. 1). It is possible that this discrepancy between results obtained by using the two precursors is a result of the fact that, whereas the formation of phosphatidylcholine from CDP-choline and diglyceride is freely reversible, the formation of phosphatidylethanolamine from CDP-ethanolamine and diglyceride is not (Bjornstad & Bremer, 1966). Phosphatidylethanolamine labelled with [32 P]orthophosphate would not therefore readily be reconverted into CDP-ethanolamine, whereas CDP-choline is readily diluted with the unlabelled pool of phosphatidylcholine. Both phenobarbitone and 20-methylcholanthrene caused small decreases in the incorporation of [32 P]orthophosphate into total phospholipids (Table 5) in liver slices incubated *in vitro*, in contradiction of the marked increases observed *in vivo* (Fig. 1). It is evident that there are changes in phospholipid metabolism that are brought about by the process of preparing liver slices. Intermediates of phospholipid synthesis other than methionine may be essential and prevent the reproduction of the changes in [32 P]orthophosphate incorporation into phosphatidylcholine and phosphatidylethanolamine *in vitro* that had been observed *in vivo*.

Experiments with slices from untreated animals demonstrated that [^{32}P]orthophosphate was incorporated more rapidly into phosphatidylethanolamine than into phosphatidylcholine and the results would appear to support the view that phosphatidylethanolamine synthesis precedes that of phosphatidylcholine. However, as discussed above, this interpretation may not be valid and apparent rate differentials may merely reflect the different pool sizes of phosphatidylcholine and phosphatidylethanolamine.

It is unlikely that the rates of incorporation of [^{32}P]orthophosphate into individual phospholipids indicate precisely their rates of synthesis, but it would appear that the increase in the specific radioactivity of phospholipids observed 3–12h after phenobarbitone injection and the decrease in specific radioactivity after 20-methylcholanthrene injection reflect changes in the overall turnover rates of the phospholipids. 20-Methylcholanthrene is therefore considered to decrease the turnover rate of the phospholipids, and phenobarbitone is considered to increase it.

Experiments *in vitro* demonstrated that [^{32}P]orthophosphate reaches equilibrium with [^{32}P]phosphatidylethanolamine more rapidly than with [^{32}P]phosphatidylcholine and thus, initially, the ratio of [^{32}P]orthophosphate incorporated into phosphatidylethanolamine/[^{32}P]orthophosphate incorporated into phosphatidylcholine will be greater, the more slowly the phospholipids are turning over. Thus the early changes in the rates of incorporation of [^{32}P]orthophosphate into individual phospholipids

may be a consequence of changes in the overall turnover rates of the total phospholipids.

It would appear, however, that the increased rate of synthesis of phosphatidylcholine from phosphatidylethanolamine, observed 12h after 20-methylcholanthrene injection, is more likely to be a result of a transitory stimulation of this pathway.

S. C. D. thanks the Medical Research Council for a research studentship.

References

- Bjornstad, P. & Bremer, J. (1966) *J. Lipid Res.* **7**, 38–45
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
- Davison, S. C. & Wills, E. D. (1974) *Biochem. J.* **140**, 461–468
- Hill, E. E., Husbands, D. R. & Lands, W. E. M. (1968) *J. Biol. Chem.* **243**, 4440–4451
- Laser, H. (1961) in *Biochemist's Handbook* (Long, C., ed.), pp. 58–60, E. and F. N. Spon Ltd., London
- Malins, D. C. & Mangold, H. K. (1960) *J. Amer. Oil Chem. Soc.* **37**, 576–578
- McMurray, W. C. & Dawson, R. M. C. (1969) *Biochem. J.* **112**, 91–108
- Orrenius, S., Ericsson, J. L. E. & Ernster, L. (1965) *J. Cell Biol.* **25**, 627–639
- Remmer, H. & Merker, H. J. (1963) *Science* **142**, 1657–1658
- Rouser, G., Siakotos, A. N. & Fleischer, S. (1966) *Lipids* **1**, 85–86
- Young, D. L., Powell, G. & McMillan, W. O. (1971) *J. Lipid Res.* **12**, 1–8