Biochemical Pharmacology, Vol. 25, pp. 1255–1260. Pergamon Press, 1976. Printed in Great Britain.

EFFECT OF POLYCHLORINATED BIPHENYLS (PCBs) ADMINISTRATION ON PHOSPHOLIPID BIOSYNTHESIS IN RAT LIVER*

KOZO ISHIDATE and YASUO NAKAZAWA
Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

(*Received* 4 *August* 1975; *accepted* 8 *November* 1975)

Abstract—The effect of PCBs or phenobarbital on the biosynthesis of phospholipids in hepatic endoplasmic reticulum of rats was studied by the intraperitoneal injection of [^{32}P]orthophosphate, [Me-^{14}C]choline or [2-^{3}H]glycerol. Significant increases in liver microsomal phospholipid content after the administration of either PCBs or phenobarbital indicated the actual proliferation of endoplasmic reticulum membranes. The rate of both [^{32}P] and [^{14}C] incorporations into microsomal choline-containing phospholipids, such as phosphatidylcholine, sphingomyelin and lysophosphatidylcholine, was reduced to one fifth by PCBs administration compared with control animals. The incorporation of [^{32}P]orthophosphate into phosphatidylethanolamine or other phospholipid classes was less or not affected, respectively, by PCBs administration. The specific inhibitory effect of PCBs on the incorporation into choline-containing phospholipids was not observed when [2-^{3}H]glycerol was used as a precursor. Phenobarbital administration, however, increased significantly the rate of [^{32}P] incorporation into liver phospholipids, especially phosphatidylcholine. It is suggested that the increase in microsomal phospholipid content by PCBs administration is not due to the stimulation of synthesis but to the inhibition of the catabolism of membrane phospholipids and that the increase in content caused by phenobarbital is due at least in part, to the stimulation of synthesis. The possible site(s) of PCBs-induced inhibition of phospholipid biosynthesis in rat liver is discussed.

Induction of hepatic drug-metabolizing enzymes by administration of many kinds of lipophilic substances is accompanied by proliferation of the membranes of the endoplasmic reticulum [1, 2]. The proliferation of the membranes is characterized by an increase in both phospholipid and protein content.

Whether such lipophilic substances cause these effects mainly by enhancing synthesis or slowing catabolism, however, remains obscure with respect to membrane phospholipids.

Orrenius and co-workers [3–5] first found that phenobarbital markedly increased the rate of incorporation of [^{32}P]orthophosphate into phospholipids of rat liver microsomal fraction, and they suggested that enhanced phospholipid synthesis was an early step in the phenobarbital-induced proliferation of liver endoplasmic reticulum. Furthermore, Young *et al.* [6] and recently Davison and Wills [7] reported that the increased synthesis of phospholipid in the liver of phenobarbital-treated rats resulted from the stimulated synthesis of phosphatidylcholine via transmethylation of phosphatidylethanolamine. Holtzman and Gillette [8], however, failed to confirm these results and found that phenobarbital increased hepatic microsomal phospholipid by inhibiting phospholipid catabolism.

On the other hand, the important role that phospholipid, especially phosphatidylcholine, plays in the microsomal drug metabolizing system has been demonstrated by the finding that the preparation of solubilized components cannot function in a reconstituted system unless phospholipid is added [9, 10]. The fact that treatment of liver microsomes with phospholipases [11, 12] or with organic solvents [13, 14] causes a significant decrease of drug metabolizing activity also indicates the essential role of phospholipid.

The present studies were undertaken in order to characterize the alterations in phospholipid metabolism associated with lipophilic substance-induced proliferation of hepatic endoplasmic reticulum. Polychlorinated biphenyls (PCBs) was chosen as the substance because this highly lipophilic compound is known to be widely distributed in the environment and its toxic effects to animals, including man, have recently been documented [15]. Since PCBs has been shown to induce the activity of the hepatic mixed-function oxygenase system in a manner similar to phenobarbital [16–18], comparative studies were done with respect to the effects of both compounds on hepatic phospholipid metabolism.

The results indicate that the proliferation of endoplasmic reticulum membranes caused by the administration of lipophilic substances to rats is not necessarily accompanied by the stimulated synthesis of phospholipids in membranes.

*This work was supported in part by a scientific research grant from the Ministry of Education, Japan.

Abbreviations: PL, phospholipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Sph, sphingomyelin; DPG, diphosphatidylglycerol (cardiolipin); LPC, lisophosphatidylcholine; NL, neutral lipids; PB, phenobarbital.

EXPERIMENTAL

[Me-^{14}C]Choline (sp. act. 10 mCi/m-mole) and [2-^{3}H]glycerol (sp. act. 500 mCi/mM) were purchased from Daiichi Pure Chemicals Co. Ltd., Japan, and

the Radiochemical Centre, Amersham, England, respectively. Carrier free $H_3[^{32}P]O_4$ was purchased from Japan Atomic Energy Research Institute.

Male Wister rats weighing 80–100 g were used for all experiments. Rats were housed two per cage, fed Clea CE-2 Laboratory Chow, and allowed free access to water.

For oral administration, PCBs (Polychloro Biphenyl, tetra, obtained from Wako Pure Chem. Indust. Co. Ltd., Japan) and phenobarbital were made homogeneous in 2% (w/v) sodium carboxymethyl cellulose (CMC) solution by sonic treatment or by mixing in a glass mortar, respectively. Experimental animals were given 100 mg/kg/day of PCBs or 80 mg/kg/day of phenobarbital by gastric intubation once a day in the morning, and control animals were given equivalent volumes of 2% CMC. In order to assure equal food intake and negate the possible effect of altered feeding habit owing to the administration of the substance, food was removed from the cages of all rats after the morning administration, and returned in the evening.

At various times after the administration of the drugs, radioactive precursor (250 μCi $H_3[^{32}P]O_4$ in 0.5 ml of 0.9% NaCl solution containing phosphate, pH 7.4, 10 μCi [Me-^{14}C]choline chloride or 100 μCi [2-^{3}H]glycerol, each in 0.5 ml of 0.9% NaCl) was injected intraperitoneally into the control and the treated animals.

At different time intervals after the injection of precursor, the rats were killed by decapitation. The livers and kidneys were removed, washed twice with ice-cold 0.9% NaCl, weighed, chilled on ice, homogenized in 20 vol. of chloroform–methanol (2:1, v/v) containing a trace amount of butylated hydroxytoluene (BHT), and the lipids extracted according to the method of Folch *et al.* [19].

For the preparation of microsomes, animals were starved overnight before they were killed and the livers were excized and homogenized in 4 vol. of 1.15% (w/v) KCl containing 1 mM EDTA at 4°. The post-mitochondrial supernatant prepared by centrifuging the homogenate twice for 20 min at 9,000 *g* was recentrifuged for 60 min at 105,000 *g*. The pellet was used as the source of the liver endoplasmic reticulum. The microsomal lipids were extracted by the procedure mentioned above.

Phospholipids were fractionated either by DEAE-cellulose column chromatography combined with quantitative thin layer chromatography (t.l.c.) according to the method of Rouser *et al.* [20] or by two-dimensional t.l.c. developed with chloroform–methanol–28% aqueous ammonia (65:35:5), followed by chloroform–acetone–methanol–acetic acid–water (5:2:1:1:0.5) according to the method of Rouser, Simon & Kritchevsky [21], except that magnesium silicate-free adsorbent (Silica Gel H, Merck No. 7736) was used. The spots were identified by iodine vapor, scraped off and extracted with methanol and chloroform–methanol–acetic acid (50:40:2).

Phospholipid phosphorus was determined by the molybdate–ascorbic acid method after digesting with 70% (w/v) perchloric acid [22].

The radioactivity incorporated into the phospholipid fraction was counted in a Packard Tri-Carb model 2002 scintillation spectrometer using 10 ml of toluene with 0.4% (w/v) 2,5-diphenyl-oxazole (PPO) and 0.01% (w/v) 2,2′-*p*-phenylene-bis-(5-phenyl-oxazole) (POPOP).

RESULTS

Effect of administration of PCBs on the incorporation of [^{32}P]orthophosphate into phospholipids. Table 1 shows the sp. act. of individual phospholipids of rat liver and kidney. The liver weight and phospholipid content increased in PCBs-treated animals, but the incorporation of [^{32}P]orthophosphate into total liver phospholipids decreased to about one half of that in control animals. The sp. act. of individual phospholipids decreased; the greatest decrease was observed in the choline-containing phospholipids, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine.

In kidney, the phospholipid content and the sp. act. of total phospholipids were not significantly different between the treated and control animals, but the distribution of [^{32}P]orthophosphate in both phosphatidylethanolamine and phosphatidylinositol was somewhat lower in PCBs-treated animals.

Time course of effect of PCBs and phenobarbital on [^{32}P]orthophosphate incorporation. The sp. act. of total phospholipids, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in the liver of control animals during the experimental periods were always within the ranges of 354 ± 41, 291 ± 66, 527 ± 57 and 216 ± 9, respectively.

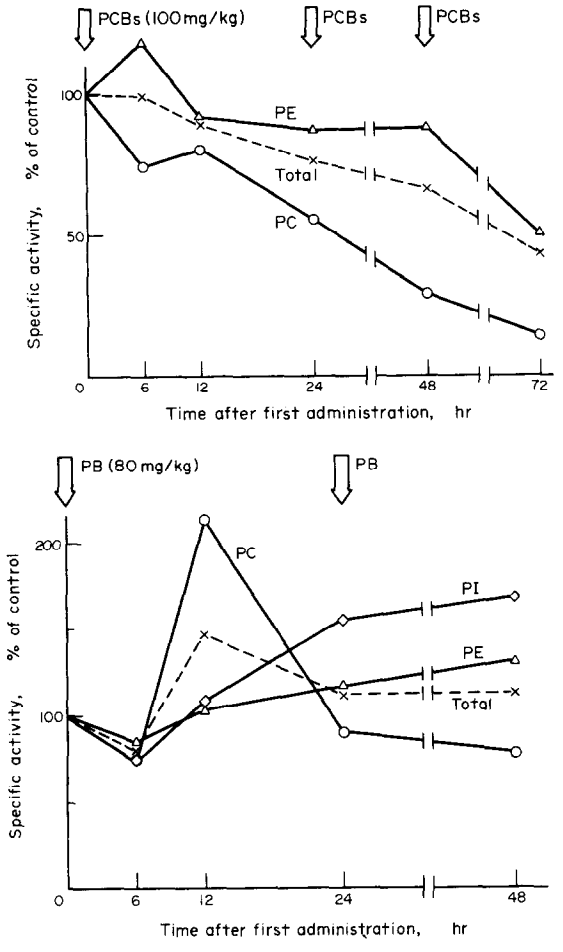
Table 1. Effect of administration of PCBs for 3 days on the incorporation of [^{32}P]orthophosphate into the phospholipids of rat liver and kidney

	Body wt (g)	Tissue (g)	PLs / g Tissue (%)	Sp. act. (cpm/μg lipid-P) Total-PL	PC	PE	PI	PS*	Sph + LPC	DPG
Liver										
Control	91 ± 9	4.6 ± 0.9	3.3 ± 0.2	690 ± 74	615 ± 97	1161 ± 154	422†	685 ± 135	319 ± 49	[illegible]
Treated	100 ± 4	7.4 ± 0.6	3.8 ± 0.2	298 ± 16	88 ± 20	585 ± 48	375†	443 ± 97	73 ± 21	[illegible]
Kidney										
Control		1.0 ± 0.2	2.8 ± 0.1	418 ± 18	526 ± 75	222 ± 22	910 ± 80	524 ± 41	390 ± 56	64 ± 4
Treated		1.2 ± 0.1	2.9 ± 0.1	414 ± 22	584 ± 80	153 ± 12	631 ± 132	523 ± 18	370 ± 30	57 ± 7

PCBs (100 mg/kg) was administered orally daily for 3 days. On the fourth day, [^{32}P]orthophosphate (250 μCi) was injected 1 hr before the animals were killed. Other experimental details are described in the text. The values are means ± S.E.M. of duplicate measurements from three animals.

* Fraction eluted by acetic acid through DEAE-cellulose column.

† The values are averages from two animals.



Figs. 1 and 2. Effect of PCBs or phenobarbital administration on the incorporation of [^{32}P]orthophosphate into rat liver phospholipids. PCBs (100 mg/kg) or phenobarbital (80 mg/kg) was administered daily and the animals were killed at the times specified. At 1 hr before being killed, they were injected with 200 μCi of [^{32}P]orthophosphate. Other experimental procedures are described in the text. x----x, sp. act. (cpm/μg lipid-P) of total liver phospholipids; O——O, sp. act. of phosphatidylcholine; △——△, sp. act. of phosphatidylethanolamine; ◇——◇, sp. act. of phosphatidylinositol.

At 24 hr after a single dose of PCBs, there was a significant decrease (45%) in the rate of [^{32}P] incorporation into phosphatidylcholine, as shown in Fig. 1. Repeated administration of PCBs caused a further decrease as seen at 48 and 72 hr after the first administration. The sp. act. of phosphatidylethanolamine, on the other hand, did not decrease until 48 hr after the first administration, but a rapid decrease (50%) was seen at 72 hr.

Figure 2 shows the effect of phenobarbital administration on [^{32}P] incorporation into the main phospholipid classes in rat liver. At 12 hr after the first administration of phenobarbital, the [^{32}P] incorporation into total phospholipids and phosphatidylcholine was maximum, showing a 47% and 113% increase in sp. act., respectively. At 24 hr, however, both the sp. act. returned to the control levels. The incorporation into phosphatidylethanolamine, on the other hand, was not affected by phenobarbital administration during experimental periods and that into phosphatidylinositol increased gradually 24 and 48 hr after the first administration.

Effect of PCBs and phenobarbital on [^{32}P]*orthophosphate incorporation into liver microsomal phospholipids.* The microsomal phospholipid content increased in rats given either PCBs or phenobarbital (Table 2), suggesting a marked proliferation of endoplasmic reticulum membranes.

The administration of PCBs caused a significant decrease in the rate of [^{32}P] incorporation into total microsomal phospholipids, mainly due to the decrease in incorporation into choline-containing phospholipids such as phosphatidylcholine and sphingomyelin, as was seen in the whole liver experiments (Table 1).

Phenobarbital, however, had no significant effects on the incorporation rate of [^{32}P]orthophosphate into microsomal phospholipid classes.

Incorporation of [Me-^{14}C]*choline into phospholipids of liver microsomes of rats pre-treated with PCBs or phenobarbital.* Table 3 shows the sp. act. of individual phospholipid classes. In all animals, more than 98% of radioactivity incorporated into total lipids were detected in phosphatidylcholine; the remainder, less than 2%, were detected in lysophosphatidylcholine, sphingomyelin and phosphatidylserine.

The initial incorporation of [Me-^{14}C]choline, into microsomal choline-containing phospholipids, likewise [^{32}P]orthophosphate, was strongly inhibited by pretreatment with PCBs, and the extent of the inhibitory effect of PCBs on the incorporation of the both precursors into phosphatidylcholine was similar.

Pretreatment with phenobarbital caused no significant changes in the incorporation rate of [Me-^{14}C]-choline into microsomal phospholipids.

Table 2. Effect of PCBs or phenobarbital on the incorporation of [^{32}P]orthophosphate into the phospholipids of rat liver microsomes

	Body (g)	Liver (g)	Microsomal PLs/g liver (mg)	Sp. act. (cpm/μg lipid-P)					
				Total-PL	PC	PE	PI	PS	Sph
Expt. 1									
Control	98 ± 4	4.0 ± 0.4	5.94 ± 0.53	310 ± 17	297 ± 41	454 ± 42	98 ± 11	21 ± 16	39 ± 8
PCBs	95 ± 1	6.3 ± 0.3	13.48 ± 1.48	134 ± 21	60 ± 20	263 ± 36	83 ± 7	12 ± 12	12 ± 7
Expt. 2									
Control	93 ± 6	4.7 ± 0.8	4.10 ± 0.32	329 ± 36	254 ± 84	559 ± 101	127 ± 44		
Phenobarbital	97 ± 4	5.3 ± 0.3	9.17 ± 1.51	327 ± 11	244 ± 19	525 ± 53	145 ± 31		

Either PCBs (100 mg/kg/day) or phenobarbital (80 mg/kg/day) was administered orally for 2 days. On the third day, [^{32}P]orthophosphate (250 μCi) was injected i.p. 1 hr before the animals were killed. Other experimental procedures are described in the text. The values are means ± S.E.M. from four animals

Table 3. Incorporation of [Me-^{14}C]choline into microsomal phospholipids of rats treated with either PCBs or phenobarbital

Treatment	Sp. act. (dpm/μg lipid-P)				
	Total-PL	PC	PS	Sph	LPC
Control	1434 ± 163	2213 ± 139	40 ± 9	87 ± 17	525 ± 129
PCBs	277 ± 79	457 ± 137	32 ± 14	19 ± 3	99 ± 45
Phenobarbital	1359 ± 150	2159 ± 308	49 ± 23	86 ± 21	460 ± 170

PCBs (100 mg/kg/day) and phenobarbital (80 mg/kg/day) were administered orally for 2 days. On the third day, [Me-^{14}C]choline (10 μCi) was injected i.p. 1 hr before the animals were killed. Other experimental details are described in the text. The values are means ± S.E.M. from five animals.

Incorporation of [2-^{3}H]glycerol into liver microsomal lipids of rats pretreated with PCBs. Fifteen min after the injection of [2-^{3}H]glycerol, the distribution of the radioactivity incorporated into microsomal lipids was not affected by pretreatment with PCBs, except that the radioactivity of phosphatidylinositol from treated rats was somewhat higher than that of the controls (Table 4).

The sp. act. of total microsomal phospholipids in PCBs-treated animals, on the other hand, decreased to 56% of that in control animals, and this decrease was mainly due to the reduction of incorporation of radioactivity into both phosphatidylcholine and phosphatidylethanolamine. It is interesting that the sp. act. of phosphatidylinositol was significantly higher in treated animals than in the control.

At 60 min after the injection of the precursor, the total radioactivity incorporated into microsomal lipids was mainly detected in phospholipid fractions (80%) in control animals, while in PCBs-treated animals, more than 30% of the radioactivity remained in the neutral lipid fraction. The differences in sp. act. of each phospholipid class between control and PCBs-treated animals were less.

Phospholipid composition of rat liver microsomal fraction after treatment with PCBs or phenobarbital. The phospholipid composition of liver microsomes of rats given either PCBs or phenobarbital for 2 days is shown in Table 5. The content of phosphatidylcholine was higher, and that of phosphatidylserine and sphingomyelin was lower after both PCBs and phenobarbital treatment.

DISCUSSION

A number of reports have demonstrated that animals given PCBs and phenobarbital, show a marked proliferation of smooth endoplasmic reticulum in the liver [23–25] associated with increased activities of microsomal drug-metabolizing enzyme [16–18, 26–28].

The present studies showed that the possible proliferation of endoplasmic reticulum in rat liver following PCBs administration, which was estimated by an increase in microsomal phospholipid content per weight of liver (Table 2), was not always accompanied by an enhancement of *de novo* synthesis of hepatocyte phospholipid.

The incorporation of [^{32}P]orthophosphate into both whole liver and microsomal phospholipids was strongly inhibited by the administration of PCBs (Tables 1 and 2, Fig. 1). This decrease was found to be mainly due to the inhibition of the incorporation into phosphatidylcholine and other choline-containing phospholipids such as sphingomyelin and lysophosphatidylcholine. The incorporation of [Me-^{14}C]-choline into microsomal phospholipid also was inhibited by the administration of PCBs (Table 3). Although the decrease of the initial incorporation of

Table 4. Incorporation of [2-^{3}H]glycerol into liver microsomal lipids of rats pretreated with PCBs

	Control		Treated		
	Count (%)	Sp. act. (dpm/μg lipid-P)	Count (%)	Sp. act. (dpm/μg lipid-P)	Sp. act. ratio (%)
15 min					
Total		431 ± 28		242 ± 39	56.1
PC	32.6 ± 1.6	252 ± 22	31.6 ± 1.9	132 ± 27	52.4
PE	17.5 ± 1.3	282 ± 38	17.8 ± 1.5	150 ± 20	53.2
PI	1.2 ± 0.1	47 ± 5	3.9 ± 0.8	77 ± 5	163.8
PS	0.1	15 ± 3	0.2 ± 0.1	12 ± 5	80.0
Sph	0.2	14 ± 3	0.2 ± 0.1	15 ± 6	107.1
NL	48.3 ± 2.6		46.0 ± 1.0		
60 min					
Total		492*		436 ± 42	88.6
PC	49.9*	368	40.4 ± 1.3	275 ± 38	74.7
PE	24.2	468	18.6 ± 2.1	338 ± 58	72.2
PI	3.0	145	4.0 ± 0.9	163 ± 26	112.4
PS	0.2	26	0.3	43 ± 28	165.4
Sph	0.9	60	0.7 ± 0.3	81 ± 40	135.0
LPC	0.6	106	0.5 ± 0.1	133 ± 40	125.5
NL	21.3		35.4 ± 2.0		

PCBs (100 mg/kg/day) was administered orally for 2 days. On the third day, 100 μCi of [2-^{3}H]glycerol was injected i.p. 15 or 60 min before the animals were killed. The values are means ± S.E.M. from four animals.

* The values in these columns are means of two animals.

Table 5. Phospholipid composition of rat liver microsomal fraction after treatment with either PCBs or phenobarbital

Treatment	Composition (% of total phospholipid)						Yield (%)
	PC	PE	PI	PS	Sph	Others	
Control	50.9 ± 0.4	23.6 ± 0.8	11.1 ± 0.6	4.6 ± 0.4	5.8 ± 0.3	3.7 ± 0.5	97.3 ± 2.2
PCBs	53.6 ± 0.6	25.6 ± 0.5	11.7 ± 0.1	3.1 ± 0.1	3.4 ± 0.4	2.7 ± 0.9	97.0 ± 3.5
Phenobarbital	53.0 ± 1.9	23.8 ± 1.9	11.6 ± 0.3	3.6 ± 0.2	4.0 ± 0.6	3.8 ± 0.3	98.4 ± 1.5

Either PCBs (100 mg/kg/day) or phenobarbital (80 mg/kg/day) was administered for 2 days. On the third day, the animals were killed by decapitation and microsomal lipids were prepared and fractionated as described in the text. The values are means ± S.E.M. from four animals.

[^{32}P]orthophosphate was recognized in the phosphatidylethanolamine fraction of whole liver and the microsomes, the inhibitory rate was far smaller than choline-containing phospholipids and the incorporation into phosphatidylinositol and phosphatidylserine was almost unaffected.

These findings suggest that PCBs or its metabolites cause a severe inhibition of hepatic phospholipid synthesis at the level of CDP-choline formation via phosphorylcholine, namely inhibition of choline kinase [ATP:choline phosphotransferase, EC 2.7.1.32] and/or cholinephosphate cytidyltransferase [CTP:cholinephosphate cytidyltransferase, EC 2.7.7.15]. The smaller extent of inhibition observed in phosphatidylethanolamine could be explained by assuming that ethanolamine kinase [EC 2.7.1.?] and ethanolaminephosphate cytidyltransferase [EC 2.7.7.14] are less sensitive to the administration of PCBs.

The activity of choline phosphotransferase [CDP-choline:1,2-diacyl-*sn*-glycerolcholinephosphotransferase, EC 2.7.8.2] is unaffected for the following reasons; the incorporation of both [^{32}P]orthophosphate and [Me-^{14}C]choline into membranous sphingomyelin and phosphatidylcholine decreased to a similar extent in PCBs-treated animals. In addition, the incorporation of [2-^{3}H]glycerol, injected 1 hr before decapitation, into microsomal phosphatidylcholine was not significantly affected by PCBs pretreatment.

Bjørnstad and Bremer [29], Kanoh and Ohno [30, 31] and recently Sundler *et al.* [32] reported that the formation of phosphatidylcholine from CDP-choline and diglyceride is freely reversible, whereas this is not the case with phosphatidylethanolamine. Therefore, it would be expected that, if CDP-choline synthesis is impeded, the back reaction of phosphotransferase would proceed to retain the CDP-choline level. Thus, the fact that the incorporation of [2-^{3}H]glycerol into microsomal phosphatidylcholine was not significantly affected, at least 1 hr after injection of the precursor, by pretreatment with PCBs (Table 4) would not conflict with the fact that PCBs caused an inhibition in the process of CDP-choline synthesis via phosphorylcholine.

Another possible site could be in the process of permeation of choline into liver cells. A number of reports have shown that choline entry into erythrocytes [33, 34], kidney slices [35, 36], synaptosomes [37, 38], hepatoma [39] and ascites tumor cells [40] is dependent on a carrier-mediated mechanism (choline permease) within the range of its physiological concentration. Thus inhibition of the choline permease in the liver, but not in the kidney, by PCBs or its metabolites may be another explanation.

The initial rate of incorporation of [2-^{3}H]glycerol, when injected 15 min before the animals were killed, into liver microsomal phosphatidylcholine and phosphatidylethanolamine was decreased by PCBs pretreatment to about one half of that in control animals (Table 4), indicating that the metabolic process from glycerol to diglyceride via phosphatidic acid also is affected by the administration of PCBs. Inhibition of phosphatidate phosphatase [L-α-phosphatidate phosphohydrolase, EC 3.1.3.4] may be a plausible explanation for these results, because of the fact that the incorporation rate of [2-^{3}H]glycerol into microsomal phosphatidylinositol, which is formed from phosphatidic acid via CDP-diglyceride, did not decrease but somewhat increased in PCBs-treated rats.

It has been demonstrated that the phosphatidylcholine content in rat liver endoplasmic reticulum membranes increases significantly after the injection of phenobarbital for several days [4, 6, 41, 42], and it has been suggested also that this increase is mainly due to the stimulation of phosphatidylcholine synthesis via transmethylation from phosphatidylethanolamine, since the activity of *S*-adenosylmethionine-phosphatidylethanolamine methyltransferase increases at an early time after the injection of phenobarbital [6, 7]. Holtzman and Gillette [8], on the other hand, found that phenobarbital enhances the synthesis of microsomal phospholipid in male rats but not in females, and that it reduces the catabolism of microsomal phospholipid in both male and female rats.

In the present study, the concentration of microsomal phospholipids significantly increased 48 hr after the first administration of phenobarbital (Table 2), and the rate of the incorporation of [^{32}P]orthophosphate into the phospholipids, especially into phosphatidylcholine, was markedly stimulated 12 hr after a single dose of phenobarbital (Fig. 2). This suggests that the increase in microsomal phospholipid content is probably attributable, at least in part, to an actual increase in hepatic phosphatidylcholine synthesis.

The increase of microsomal phospholipid content was observed also in liver of rats pretreated with PCBs. However, the rate of incorporation of [^{32}P]orthophosphate into microsomal phospholipids was reduced by the administration of PCBs and this decrease was greatest in the case of phosphatidylcholine. The decrease in the sp. act. of phosphatidylcholine caused by PCBs administration was also recognized in the incorporation of [Me-^{14}C]choline.

These results indicate that the increase of microsomal content of phospholipid, particularly phosphatidylcholine, is not due to the stimulation of the synthesis but is due entirely to a decrease in catabolism, thus differing from the effect of phenobarbital.

REFERENCES

1. H. Remmer and H. J. Merker, *Science* **142**, 1657 (1963).
2. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
3. S. Orrenius, J. L. E. Ericsson and L. Ernster, *J. Cell Biol.* **25**, 627 (1965).
4. S. Orrenius, *J. Cell Biol.* **26**, 725 (1965).
5. S. Orrenius and J. L. E. Ericsson, *J. Cell Biol.* **28**, 181 (1966).
6. D. L. Young, G. Powell and W. O. McMillan, *J. Lipid Res.* **12**, 1 (1971).
7. S. C. Davison and E. D. Wills, *Biochem. J.* **142**, 19 (1974).
8. J. L. Holtzman and J. R. Gillette, *J. biol. Chem.* **243**, 3020 (1968).
9. H. W. Strobel, A. Y. H. Lu, J. Heidema and M. J. Coon, *J. biol. Chem.* **245**, 4851 (1970).
10. A. Y. H. Lu and W. Levin, *Biochim. biophys. Acta* **344**, 205 (1974).
11. M. D. Chaplin and G. J. Mannering, *Molec. Pharmac.* **6**, 631 (1970).
12. T. E. Eling and R. P. DiAugustine, *Biochem. J.* **123**, 539 (1971).
13. K. C. Leibman and R. W. Estabrook, *Molec. Pharmac.* **7**, 26 (1971).
14. M. Vore, J. G. Hamilton and A. Y. H. Lu, *Biochem. biophys. Res. Commun.* **56**, 1038 (1974).
15. L. Fishbein, *A. Rev. Pharmac.* **14**, 139 (1974).
16. C. L. Litterst and E. J. van Loon, *Bull. Environ. Contam. Toxicol.* **11**, 206 (1974).
17. D. R. Bickers, A. Kappas and A. P. Alvares, *J. Pharmac. exp. Ther.* **188**, 300 (1974).
18. S. Fujita, H. Tsuji, K. Kato, S. Saeki and H. Tsukamoto, *Fukuoka Acta med., Japan* **62**, 30 (1971).
19. J. Folch, M. Lees and G. H. Sloane-Stanley, *J. biol. Chem.* **226**, 497 (1957).
20. G. Rouser, C. Galli and E. Lieber, *J. Am. Oil Chem. Soc.* **41**, 836 (1964).
21. G. Rouser, G. Simon and G. Kritchevsky, *Lipids* **4**, 599 (1969).
22. G. Rouser, A. N. Siakotos and S. Fleischer, *Lipids* **1**, 85 (1966).
23. M. Nishizumi, *Archs Environ. Health* **21**, 620 (1970).
24. R. D. Kimbrough, R. E. Linder and T. B. Gaines, *Archs Environ. Health* **25**, 354 (1972).
25. J. G. Vos and E. Notenboom-Ram, *Toxic. appl. Pharmac.* **23**, 563 (1972).
26. J. V. Bruckner, K. L. Khanna and H. H. Cornish, *Toxic. appl. Pharmac.* **28**, 189 (1974).
27. G. J. Johnstone, D. J. Ecobichon and O. Hutzinger, *Toxic. appl. Pharmac.* **28**, 66 (1974).
28. C. L. Litterst and E. J. van Loon, *Proc. Soc. exp. Biol. Med.* **141**, 765 (1972).
29. P. Bjornstad and J. Bremer, *J. Lipid Res.* **7**, 38 (1966).
30. H. Kanoh and K. Ohno, *Biochim. biophys. Acta* **306**, 203 (1973).
31. H. Kanoh and K. Ohno, *Biochim. biophys. Acta* **326**, 17 (1973).
32. R. Sundler, B. Åkesson and Å. Nilsson, *Biochim. biophys. Acta* **337**, 248 (1974).
33. K. Martin, *J. gen. Physiol.* **51**, 497 (1968).
34. A. Askari, *J. gen. Physiol.* **49**, 1147 (1966).
35. C. P. Sung and R. M. Johnstone, *Can. J. Biochem.* **43**, 1111 (1965).
36. C. P. Sung and R. M. Johnstone, *Biochim. biophys. Acta* **173**, 548 (1969).
37. R. M. Marchbanks, *Biochem. J.* **110**, 533 (1968).
38. I. Diamond and E. P. Kennedy, *J. biol. Chem.* **244**, 3258 (1969).
39. P. G. W. Plagemann, *J. Lipid Res.* **12**, 715 (1971).
40. E. W. Haeffner, *Eur. J. Biochem.* **51**, 219 (1975).
41. S. D. Cooper and G. Feuer, *Can. J. Physiol. Pharmac.* **50**, 568 (1972).
42. S. C. Davison and E. D. Wills, *Biochem. J.* **140**, 461 (1974).