Lecithin fatty acid composition in bile and plasma of man, dogs, rats, and oxen

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SUMMARY The lecithins of bile and plasma from nine patients, three dogs, four rats, and two steers (oxen) were extracted and their fatty acid patterns were determined. In all four species the lecithin of bile had a higher concentration of palmitic acid than did that of plasma. The concentration of stearic and arachidonic acids was higher in plasma lecithin. These differences were statistically significant.

The work of others shows that biliary and plasma lecithins are both derived from the liver. Plasma lecithin fatty acid composition was not noticeably changed on passage through the tissues. We conclude, therefore, that the different patterns indicate either the presence of two functionally distinct pools of lecithin in the liver, or selection from a single heterogeneous pool.

KEY WORDS lecithin · fatty acid composition · bile · plasma · man · dog · rat · ox · hepatic synthesis · metabolic pools

THE WORK of several investigators has shown that the lecithins of both plasma and bile are derived from the lecithins of the liver (1–4). In the course of investigations in this laboratory of the digestion and absorption of fat in man, striking differences in the fatty acid composition of the lecithins of bile and plasma were observed. This observation prompted the present investigation of the fatty acid pattern of the lecithins of bile and plasma in man, dogs, rats, and oxen.

METHODS

The lipids of bile and plasma were extracted in 20 volumes of chloroform–methanol 2:1 (v/v), washed with 0.15 M saline, and dried over sodium sulfate. The lecithin fraction from each extract was separated by silicic acid column chromatography as previously described (5). The purity of the lecithins obtained was checked by thin-layer chromatography with chromatographically pure standards by the method of Skipski et al. (6). Total phosphorus was determined by Bartlett's procedure (7), and esters by a modification (8) of the methods of Rapport and Alonzo (9) and Snyder and Stephens (10). Ester to phosphorus ratios ranged from 1.71 to 2.01 (mean 1.88). In some cases the purity of the lecithin fraction was further checked by chromatography, again with appropriate standards, on silicic acid-impregnated Whatman No. 1 paper by the method of Marinetti and Stotz (11). The isolated lecithins were identified on the paper with phosphomolybdic acid and stannous chloride. No contamination of the lecithins was detected using samples containing 10–15 μg of lipid P. The fatty acid methyl esters of the purified lecithins were prepared as previously described (5) and analyzed in a 6 ft column of 15% diethylene glycol succinate polyester on Chromosorb W in a model 10 Barber-Colman gas chromatograph. The columns were checked for quantitative accuracy each day with standard mixtures (Applied Science Laboratories, State College, Pa.) Peaks were identified by their retention times relative to known standards and quantified by triangulation. Statistical analysis of the differences between the fatty acid composition of the lecithins of bile and plasma was performed using the U test of Mann and Whitney (12). The "t" test of Student gave similar results.

For the studies in dogs, gallbladder bile and blood were collected under barbital anesthesia in the course of various surgical procedures in three animals. Ox bile and blood were obtained at the slaughter house at the moment of death in two steers. For the studies in rats, biliary cannulae were inserted under sodium amytal

TABLE 1 BILE AND PLASMA LECITHIN FATTY ACID PATTERNS IN MAN

Patient or Other Source	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
				% of total fatty acids				
Bile								
1, Gallbladder	Tr.	56.7	3.0	3.3	12.5	23.4	Tr.	1.0
2, Gallbladder	Tr.	39.9	7.6	6.3	16.5	24.1	Tr.	5.7
3, T-tube, fasting	Tr.	46.3	4.2	3.2	16.7	25.5	Tr.	4.9
3, T-tube, postprandial*	Tr.	48.2	3.8	2.5	15.3	23.9	Tr.	4.3
4, T-tube	Tr.	45.9	5.1	4.3	16.7	20.8	Tr.	3.8
5, T-tube	Tr.	39.0	2.4	3.4	13.7	32.1	1.4	7.9
Plasma								
Blood Bank	Tr.	25.6	1.7	14.5	23.6	22.6	Tr.	9.0
6,† Fasting	Tr.	36.6	Tr.	13.9	13.9	16.7	Tr.	10.6
6, Venous, post-absorptive*	Tr.	37.5	Tr.	18.9	17.7	19.6	Tr.	14.3
7,† Venous	Tr.	40.2	Tr.	11.4	13.7	16.6	Tr.	11.6
5, Venous	Tr.	33.5	2.5	12.5	16.5	17.5	Tr.	17.5
P	—	<0.009	<0.004	<0.002	N.S.	<0.004	N.S.	<0.002

* One hour after breakfast for bile, 4 hr after breakfast for plasma.
† Duodenal ulcer.

anesthesia in 300 g male Wistar rats. Twenty-four hours after recovery from the surgical procedure, bile was collected for 6 hr and blood was obtained by cardiac puncture under ether anesthesia at the end of the collection period. The bile and blood samples from three of the rats were pooled for analysis; approximately equal volumes were taken from each animal. Bile only was obtained from the fourth rat. For the studies in man, bile was obtained by gallbladder puncture under general anesthesia (for nongallbladder surgery) in patients 1 and 2 (T. J. and R. F.) and by T-tube drainage 10 days after cholecystectomy in patients 3–5 (H. B., K. N., and A. H.). In patient 3 bile was collected both before and after a normal breakfast. In patient 5 bile from a T-tube and blood were obtained simultaneously.

The concentration of lecithin in bile in the four species studied was roughly similar. In man, T-tube bile contained 4.1–9.3 μmoles/ml; gallbladder bile, 7.2–47.2 μmoles/ml. In rats, dogs, and oxen concentrations varied from 3.0 to 5.0 μmoles/ml. In man, 15–30 ml; in dogs, 12–27 ml; in rats, 5 and 15 ml; and in oxen about 500 ml of bile were obtained; 5–6 ml of plasma were taken in man, dogs, and rats, and 10–15 ml in oxen.

Fasting and post-absorptive blood was obtained in patient 6 (C. R. T.) and fasting blood in patient 7 (R. S.). A larger sample of 4-week old blood bank blood was also analyzed. In order to ascertain what changes may occur in plasma lecithin in the course of passage through the tissues, peripheral venous and arterial blood was collected simultaneously in patient 8 (M. H.) in the fasting state, and hepatic venous and superior vena caval blood from patient 9 (D. W.) in the course of a cardiac catheterization (which eliminated the possibility of congenital heart disease). All blood samples were collected in heparinized tubes and the plasma was immediately separated by centrifugation and mixed with chloroform–methanol within 40 min of collection.

RESULTS

In man, the proportion of palmitic (16:0), palmitoleic (16:1), and linoleic (18:2) acids was significantly higher in the lecithin of bile than in that of plasma, whereas stearic (18:0), and arachidonic (20:4) acids were present in significantly lower concentration in bile than in plasma lecithin (Table 1). No differences were observed in the proportions of myristic (14:0), oleic (18:1), and linolenic (18:3) acids. Ingestion of a normal breakfast did not change the fatty acid pattern of biliary lecithin in patient 3 nor that of plasma lecithin in patient 6. Examination of Table 2 shows that plasma lecithin from arterial and peripheral venous blood, and hepatic

TABLE 2 LACK OF SELECTIVE UTILIZATION OF PLASMA LECITHIN FATTY ACID BY TISSUES

Patient and Source	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
				% of total fatty acids				
8, Venous	Tr.	18:3	2.4	13.8	15.0	27.4	Tr.	15.1
8, Arterial	Tr.	14.1	1.6	14.9	16.2	25.6	Tr.	19.6
9, Hepatic vein	Tr.	18.5	1.7	15.6	18.2	26.3	1.2	11.5
9, Superior vena cava	Tr.	25.5	1.0	13.1	16.5	25.8	Tr.	10.9

vein and superior vena cava, has a similar fatty acid pattern. The only finding of possible significance was the larger amount of palmitic acid in lecithin from peripheral or mixed venous blood as compared to arterial or hepatic vein blood. Whether this difference is significant will need to be determined by further studies.

In dogs (Table 3), as in man, palmitic and linoleic acids were present in significantly higher concentration in the lecithin of bile than in that of plasma. Stearic and arachidonic acids accounted for significantly larger proportion of the fatty acids of the lecithin of plasma than of bile. By contrast with the results in man, oleic acid was found to be present in larger concentration in bile than in plasma lecithin. No significant differences were observed in the proportions of myristic, palmitoleic, and linolenic acids in the two lecithins.

Data from rats and oxen (Table 3) show that in both these species the concentration of palmitic acid was higher, and that of stearic and arachidonic acid lower, in the lecithin of bile than in that of plasma. Rats resembled man and the dog in that biliary lecithin contained more linoleic acid than did plasma lecithin. The reverse was true in the ox. Insufficient data are available in these two species for statistical analysis.

DISCUSSION

The results of the investigations reported here demonstrate significant differences in the fatty acid compositions of the lecithins of bile and plasma. In all four species studied, bile lecithin contained significantly more palmitic acid and less stearic and arachidonic acid than did plasma lecithin. Other differences were also observed but were not consistent in all species. The possibility that the differences between the lecithins of bile and venous plasma could be a reflection of peripheral utilization of specific subfractions of plasma lecithin would seem to be ruled out by the finding that the fatty acid pattern of lecithin from arterial and hepatic venous blood plasma did not differ significantly from that seen in peripheral or mixed venous blood in man.

Fishler et al. (1) demonstrated in 1943 that following hepatectomy virtually no P^{32} was incorporated into the plasma phospholipids of dogs 6 hr after the injection of phosphate-P^{32}. They therefore concluded that plasma phospholipids were derived from the liver. Popják and Muir (2) confirmed these findings. Borgström and Olivecrona (3) showed that, after ligation of the blood vessels of the porta hepatis in rats, injected palmitic acid-1-C^{14} was not recirculated, and concluded from this that the liver was the chief site of plasma lipoprotein synthesis. Zilversmit and Van Handel (4) showed that after intravenous injection of P^{32} in dogs, biliary lecithin specific activity reached a peak at 8–12 hr and then fell rapidly until it coincided with the plasma lecithin specific activity at about 24 hr. Both activities then declined together. The authors concluded that both plasma and bile lecithin were derived from the same source, presumably the liver. Current evidence, therefore, indicates that the lecithins of both bile and plasma are produced in the liver.

It has been shown by Glomset (13, 14) that transesterification of cholesterol by fatty acids from lecithin occurs in plasma and that these fatty acids are derived from the β-position of the lecithins. Menzel and Olcott (15) have shown that this position is occupied almost exclusively by unsaturated fatty acids. Transesterification could thus account for differences in linoleic and arachidonic acids

TABLE 3 BILE AND PLASMA LECITHIN FATTY ACID PATTERNS IN DOGS, RATS, AND OXEN

Tissue	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
				% of total fatty acids				
Dogs								
Bile A	Tr.	22.1	1.9	12.5	14.5	35.3	Tr.	13.3
Bile B	Tr.	21.8	3.1	15.5	15.9	26.3	Tr.	16.6
Bile C	Tr.	21.2	4.4	15.8	15.9	21.7	Tr.	18.8
Plasma A	Tr.	16.4	Tr.	29.4	10.2	18.2	Tr.	25.6
B	Tr.	16.4	1.4	32.4	10.3	12.5	0	25.1
C	Tr.	13.3	1.9	30.3	9.0	10.9	0	33.2
P	N.S.	<0.05	N.S.	<0.05	<0.05	<0.05	N.S.	<0.05
Rats								
Bile 1 (3 pooled)	Tr.	26.9	1.0	11.3	8.0	32.1	Tr.	18.3
Bile 2 (1 rat)	Tr.	31.4	0.8	10.0	6.2	33.4	0	17.7
Plasma (3 pooled)	0	17.3	0	29.9	6.6	16.6	0	29.6
Oxen								
Bile A	Tr.	44.4	4.7	14.1	19.7	6.9	1.9	Tr.
Bile B	Tr.	26.6	3.4	19.1	24.5	12.2	Tr.	3.0
Plasma A	1.4	16.8	4.3	18.8	13.7	23.9	6.0	8.8
Plasma B	Tr.	19.4	2.6	29.2	14.1	19.6	2.5	4.9

between bile and plasma lecithins, but not for difference in the saturated acids, palmitic and stearic. Furthermore, such a reaction would lower the levels of the unsaturated acids in plasma lecithin, yet in all species studied it had a higher concentration of arachidonic acid than did biliary lecithin. In oxen, plasma lecithin contained more linoleic acid than did the lecithin of bile, though the opposite was found in man, dogs, and rats. No cholesterol ester was detectable in the bile of man, dog, or ox. Only traces were found in rat bile (unpublished data). Therefore transesterification of cholesterol by the β fatty acid of lecithin in plasma cannot account for the difference reported here.

In view of these considerations and the fact that the lecithins of both bile and plasma are of hepatic origin, our findings indicate the presence of two separate pools of lecithin in the liver or selection from one heterogeneous pool. The presence of more than one pool of lecithin in the liver was suggested by Zilversmit and Van Handel (4) some years ago. Further evidence to support this view was reported by Collins (16) who showed that radioactive phosphate was incorporated preferentially into palmitoyl lecithin as opposed to stearoyl lecithin in rat liver in vivo, indicating a more rapid turnover of palmitoyl lecithin. Zilversmit and Van Handel (4) reported that in dogs given phosphate-P^{32} intravenously the specific activity of biliary lecithin rose faster than that of plasma lecithin. As bile lecithin has been shown to be predominantly of the palmitoyl type the results reported by Collins (16) and Zilversmit and Van Handel (4) suggest a more rapid turnover of biliary than of plasma lecithin. However, at least part of the difference in specific activity between the lecithins of bile and plasma might have been due to dilution of plasma lecithin-P^{32} with preexisting lecithin.

Preliminary data from this laboratory in dogs and rats indicate that total liver lecithin fatty acid patterns differ from those of both plasma and bile. Comparison of the data here reported on the fatty acids of rat bile and plasma lecithin with those of Glenn, Opalka, and Tischer (17) for rat liver lecithin confirm this view. If this proves to hold true for larger series it would suggest the presence of yet a third, perhaps structural, pool of lecithin in the liver as suggested by others (4). Two groups of investigators have, however, been unable to show any difference between the lecithins of rat liver microsomes and mitochondria (18, 19).

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