Short Communications

SC 2359

The phospholipids of ruminant bile

The concentration of phospholipids in human bile may be as high as 800 mg/100 ml (see ref. 1); the major component is phosphatidyl choline, with small amounts of lysophosphatidyl choline and phosphatidyl ethanolamine[2]. It was thought[3] that palmitic and oleic acids were the only fatty acids present in phosphatidyl choline from human bile, but more recent analyses using gas–liquid chromatography have shown that a much wider variety of fatty acids are associated with the bile phospholipids[1,4]. The concentration and composition of phospholipids in bile from sheep and cattle have been studied in the present investigation.

Samples of bile, which were free from contamination by pancreatic juice, were collected from fistulae of the bile ducts of merino sheep and merino lambs[5] and from the gall-bladders of merino sheep and shorthorn cattle after they were slaughtered. The ages of the sheep and cattle were estimated to be from 4–6 years and from 1–2 years respectively; the lambs were about 2 weeks old. The phospholipids were extracted from samples of bile into ethanol–diethyl ether (3:1) and the concentration of lipid phosphorus was estimated[6]. The concentration of phospholipid was calculated by multiplying the concentration of lipid phosphorus by 25. In other experiments, the phospholipids were extracted from the bile into chloroform–methanol (2:1) and were separated from the other lipid fractions on silicic acid columns. The non-phospholipid fractions were removed from the columns with chloroform and the phospholipids were eluted with methanol. The different phospholipids in the methanol eluate were separated by thin-layer chromatography[7] and the fractions were visualised on the silicic acid plates with iodine vapour or by spraying the plates with Dragendorff reagent[8] or ninhydrin. The content of phosphorus in each of the phospholipid fractions was estimated after they had been eluted from the plates with methanol. The techniques used for gas–liquid chromatography have been described in another paper[9].

The mean concentrations of phospholipids in samples of bile collected for periods of 12 h from fistulae in 4 sheep and 4 lambs were 1370 $\pm$ 90 mg/100 ml and 1520 $\pm$ 300 mg/100 ml respectively. When the animals were deprived of bile the flow rate of the bile decreased significantly but the concentration of phospholipids in the bile did not change. Samples of bile collected from the gall-bladders of 2 sheep contained 1090 and 1430 mg of phospholipid/100 ml and samples of gall-bladder bile collected from 2 cattle contained 1340 and 1150 mg of phospholipid/100 ml. When bile was returned to the duodenum of sheep and lambs with bile fistulae, the average rate of bile flow in the sheep was 37.1 $\pm$ 4.9 ml/h and in the lambs 4.6 $\pm$ 0.93 ml/h; in sheep and lambs, therefore, about 10–15 g and 1–2 g respectively of phospholipid enters the gut each day in the bile.

The bile phospholipids obtained from sheep and cattle contained about 90% of phosphatidyl choline; sheep bile, however, contained almost equal proportions of phosphatidyl choline and lysophosphatidyl choline (Table I).

The lysophosphatidyl choline in a sample of phospholipid obtained from sheep bile was isolated by silicic acid column chromatography. The phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin were eluted with chloroform–methanol (1:4) and lysophosphatidyl choline was eluted with methanol. When the lysophosphatidyl choline fraction was examined with thin-layer chromatography, it was found to move as a single spot with an R_F value identical to that of a sample of

TABLE I

THE PHOSPHOLIPID FRACTIONS IN RUMINANT BILE

Phospholipid fraction	*Per cent of total phospholipids in bile from*					
	Sheep 1	*Sheep 2*	*Lamb 1*	*Lamb 2*	*Steer 1*	*Steer 2*
Phosphatidyl choline	53	49	90	95	89	95
Lysophosphatidyl choline	39	45	Trace	Trace	Trace	Trace
Phosphatidyl ethanolamine	Trace	5	5	2	4	3
Sphingomyelin	8	Trace	5	3	8	2

lysophosphatidyl choline isolated from rat liver. The lysophosphatidyl choline isolated from the bile contained esterified fatty acids[10] and phosphorus in a molar ratio of 0.87:1 and caused haemolysis of sheep red blood cells. When 4 ml of a 1% suspension of washed sheep red cells containing 0.2 μM of bile lysophosphatidyl choline were incubated for 30 min at 37°, all of the red cells were haemolysed. When the red cells were incubated with 0.06 μM of lysophosphatidyl choline, approx. 12% of the cells were haemolysed.

The composition of the fatty acids in the bile phospholipids of sheep, lambs and cattle is shown in Table II. In these animals, palmitic and oleic acids were the major constituents, but relatively high proportions of stearic and linoleic acids were also present. The fatty acid composition of the phosphatidyl choline and lysophosphatidyl choline fractions from sheep bile were not significantly different (Table II).

TABLE II

THE FATTY ACIDS IN PHOSPHOLIPIDS FROM RUMINANT BILE

Animal number	*Per cent of total fatty acids**					
	16:0	*16:1*	*18:0*	*18:1*	*18:2*	*18:3***
Sheep 1 (lysophosphatidyl choline)	31.1	4.8	12.1	31.0	6.9	1.7
Sheep 1 (phosphatidyl choline)	33.6	6.7	12.2	24.2	8.5	2.5
Sheep 2 (total)	28.4	5.6	13.2	28.7	10.2	6.1
Sheep 3 (total)	29.3	6.0	13.6	34.5	5.5	1.7
Lamb 1 (total)	29.8	9.2	6.8	19.4	17.2	6.0
Lamb 2 (total)	32.0	4.5	10.2	19.7	14.4	9.0
Steer 1 (total)	36.7	6.0	14.7	20.2	8.0	4.7
Steer 2 (total)	33.2	6.4	11.8	24.5	7.4	4.4

* Small amounts of saturated and unsaturated fatty acids with 12, 14, 15, 17, 20 and 22 carbon atoms were generally present.

** The fatty acids are designated by the number of carbon atoms and the number of double bonds: thus 18:0 represents stearic acid, 18:2 represents linoleic acid etc.

HOFMANN[11] has suggested that lysophosphatidyl choline, in addition to bile salts, may aid the absorption of fat by forming soluble micelles with fatty acids and monoglycerides in the intestinal lumen. In sheep, the absorption of fat is absolutely dependent on the presence of bile in the gut[5] and it is possible that the large amounts of phospholipid which enter the duodenum in the bile may facilitate the uptake of fat into the cells of the intestinal mucosa.

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The origin of hydrogen in fatty acids formed by lactating rat mammary gland

It is generally accepted that, of the 4 hydrogens in each ($-CH_2-CH_2-$) group in fatty acids, the two on the odd-numbered carbons are derived from reduced pyridine nucleotides, predominantly TPNH[1]. Of the 2 hydrogens on the even-numbered carbons, one is derived from acetyl-CoA and the other from water[1].

FOSTER AND BLOOM[2,3] have shown that more than half of the hydrogens on the even-numbered carbons of the fatty acids formed by rat-liver slices is derived from water, indicating an exchange between the methyl hydrogen atoms of acetyl-CoA and the protons of water.

The present report deals with incorporation of ^3H and carbon from acetate and glucose into fatty acids by lactating rat mammary gland preparations. In this tissue, labilization of the methyl hydrogen (that derived from acetate or glucose, and appearing on the even-numbered carbons of the fatty acid) also occurs. But in this case the exchange with the protons of water is less than that observed with liver slices. The value for the $^3H/^{14}C$ ratio was about 0.3 (Table I) in fatty acids synthesized by mammary gland slices from [2-^3H,^{14}C]acetate in the presence of glucose or from [6-^3H,^{14}C]glucose in the presence of acetate. The values that have been reported for liver slices, however, are 0.2 for acetate and 0.1 for glucose[2,4]; the difference between the acetate and glucose ratios for liver was attributed by FOSTER AND BLOOM[2,4] to labilization of the 6-^3H from glucose at the phosphoenol pyruvate stage[5]. The reason