

## Polyene Phosphatidylcholine: Pharmacokinetics After Oral Administration—A Review

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Polyene phosphatidylcholine (PPC) constitutes a subgroup of special phosphatidylcholines (or lecithins) having polyunsaturated fatty acids in the side chains of the molecule (7). The prototype is 1,2-dilinoleoyl phosphatidylcholine (dilinoleoyl-PC). Polyene phosphatidylcholine occurs naturally in plants (e.g., soybeans, safflower), or can be obtained by synthetic or semisynthetic processes. Investigation of the pharmacokinetics of PPC especially has to provide evidence on the preservation of the integrity of this particular molecule (among the "animal" phosphatidylcholines) during absorption and organ distribution—particularly if therapeutic aims, such as the treatment of atherosclerosis, require the use of this special phosphatidylcholine.

The present review is based on experiments mainly using synthetic radiolabeled dilinoleoyl-PC. The evidence accumulated thus far has been gained mostly from experiments in animals (mainly rats, but also dogs and monkeys). Only recently a study using radiolabeled dilinoleoyl-PC provided reliable human data (31).

### METHODOLOGICAL REMARKS

Phosphatidylcholine is found ubiquitously in mammals. Polyene phosphatidylcholine, if orally administered, therefore, has to be distinguished from endogenous lecithins when investigating its absorption and metabolic fate. Quantitative conclusions thus may be drawn only from experiments using radiolabels in the fatty acids and choline moieties of the molecule. A further restriction has to be taken into account since label exchange may occur, particularly if a tritium label is used in the fatty acid moiety. It was observed (6) that a considerable amount of tritium from the fatty acids exchanges for nonradioactive hydrogen with body water. It appears, therefore, desirable for most experiments that a  $^{14}\text{C}$  label be used in the fatty acid moiety and a tritium label in the methyl groups of the choline, if double

labeling is required. For positional analysis, single-label PPC molecules were synthesized carrying a  $^{14}\text{C}$  label either in the 1-, 2-, or 3-position (13). Detailed descriptions of the individual techniques are in the below cited reports. Synthesis of radiolabeled PPC was described by Lekim and Betzing and Stoffel et al. (14,15,27).

### ABSORPTION OF POLYENE PHOSPHATIDYLCHOLINE

#### Total Absorption from the Gut

Polyene phosphatidylcholine (orally administered to rats, rhesus monkeys, or humans as a single dose) was more than 90% absorbed, since there was only little excretion with the feces during the first 5 or 7 days (Table 1). Similar results were obtained in males as well as in females (5,6,31). In rats the absorption as measured by the disappearance of radiolabel from the gut showed a rapid initial phase (65% during the first 4 hr) and a slow final phase (approximately a further 25% from 4 to 24 hr). The time course of disappearance from the gut and the total amount of absorption was similar for the fatty acids and for the choline moieties (8).

#### Integrity of the Absorbed PPC

The pharmacokinetics of orally ingested PPC involve the question of bioavailability of the intact molecule. Therefore, the primary step, the mechanism of intestinal absorption, has been of particular research interest (1-3,8,11-13,17-21, 25,28). It has been established that a major portion of phosphatidylcholine is hydrolyzed to 1-acyl-lysophosphatidylcholine and is reacylated by the mucosal cells to phosphatidylcholine after absorption. However, some evidence is available that a minor portion of the molecule might be absorbed intact (1,2). This view was supported in particular by recent investigations in humans (31).

TABLE 1. *Fecal excretion of radioactivity accumulated during a period of 5 or 7 days\**

	% administered dose		
	Rats	Rhesus monkey	Human
PPC-FA moiety	6.1 <sup>b</sup>	8.0 <sup>b</sup>	5.7
PPC-choline moiety	3.2	3.6	2.2
N (males/females)	4/4	3/3	4/1
Reference	(5)	(6)	(31)

\*After a single oral dose of 1,2- $^3\text{H}$ -dilinoleoylphosphatidyl- $^{14}\text{C}$ -choline (rat, rhesus) or 1,2- $^{14}\text{C}$ -dilinoleoylphosphatidyl- $^3\text{H}$ -choline. Mean values of *N* animals or patients. PPC, polyene phosphatidylcholine; FA, fatty acid.

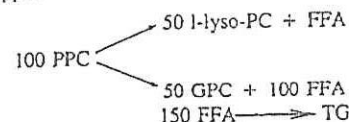
<sup>b</sup>Values for nonvolatile  $^3\text{H}$ , i.e., non- $^3\text{H}_2\text{O}$  (see methods section).

Lymph cannulation experiments were performed in rats to avoid interchange of exogenous PPC with endogenous PC (4,13). The lymph was collected immediately after production from the thoracic duct for different periods after giving doses of dilinoleoyl-PC, labeled with  $^3\text{H}$  in the fatty acids and with  $^{14}\text{C}$  in the choline moiety. (Under these circumstances  $^3\text{H}$ -label exchange with body water did not occur considerably.)

Table 2 displays the recovery of the radiolabel in the lymph. More than 88% of the tritium but only 50% of the carbon label were recovered in the lymph chylomicrons. A small amount of tritium (fatty acids) and almost half of the carbon (choline moiety) could bypass the lymph. Apparently, a certain portion of the molecules was degraded, and the degradation products (mainly nonlipids) entered the liver directly via the portal vein. The other portion (the lipids) was recovered with the lymph. These percentages of recovery were independent of time after dose administration (13). They represent, therefore, the average absorption process.

A further step was to investigate where in the lipid fractions of the lymph the labels were located. No labeled free fatty acids (FFA) and no labeled lysophosphatidylcholine were detected. The  $^{14}\text{C}$  radioactivity (the choline label) was 100% in phosphatidylcholine; the fatty acid label (tritium) was 25% in phosphatidylcholine and 75% in triglycerides (TG) (Table 3). The tritium/carbon ratio was almost half of the original ratio. All these figures were independent of time, again representing the average absorption process.

Phospholipase A is present in the intestinal lumen. The data suggest that the following might happen:



The free fatty acids are incorporated into triglycerides in the mucosa and the glycerylphosphatidylcholine (GPC) bypasses the lymph and is found in the liver

TABLE 2. Distribution of radioactivity in the intestinal tract, in the liver and in the lymph chylomicrons of the rat\*

	$^3\text{H}$ Radioactivity		$^{14}\text{C}$ Radioactivity	
	% Dose	% Absorption	% Dose	% Absorption
Lymph chylomicrons	24.8	88.2	17.4	51.6
Liver	0.8	2.9	12.5	36.3
Other organs (difference)	2.5	8.9	4.3	12.3
Total absorption	28.1	100.0	34.2	100.0
Intestinal tract	71.9	0	65.8	0

\*At 6.5 hr after oral administration of 1,2-(9,10,12,13- $^3\text{H}$ )-dilinoleoyl-sn-glycero-phospho-(N-( $^{14}\text{C}$ )-choline).

TABLE 3. Distributions of  $^3\text{H}$  and  $^{14}\text{C}$  radioactivities\*

Time after dose (hr)	Phosphatidylcholine		Neutral lipids % $^3\text{H}$
	% $^3\text{H}$	$^3\text{H}/^{14}\text{C}$	
1.5	23.5	24.3	77.5
2.5	24.0	17.2	76.0
3.5	24.5	20.4	75.5
4.5	25.0	19.7	75.0
5.5	24.0	17.9	76.0
6.5	24.5	20.9	75.5

\*Radioactivities in lipid fractions of the lymph chylomicrons in lymph-cannulated male rats ( $N = 7$ ) after a single oral dose of 70 mg/kg polyunsaturated phosphatidylcholine labeled as in Table 1 ( $^3\text{H}/^{14}\text{C} = 59:1$ ). After 0.5 hr the lymph was collected for periods of 1 hr. The only labeled lipid fractions were phosphatidylcholine and triglycerides. No labeled lysophosphatidylcholine and no labeled free fatty acids were detected; 100% of the  $^{14}\text{C}$  radioactivity was located in the phosphatidylcholine fraction, and practically none was found in the triglyceride fraction.

and in the other organs. According to this scheme the  $^3\text{H}$ -label distribution (fatty acids) in the lymph is expected to be 25% in the PC fraction and 75% in the TG fraction. The values observed were 23.5 to 25.0 and 75.0 to 76.5, respectively. The  $^{14}\text{C}$ -label distribution between the lymph and the liver plus other organs should be equal. The actual values were 52% and 48% (see Table 2). Further, in this scheme the  $^3\text{H}/^{14}\text{C}$  ratio in the PC fraction should be half of the original ratio, i.e., 26. The observed values were 17.2 to 24.3.

Apparently, phosphatidylcholine was absorbed after hydrolyzation as lysophosphatidylcholine and was reacylated in the mucosa again to phosphatidylcholine since no radioactive lyso-PC has been detected. These data confirm on a quantitative basis the view of the absorption of PC, as it was derived from earlier investigations (2,15,20). There are reports that PC may also be absorbed to a certain extent as intact molecules (1,2). Although this possibility has not been ruled out completely, the present data show that intact PPC absorption (in rats) accounts only for a minor portion.

#### Identity of the Absorbed PPC

After absorption, approximately 50% of the orally administered PPC was available in the lymph (and blood stream) intact or via reacylation. Positional analysis of the fatty acids had to clarify if the identity of the PPC, in contrast to the body-owned PC, was preserved. The lymph phosphatidylcholine was cleaved by using phospholipase  $\text{A}_2$ . Only 10% of the  $^3\text{H}$  label (linoleic acid) was found in the 2-position, but 90% was found in the 1-position (13).

A further experiment was performed using single-carbon labels separately in each of the three positions of the PC molecule and then collecting the  $\text{CO}_2$  of the expired air (Fig. 1). Cleaving of PC in only the 2-position should result in the occurrence



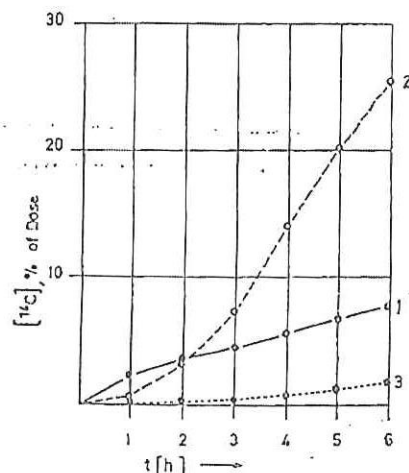


FIG. 1. Cumulative amounts of respiratory  $^{14}\text{CO}_2$  collected after a single oral dose of 70 mg/kg labeled with  $^{14}\text{C}$  in the acyl-1 position (1), the acyl-2 position (2), and the 3-position (choline) (3). Mean values in percent of the administered dose measured in two male plus two female animals for each labeled phosphatidylcholine species. The standard deviations for the cumulated values after 6 hr were  $\pm 4\%$  (1),  $\pm 7\%$  (2), and  $\pm 11\%$  (3) of the mean. The exact labeling was as follows:

1. 1-( $^{14}\text{C}$ )-linoleoyl-2-(9,10,12,13- $^3\text{H}_4$ )-linoleoyl-*sn*-glycero-3-phosphorylcholine: 37,300,000 dpm  $^3\text{H}$  and 620,000 dpm  $^{14}\text{C}$
2. 1-(9,10,12,13- $^3\text{H}_4$ )-linoleoyl-2-( $^{14}\text{C}$ )-linoleoyl-*sn*-glycero-3-phosphorylcholine: 66,900,000 dpm  $^3\text{H}$  and 1,730,000 dpm  $^{14}\text{C}$
3. 1,2-(9,10,12,13- $^3\text{H}_4$ )-dilinoleoyl-*sn*-glycero-3-phosphoryl-( $\text{N}-^{14}\text{CH}_3$ )-choline: 186,000,000 dpm  $^3\text{H}$  and 5,500,000 dpm  $^{14}\text{C}$ .

(Data according to Lokim, ref. 13.)

of  $^{14}\text{C}$  in the expired  $\text{CO}_2$  if the label was introduced in the 2-position. By far, the highest amount of  $^{14}\text{C}$  label in the expired  $\text{CO}_2$  was found in this case.

Obviously, in rats, hydrolyzation of PPC during absorption took place mostly at the 2-position resulting in 1-lyso-PC, which is reacylated predominantly with an unsaturated fatty acid because of the specificity of lyso-PC-acyltransferase (EC 2.3.1.2.3). Consequently, a polyene phosphatidylcholine was made available to the organism. This is particularly important since the mammalian endogenous PC contains practically 100% palmitic acid in the 1-position. According to Whyte et al. (29) this cannot be changed by ingesting unsaturated free fatty acids. As during absorption of PC the 1-position of phosphatidylcholine is usually not attacked; the unsaturated type of a polyunsaturated phosphatidylcholine will not be changed and can thus be introduced into the organism by oral administration of PPC.

This was further proved by a study of Rosseneau and co-workers (23) in chimpanzees. The experimental setup was as follows. The chimpanzees received by mouth PPC, then a control diet, and finally saturated lecithin for three 1-month

periods. The fatty acid distribution in the lipoprotein fractions was determined by gas chromatography. The result is shown in Table 4, where for the three dietary regimens the ratio of oleic/linoleic acid (i.e., the  $\text{C}_{18:1}/\text{C}_{18:2}$  ratio) is presented as it is found in the phospholipids, cholesteryl esters, and triglycerides of the three lipoprotein classes. The data show that after a PPC diet the ratio of oleic/linoleic acids in the phospholipids was significantly decreased; whereas, an increase was seen for saturated lecithin. This is also true for the cholesteryl esters in all three lipoprotein classes and, of course, for the triglycerides since part of the fatty acids of the ingested polyene phosphatidylcholine is transferred to triglycerides.

The results of recent pharmacokinetic studies in humans (31) using radiolabeled PPC are completely in line with these findings with one exception: the specificity of hydrolyzation of PPC at the 2-position appears to be less marked in humans, meaning that 1-lyso-PC and 2-lyso-PC are formed during absorption in almost equal amounts; both entities however are reacylated as in other animals. Nevertheless, these authors emphasize that oral administration of PPC allows the introduction of the special PPC type of PC into the human organism, particularly since the absorption rate of intact (reacylated) PPC appears to be higher in humans than in other animals (see below).

#### Influence of Media on PPC Absorption

The medium in which PPC is dissolved or suspended may influence its absorption. Therefore, comparative studies in dogs were performed applying radiolabeled PPC

TABLE 4. Changes in fatty acid compositions of phospholipids, cholesteryl esters, and triglycerides in the lipoprotein classes VLDL, LDL, and HDL, of chimpanzees<sup>a</sup>

Lipid	Lipoprotein class	$\text{C}_{18:1}/\text{C}_{18:2}^b$		
		PPC	Saturated lecithin <sup>c</sup>	Control diet
Phospholipids	VLDL	0.5	1.0	0.8
	LDL	0.3	0.8	0.4
	HDL <sub>2</sub>	0.3	0.8	0.4
Cholesteryl esters	VLDL	0.3	1.1	0.4
	LDL	0.2	0.8	0.3
	HDL <sub>2</sub>	0.2	0.8	0.3
Triglycerides	VLDL	0.4	2.5	1.5
	LDL	0.7	2.9	1.5
	HDL <sub>2</sub>	0.7	2.4	1.4

<sup>a</sup>After 4 weeks of oral treatment with polyene phosphatidylcholine (PPC), saturated lecithin (20 g/day), or a control diet. VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

<sup>b</sup>Ratio of oleic/linoleic acid ( $\text{C}_{18:1}/\text{C}_{18:2}$ ), as derived from data of Rosseneau et al. (23).

<sup>c</sup>The saturated lecithin was obtained by hydrogenation of PPC, so that the chain length distribution was identical.

dissolved either in oil/monoglyceride/diglyceride, in 11% ethanol, or suspended in mannitol/water (8). The label was  $^{14}\text{C}$  in the fatty acids (to avoid label-exchange with body water) and tritium in the choline moiety. Figure 2 shows the plasma radioactivity originating from the choline moiety as given in percent of the administered oral dose. The time course (i.e., the kinetics) as well as the radioactivity levels did not differ significantly from each other for the different media. The same is true for the tritium label (Fig. 3) originating from the fatty acids. Thus, there is no great influence on absorption by the media in which the phosphatidylcholine is offered.

Stein and Stein (26) reported a higher absorption of phosphatidylcholine if it is ingested together with large amounts of oil. Whether this is an effect of an accelerated rate of absorption, because of the higher need of phosphatidylcholine to build up more chylomicrons, or whether the recylated portion is increased is not clear at present.

#### KINETIC DATA: TIME COURSE OF BLOOD LEVELS

Figure 4 presents comparative data of total plasma levels of radioactivity (percent of ingested dose) originating from the choline moiety after absorption of orally

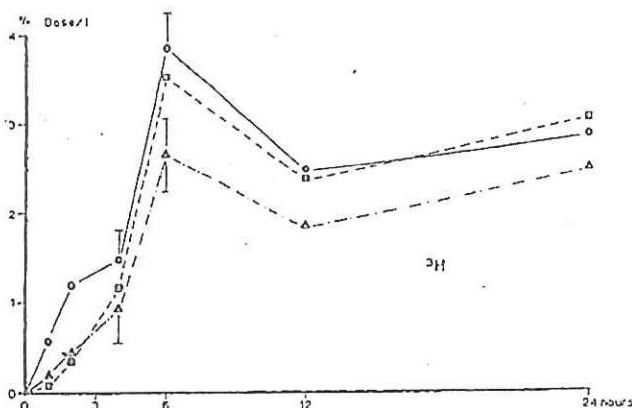


FIG. 2. Time course of blood  $^3\text{H}$  radioactivity (choline moiety) in dogs after a single oral dose of polyunsaturated phosphatidylcholine (150 mg/kg) dissolved in either oil/monoglyceride/diglyceride, 11% ethanol or suspended in mannitol/ $\text{H}_2\text{O}$ . Blood levels calculated as percentage of dose/liter whole blood (mean  $\pm$  SD; noninserted error bars are of the same magnitude as the inserted ones). Label: 1,2- $^{14}\text{C}$ -dipalmitoyl-3-sn-phosphatidyl-(N- $^3\text{H}$ )choline.  $\bullet$ , ethanol/ $\text{H}_2\text{O}$  solution;  $\square$ , mannitol/ $\text{H}_2\text{O}$  suspension;  $\triangle$ , mono/diglyceride solution;  $N = 6$ . (Data according to Fox et al., ref. 8.)

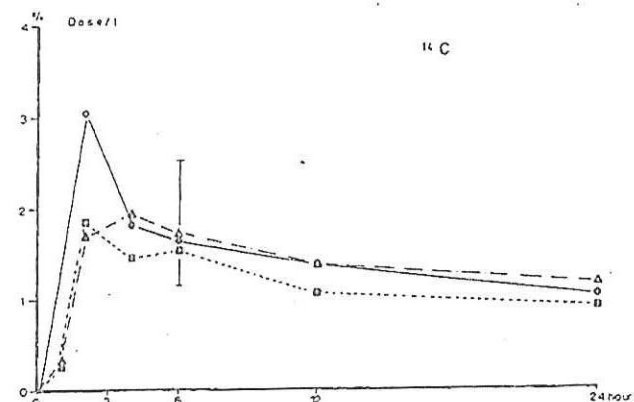


FIG. 3. Time course of blood  $^{14}\text{C}$  radioactivity (fatty acid moieties) in dogs after a single oral dose of polyunsaturated phosphatidylcholine (150 mg/kg) in different media (see Fig. 2).  $\bullet$ , ethanol/ $\text{H}_2\text{O}$  solution;  $\square$ , mannitol/ $\text{H}_2\text{O}$  suspension;  $\triangle$ , mono/diglyceride solution;  $N = 6$ . (Data according to Fox et al., ref. 8.)

administered PPC in dogs, rats, and monkeys. The peak levels of absorbed PPC in plasma were approximately 5% of the oral dose in rhesus monkeys and rats, and approximately 1.8% in dogs. A recent investigation in humans (31) revealed peak values in total blood  $19.9 \pm 3.9\%$  of the administered  $^3\text{H}$  label (choline moiety) and of  $27.9 \pm 4.4\%$  of the administered  $^{14}\text{C}$  label (fatty acid moiety). Since approximately three-fourths of the radioactivity was detected in the plasma and only one-fourth in the erythrocytes, these data suggest that the absorption rate of PPC (intact or recylated) is approximately three times higher than in rats or rhesus monkeys.

The time course of plasma radioactivity was found to be similar in all species (see Fig. 4) as well as in humans. The peak radioactivity was observed at 6 hr after dose administration; whereas, there was an indication for a lag time of 1 or 2 hr in dogs. (Early data for rhesus monkeys or rats are not available.) In humans the  $^3\text{H}$  label (choline moiety) peaked between 6 and 24 hr, whereas the  $^{14}\text{C}$  label (fatty acid moiety) peaked between 4 and 12 hr. A lag time of 2 hr was observed in the beginning. The half-life of decay of PC radioactivity between 24 and 96 hr averaged 65.7 hr for  $^3\text{H}$  and 37.8 hr for  $^{14}\text{C}$ . Similar values were obtained in rats (6,17) and in rhesus monkeys (5). It has to be emphasized that the decay is overlapped by reformation of PC from GPC in the liver originating from the portion of PPC being disintegrated during absorption (see above).



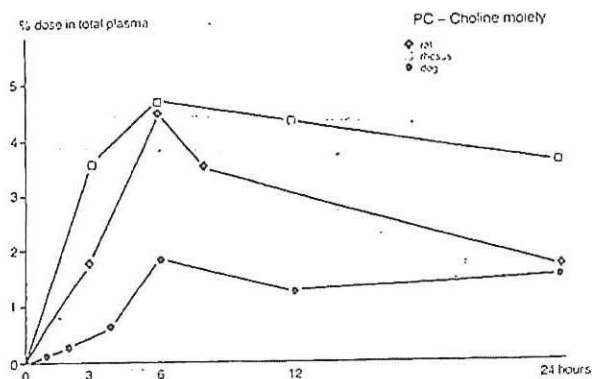


FIG. 4. Absorption in different species of polyunsaturated phosphatidylcholine (PC) suspended in H<sub>2</sub>O as measured by the time course of plasma radioactivity originating from the label of the choline moiety after a single oral dose. Monkeys and rats: 1,2-(<sup>3</sup>H)-dilinoleoyl-3-sn-phosphatidyl-(N-C<sup>14</sup>H<sub>3</sub>) choline; dog: as in Fig. 2. (Data according to Fox et al., ref. 6.)

## ORGAN DISTRIBUTION

### Tissue Concentrations

Experiments in rats (6) were performed using 1,2-<sup>3</sup>H-dilinoleoyl-3-sn-phosphatidyl-<sup>14</sup>C-choline. Because of exchange of the tritium label with body water, only the values of the <sup>14</sup>C-radioactivity (choline moiety of PPC) gave representative results.

After a single oral dose of radiolabeled PPC, <sup>14</sup>C radioactivity was accumulated in the liver, with maximal concentrations occurring at 24 hr after dose administration (24.5% dose); these declined slowly during 8 days (4.0% dose). Significant amounts of radioactivity were detected in the striated muscle (5.8% dose during 6 hr), which increased during 8 days after dose administration (up to 25.0% dose). Limited amounts of <sup>14</sup>C radioactivity were detected in the kidneys after 24 hr (2.7% dose). Radioactivity was detected in the lungs (maximal 0.9% dose at 24 hr) and myocardium (0.3% dose at 24 hr, which was retained during the 8 days).

Concentrations of radioactivity (expressed in terms of tissue weight) were highest in the liver (1,196 µg/g) and <sup>14</sup>C radioactivity was detected in the brain (0.20% dose/g, 101 µg/g, after 4 hr). <sup>14</sup>C Radioactivity associated with the blood after a single oral dose of radiolabeled PPC was associated almost entirely with the plasma for 48 hr. At 96 hr, some <sup>14</sup>C radioactivity (approximately 22%) was associated with the cells; after 8 days up to 54% was located in the cells. [In humans three-

fourths of the blood radioactivity was associated with the plasma during the first period (31).]

Radioactivity in the tissues of rats after five daily oral doses of <sup>3</sup>H/<sup>14</sup>C-PPC was calculated in terms of the estimated cumulative dose retained (Table 5). These values were estimated from the rates of excretion in the single-dose study (see below). Concentrations of <sup>14</sup>C radioactivity in the tissues of rats after the first four daily oral doses of <sup>3</sup>H/<sup>14</sup>C-PPC showed that liver, depot fat, striated muscle, and bone had accumulated significant amounts of <sup>14</sup>C radioactivity (18.6, 7.0, 31.4, and 5.6%, respectively, of the estimated dose retained). Limited amounts of <sup>14</sup>C radioactivity were detected in the lungs, kidneys, testes, small and large intestine, blood, and plasma (1.3, 3.0, 1.3, 1.9, 1.6, 2.7, and 1.4% dose, respectively).

After the fifth dose of radiolabeled PPC, some <sup>14</sup>C radioactivity had accumulated in the liver at 6 hr (22.9% dose, 3718 µg/g), and this declined during the next 16 days (2.3% dose; 245 µg/g). The total <sup>14</sup>C radioactivity in the striated muscle increased after the fifth dose to a maximum after 4 days (36.9%, 545 µg/g). Some <sup>14</sup>C radioactivity was associated with the bone (up to 4.7% dose; 653 µg/g) and depot fat (up to 5.6% dose; 560 µg/g) at 24 hrs after the fifth dose. <sup>14</sup>C Radioactivity (expressed in terms of tissue weight) showed that radioactivity was concentrated into the adrenals at 6 hr after the fifth dose (1.75% dose/g). <sup>14</sup>C Radioactivity associated with the liver was maximal at 6 hr after fifth dose (2.0% dose/g) and that associated with the kidneys was also maximal (1.33% dose/g) at this time. The <sup>14</sup>C radioactivity in the blood was approximately equally distributed between cells and plasma immediately before and during 4 days after the fifth daily dose, and was concentrated (up to 65% of the total blood radioactivity) into the cells at 8 days after the fifth daily dose. After 16 days <sup>14</sup>C radioactivity in the blood was located mainly in the cells (76%). Rather similar results after a single dose, as well as after repeated doses, were obtained in rhesus monkeys using identical methods and radiolabeled PPC as in rats (5).

TABLE 5. Estimated cumulative retention of <sup>14</sup>C-radioactivity (choline moiety) during five daily oral doses of <sup>3</sup>H/<sup>14</sup>C-PPC to rats and rhesus monkeys.<sup>a,b</sup>

Day	Percent of cumulative dose retained			
	Male rat	Female rat	Male rhesus monkey	Female rhesus monkey
1	63.0	55.0	53.9	57.8
2	64.3	56.5	54.2	58.0
3	65.8	58.2	54.9	58.4
4	67.5	60.4	57.4	59.9
5	74.0	68.3	65.9	67.9

<sup>a</sup>References 5 and 6.

<sup>b</sup>Estimation was based on single-dose excretion rates. Four rats of each sex and three rhesus monkeys of each sex were used.

The situation in rhesus monkeys was comparable to that in rats (5). Hours after the last daily dose, radiolabeled PPC in the liver and depot fat contained 11 and 7% dose, respectively; this declined to 1.7 and 4.5% dose during the next 16 days. The total  $^{14}\text{C}$  radioactivity in striated muscle was between 16.2 and 20.3% dose at 6 hr after five repeated daily oral doses. This value was observed between 10 and 14% after the next 5 days and between 17 and 19% after the next 16 days. The  $^{14}\text{C}$  radioactivity in the blood at 6 hr after the last dose was associated mainly with the plasma (74–85%). After 5 days, between 69 and 72% was present in the plasma, and this had declined further after 17 days to 46 to 48%.

#### Whole-Body Autoradiography

Whole-body autoradiography was performed in rats using 1,2- $^3\text{H}$ -dilinoleoyl-3-sn-phosphatidyl- $^{14}\text{C}$ -choline (6,13). In both experiments, essentially the same results were obtained.

During 6 hr after a single oral dose of  $^{14}\text{C}$ -PPC, radioactivity was located mainly in the liver, kidneys, and the intestinal mucosa. At this time, small amounts of radioactivity were present in the secretory glands (thymus, thyroid, and salivary glands) and the lymph nodes. Only limited amounts of radioactivity were present in the gut contents.

After 12 hr, the distribution of radioactivity was more general, but was mainly located in the liver, kidneys, and intestinal mucosa. Some radioactivity was associated with the secretory glands, especially in the salivary glands and in the testes and epididymis. Limited amounts were present in the gut contents, and some was associated with a fur or skin (possibly with sebaceous glands).

After 24 hr, radioactivity was still mainly associated with the liver, kidneys, and intestinal mucosa. Radioactivity was associated with the bone marrow, lungs, spleen, testes, epididymis, secretory glands, and fur or skin (sebaceous glands) in limited amounts. Higher concentrations were associated with the seminal vesicles and the preputial glands.

The autoradiographs after 6 hr showed part of the intestinal radioactivity in the gut contents and part in the intestinal wall. After 12, 24, and 48 hr practically no radioactivity was observed in the lumen, but quite high concentrations (still after 48 hr) were associated with the intestinal mucosa. These findings are in line with (a) the human data suggesting a protracted absorption (31) and (b) animal results (22) of PPC secretion into the bile, thus undergoing enterohepatic circulation.

After 48 hr, radioactivity was generally distributed but remained mainly associated with the liver and intestinal mucosa. High concentrations were located in the kidneys, testes, and epididymis, together with the secretory glands, notably the salivary, thyroid, and thymus glands. Radioactivity was associated in limited amounts in the bone marrow, lachrymal glands, and lymph nodes of the head region. A general distribution of radioactivity into the striated muscle was evident at this time.

After 96 hr, the concentration of radioactivity had declined generally, with no major concentrations present in any particular organ. Some radioactivity was located

in the liver, kidneys, and gastric mucosa with local concentrations in the thymus, lachrymal glands, lymph nodes of the head region, epididymis, coagulating glands, and preputial glands. A general distribution of low concentrations of radioactivity was evident in the other tissues.

After 8 days, the concentrations of radioactivity were low but generally distributed. Local concentrations were evident in the gastric mucosa, epididymis, testes, and seminal vesicles with some radioactivity present in the thymus and lachrymal glands.

Whole-body autoradiographs taken immediately before the fifth and final daily dose of  $^{14}\text{C}$ -PPC indicated general distribution of radioactivity throughout the tissues. Major concentrations were located in the liver, spleen, kidneys, adrenal glands, gastric and intestinal mucosae, and the salivary glands. High concentrations were evident in the lachrymal glands, thymus, lymph nodes, seminal vesicles, epididymis, preputial glands, and bone marrow. Low concentrations of radioactivity were located in the striated muscle and brain. At 12 hr after the fifth daily dose, the radioactivity was mainly concentrated into the liver, kidneys, spleen, and intestinal and gastric mucosae with local high concentrations in the lymph nodes, thymus, bone marrow, epididymis, seminal vesicles, and testes. Some radioactivity was present in the brown fat, and there was a general distribution of radioactivity at low concentrations in the striated muscle.

At 48 hr after the fifth dose, higher concentrations of radioactivity were evident in the lymph nodes, gastric mucosa, epididymis, seminal vesicles, and testes with lower concentrations in the brain, spinal cord, and striated muscle. Ninety-six hours after the last dose the pattern of distribution remained similar, with local concentrations in the secretory glands (lachrymal, salivary, and Harderian), adrenal gland, seminal vesicles, epididymis, and testes. Generally lower concentrations were present in the brain, spinal cord, and striated muscle.

After 8 days, the concentration of radioactivity in most tissues was low with higher concentrations occurring in the secretory glands (salivary and lachrymal). Radioactivity was distributed generally in the central nervous system and striated muscle.

#### METABOLIC FATE OF PPC: INCORPORATION INTO SERUM LIPOPROTEINS, RED BLOOD CELLS, AND HEPATOCYTES

Figure 5 represents a schematic, tentative view of the present knowledge of the pathways for absorption of PPC and for metabolism of the disintegrated portions of the molecule based on the recent absorption studies as described above. In rats around 52% of PPC are available (after intact absorption or reacylated lyso-PPC) in the chylomicrons. This percentage may be even higher in humans (31). The disintegrated portion (GPC and the polyunsaturated fatty acids) undergoes partial resynthesis to PC and triglyceride. The remaining components are transported to the liver. Here again resynthesis takes place with incorporation into hepatocytes (10,16).



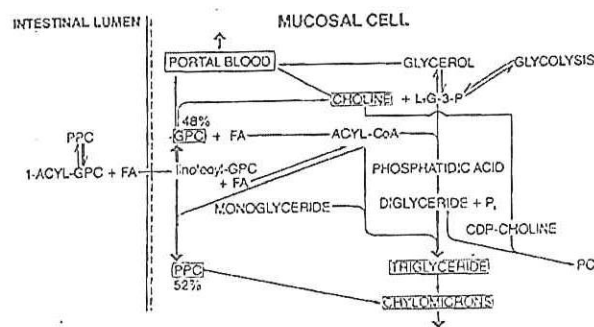


FIG. 5. Schematic, tentative view of the metabolic pathways of polyene phosphatidylcholine (PPC) in the mucosal cell after absorption. Major portion of PPC is absorbed as 1-lyso-PC, part of which (in rats, ~48%) is further disintegrated and bypasses the lymph to the liver (via portal vein); the other part is reacylated to PPC and incorporated in the lymph chylomicrons. The liberated fatty acids (FA) and part of GPC and choline are resynthesized to triglycerides and phosphatidylcholine (PC), respectively. L-G-3-P, glucose 3-phosphate; CoA, coenzyme A; P, inorganic phosphate; CDP, cytidine diphosphate; GPC, glycerylphosphatidylcholine.

A matter of great importance are the observations of a special uptake of PPC from the chylomicrons by high-density lipoprotein (HDL). These recent investigations are reported elsewhere (O. Zierenberg, *this volume* and ref. 30). They indicate that body-owned saturated PC is exchanged by PPC particularly in HDL, thus increasing the cholesterol-transporting capacity of the HDL particles. These studies were performed in rats and dogs after oral and intravenous PPC administration. The incorporation of PPC was further investigated *in vitro* using human HDL, as well as HDL from animal source. The results were basically confirmed by a recent human pharmacokinetic study (31); it appears to put forth a better understanding of the antiatherogenic effect of PPC (in contrast to saturated lecithin) as derived from animal experiments (9).

As seen from the organ distribution studies, increasing amounts of PPC with time after oral administration were detected in the blood cells. Six hours after dose administration of radiolabeled PPC in animals and in humans, approximately 75% of the radioactivity of total blood were found in the lipoproteins (particularly in HDL) and only about 25% in blood corpuscles. This ratio reverses during the next 6 days. Incorporation of polyunsaturated phosphatidylcholine into the membranes of erythrocytes enlarges the flexibility and fluidity of the red blood cells as shown by Salvio et al. (24).

No particular investigations on the metabolism of the PPC molecules have been undertaken so far. One has to assume that PPC undergoes the same metabolic pathways as endogenous PC.

### EXCRETION AND RETENTION OF PPC

The knowledge on excretion and retention of PPC is almost entirely based on radioactivity measurements after single or repeated doses of radiolabeled PPC. Since a considerable portion of PPC is already disintegrated during the absorption process, excretion and retention of radioactivity comprises the intact PPC as well as both its metabolites and the metabolites of other resynthesized material, such as triglycerides. Excretion with the feces is very low (see Table 1). Considerable radioactivity is excreted with the expired air, approximately 15% after a single oral dose in rats and rhesus monkeys (5,6) (see also Fig. 1). This almost entirely has to be attributed to the metabolism of fatty acids that are liberated during absorption and incorporated into triglycerides (19).

The renal excretion of  $^{14}\text{C}$  radioactivity after a single dose of  $^3\text{H}/^{14}\text{C}$ -PPC was 17.4% dose in rats and 17.7% in rhesus monkeys during 5 days after a single dose (5,6). From bile duct cannulation experiments (22) it is known that a considerable amount of PPC appears in the bile. Since the fecal excretion is low, PPC must undergo an effective enterohepatic recirculation.

Retention of PPC was estimated from the single-dose excretion rates in rats and rhesus monkeys (5,6) (see Table 5). These retention values again comprise intact and disintegrated PPC. Retention as such is not an important factor, since PPC and its metabolites are considered to be nontoxic.

### CONCLUSION

The knowledge of the pharmacokinetics of polyene phosphatidylcholine accumulated thus far provides evidence that more than 50% of orally administered PPC is made biologically available for the organism either by intact absorption (lesser extent) or by reacylation of absorbed lyso-PC (greater extent). This is true for several animal species (rats, dogs, rhesus monkeys, chimpanzees) and for humans. Obviously, there are some differences in the specificity of hydrolyzing PPC to lyso-PC. In rats hydrolyzation takes place mainly in the 2-position of the molecule (90%); in humans the 1- and the 2-position are almost equally attacked. Nevertheless, it is possible to exchange body-owned (endogenous) PC for the special PPC, particularly since the absorption in humans appears to be higher than in other animals. PPC is particularly incorporated into HDL particles, into the membranes of hepatocytes, and into red blood cells. These results have a specific implication for the beneficial effects of PPC in atherosclerosis and hepatic diseases.

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