planned to analyze white and gray matter separately and from different brain areas. It is the major advantage of our procedures that very small amounts of esters are required for analysis, 3 μl being sufficient for a single chromatogram, and as little as 10 μl for total analyses including hydrogenation and oxidation. Hence it is possible to analyze gray and white matter from several different brain areas from a small laboratory animal.

Summary. Gas chromatographic analysis of normal fatty acids according to chain length from C^{10} through C^{24} and even-numbered α hydroxy acids from C^{18} through C^{24} was reported for samples from human and chicken brain. Separation of 25 resolved peaks representing saturated and unsaturated normal fatty acids was achieved by chromatography of esters on a 10 foot diethylene glycol polyester succinate column. Over half of these peaks were identified, tentative identifications were given for remainder and their relative amounts in human and chicken brain fatty acids were reported.

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Fatty Acid Pattern of Human Bile under Normal and Pathological Conditions.* (25780)

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It was found(1) with the aid of gas-liquid chromatography (GLC) that total fatty acids of human bile constitute a complex mixture of several compounds. It was also shown that purified bile lecithin contains palmitic, stearic, oleic, linoleic, and arachidonic acids, and small amounts of several other fatty acids. Fatty acids of the bile lecithin are asymmetrically distributed on the molecule and represent about 90% of total fatty acids of human bile. The lecithin of the bile may play an important role together with bile acids in holding cholesterol in solution(2,3). If lecithin has a pronounced physiological action, differences in fatty acid composition of the bile lecithin might influence such a balanced system. The purpose of this study was to investigate which fatty acids are normally present in human bile and to see how these may vary in biliary disease.

Methods. Material. Specimens of gallbladder bile and hepatic bile were collected from different cases. Table I gives clinical data. Three cases with duodenal ulcer had no signs of biliary disease. The bile was collected into methanol and stored in a refrigerator until analyzed. Fractionation and Gas-Liquid Chromatography: Total fat was extracted with chloroform:methanol according to Folch *et al.*(4). Total fatty acids were isolated after saponification and acidification, then methylated and submitted to analysis by gas-liquid chromatography as described previously(1). The lecithin from 3 different bile

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TABLE I. Clinical Diagnosis of 13 Patients, Made by Routine Laboratory Methods, X-ray Examination and Clinical Findings during Operation.

Patient	Sex	Age	Clinical diagnosis	Gallbladder function
H.J.	♀	66	Duodenal ulcer	Normal gallbladder
U.L.	♂	47	*Idem*	*Idem*
A.B.	♀	36	"	"
M.S.	♀	38	Gallstone disease	Funct. gallbladder
V.J.	♀	53	*Idem*	*Idem*
J.L.	♀	27	"	"
E.N.	♀	62	"	Non-funct. gallbladder
H.S.	♀	50	"	*Idem*
O.F.	♂	75	Cancer of head of pancreas	Normal gallbladder
C.C.	♀		Gallstone disease—common duct stone with subsid. jaund.	Non-funct. gallbladder
H.P.	♀	76	Common duct stone—cholecystectomized before subsid. jaund.	
T.P.	♀	43	Gallstone disease	Gallbladder occlusion by stone
E.H.	♀	53	Common duct cancer	Gallbladder occlusion by tumor infiltration around cystic duct

specimens from cases with gallstone disease (one with common duct stone without jaundice) was purified by silicic acid chromatography as described earlier(1). The purified bile lecithin was hydrolyzed through the action of snake venom phospholipase A (*Crotalus adamanteus*). Fatty acids released by phospholipase A and those obtained after hydrolysis of the lysolecithin formed from the bile lecithin were methylated and subjected to GLC. Other experimental procedures used have been described(5). Fatty acids are numbered as suggested by Insull *et al.*(6). The category C_{20-22} refers to a group of unsaturated fatty acids whose structure has not been determined.

Results. Specimens of bile from subjects without biliary disease and from subjects with varying biliary disease were analyzed. Content of neutral fat + free fatty acids and of phospholipids in the bladder bile specimens analyzed was in the range of 0.5 to about 1% (1,2). The detailed results of analyses of fatty acids are shown in Table II and a summary of the proportions of the 3 main groups of acids is given in Table III. There is no significant difference in composition of total fatty acids of bile between patients without biliary disease and those with gallstone disease, with or without complications. The results of these analyses also indicate the very good reproducibility of the methods used for extraction and gasliquid chromatography. There is no difference in composition between total fatty acids of bile from gallbladders and those from hepatic bile. Because of the limited cases studied (especially normals) it is, however, not possible to make definite conclusions from our data.

Analyses were also made of total fatty acids of bile specimens from 2 patients with occluded gallbladder, one case caused by gallbladder stone, with beginning resorption of bile pigments, and one case with white bile due to a common duct cancer above and around the cystic duct. In both these cases there is a clear tendency to higher concentration of heptadecanoic acid and stearic acid (Table II). Total saturated fatty acids have increased from around 35 to 58% of total fatty acids. Furthermore, concentration of linoleic acid has decreased from 25 to about 8% of total fatty acids. Thus there are significant changes in proportions between saturated, mono-saturated and polyunsaturated fatty acids (Table III).

In Table IV fatty acid composition of human gallbladder bile lecithin from 3 patients with gallstone disease is shown. The lecithin was purified by silicic acid column chromatography and hydrolyzed with phospholipase A. Fatty acids released by action of snake

TABLE II. Detailed Composition of Total Fatty Acids of Gallbladder Bile and Hepatic Bile Patients without Biliary Disease and Patients with Gallstones. Clinical data given in Table I. Values expressed as percentage of total fatty acid methyl esters.* Component fatty acids are also designated by a dual symbol giving chain length and No. of double bonds.

		Duodenal ulcer			Gallstone disease with functioning gallbladder			Gallstone disease without functioning gallbladder		Cancer of panct. head (jaund.)	Common duct stones with subsiding jaund., C.C.*		Occluding gallbladder stone	"White bile" Common duct cancer above and around cystic duct
Saturated														
Lauric	12:0													
Myristic	14:0	.5	1.3	.7	.7	1.0	.7	.9	.6	.7	1.3	.7	4.1	4.6
Pentadecanoic	15:0	.5	.6	.7	.6	.6	.9	.8	.8	.4	.6	.4	4.0	2.7
Palmitic	16:0	30.7	27.2	28.3	23.3	23.4	43.8	33.2	26.9	32.1	27.5	27.8	26.6	24.1
Heptadecanoic	17:0	.3	.5	.5	.7	.4	.8	.6	.7	.3	.5	.7	7.7	15.2
Stearic	18:0	4.5	4.7	5.4	5.5	6.5	3.5	4.8	4.3	4.0	8.5	6.9	13.6	13.7
Monounsaturated														
Palmitoleic	16:1	3.4	7.4	6.4	10.0	8.4	5.1	5.9	6.7	7.4	2.4	5.0	3.6	3.4
Oleic	18:1	17.6	17.4	19.2	20.2	22.7	13.1	17.7	20.8	18.5	20.0	20.6	22.7	15.8
Diunsaturated														
Linoleic	18:2	29.7	22.4	25.4	27.2	29.8	26.5	25.6	27.0	26.4	27.8	28.1	10.4	6.7
Polyunsaturated														
Linolenic	18:3	.7	.9	1.1	.5	.5		.7	.6	.4	.6	.6	2.0	.6
Arachidonic	20:4	7.6	5.0	5.7	4.6	2.7	3.5	3.6	7.1	4.2	4.1	4.5	1.9	3.6
	20-22?	4.0	6.0	5.2	6.0	3.0	2.1	5.3	4.0	5.3	6.1	4.1	3.4	7.8

* C.C.—In this case hepatic bile was analyzed.

TABLE III. Composition of Principal Groups of Fatty Acids of Gallbladder Bile of Patients with Hepato-biliary Disease. Values expressed as percentage of total fatty acid methyl esters.

Subject	% of total fatty acids		
	N-saturated FA	Mono-unsaturated FA	Highly unsaturated FA
H.J.	36.5	21.0	42.0
U.L.	34.3	24.8	34.3
A.B.	35.6	25.6	37.4
M.S.	30.8	30.2	38.3
V.J.	31.9	31.1	36.0
L.L.	49.7	18.2	32.1
E.N.	40.3	23.6	35.2
H.S.	33.3	27.5	38.7
O.F.	37.5	25.9	36.3
C.C.	38.4	22.4	38.6
H.P.	36.5	25.6	37.3
T.P.	56.0	26.3	17.7
E.H.	60.3	19.2	18.7

venom phospholipase and fatty acids of the lysolecithin were determined with gas-liquid chromatography. In this way 2 different groups of fatty acids were obtained. Fatty acids freed by phospholipase A contained mainly unsaturated fatty acids and those of lysolecithin contained mainly saturated fatty acids. The predominating fatty acid of the lysolecithin was palmitic acid. According to new work of Tattrie(7) it seems very likely that saturated fatty acids of lecithin are attached to the C-1 (alpha) ester position and unsaturated fatty acids are attached to the C-2 (beta) position. The reasons for this concept have been discussed(1). The results give further support to the conclusion(1) that there are several different lecithins present in human bile.

It is also apparent that fatty acid composition is different in the 3 lecithin specimens analyzed. In subject U. O. there is an increased amount of polyunsaturated fatty acids especially belonging to the C_{20-22} group. Further work is necessary to identify these fatty acids. In subject L.T. (with a common duct stone but without jaundice) the proportion of oleic acid was increased from about 24 to 47%.

Discussion. Study of the fatty acid composition of total biliary fatty acids of patients without biliary disease and patients with uncomplicated gallstone disease showed no significant alteration which could indicate that fatty acids excreted *via* the bile were essentially different from those of normal individuals. Because of the limited number of normal cases studied definite conclusions can not be made.

There is some indication that in patients with occlusion of the cystic duct and absorption of the bile pigments the fatty acid pattern is definitely changed. There is a significant reduction in proportion of polyunsaturated fatty acids, especially linoleic acid, and a corresponding increase in proportion of saturated fatty acids, mostly in the stearic acid concentration. These changes may be due to metabolic impairment of the lipid metabolism of the liver before occlusion of the cystic duct is complete. A partial reabsorption of fatty acids from the bile or perhaps hydrogenation of unsaturated fatty acids by

TABLE IV. Fatty Acid Composition of Human Bile Lecithin from 3 Patients (U.O., L.T., and N.H.). Lecithin was isolated by silicic column chromatography and hydrolysed by phospholipase A *(Crotalus adamantus)*. Fatty acids were determined by gas-liquid chromatography. Values expressed as percentage of total fatty acid methyl esters respective fractions.

Fatty acid		% of total fatty acid in respective fraction of human bile lecithin					
		Fatty acids freed from bile lecithin by phospholipase A			Fatty acids obtained after hydrolysis of lysolecithin		
Saturated							
Lauric	12:0						
Myristic	14:0	.6	.9	.6	1.4	1.4	5.3
Pentadenoic	15:0		.6	.7	1.6	1.5	5.4
Palmitic	16:0	15.1	8.4	17.1	61.0	66.3	53.8
Heptadecanoic	17:0		.7	.7	2.7	2.1	2.7
Stearic	18:0	4.6		1.5	8.2	11.4	6.5
Monounsaturated							
Palmitoleic	16:1	4.5	8.5	6.4	6.7	9.8	7.9
Oleic	18:1	21.9	47.4	25.9	5.0	7.5	6.6
Diunsaturated							
Linoleic	18:2	31.0	32.0	32.1	3.1		1.7
Polyunsaturated							
Linoleic	18:3		1.5				
Arachidonic	20:4	1.8	5.1				
	20-22?	20.4	8.2	8.6			3.3

Component fatty acids are also designated by a dual symbol giving chain length and No. of double bonds. Sum of each column is less than 100% because trace amounts of unusual fatty acids detected were omitted from final tabulation.

bacterial enzymes must, however, also be considered.

Study of a few specimens of lecithin purified from the gallbladder bile of subjects with gallstone disease encourages a wider study of fatty acid composition of the lecithins of bile collected from subjects with various liver affections. It will be of interest to compare fatty acid composition of the various lipids of the liver with corresponding fatty acids in the bile. This is now under investigation.

Summary. 1) Total fatty acids of human gallbladder bile and hepatic bile were analyzed qualitatively and quantitatively by gas-liquid chromatography. The 3 major fatty acids in human bile are palmitic, oleic and linoleic acid, representing around 80% of total fatty acids. 2) Analysis of composition of total fatty acids in uncomplicated gallstone disease failed to reveal any abnormality in this disease. 3) In one subject with occluding gallbladder stone and beginning absorption of bile pigments, and one subject with white bile, a reduction of the proportion of polyunsaturated fatty acids and an excess in saturated fatty acids were observed. 4) The significance of differences in relative concentrations of fatty acids of lecithins in subjects with gallstone disease is discussed.

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Metabolism of Arginine by Mouse Fibroblast, Strain L.* (25781)

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It has recently been reported(1) that periodic additions of L-arginine to cultures of mouse fibroblast cells (Earle's strain L) growing in suspension obviated the need for a medium change for periods up to 10 days or more. During this time growth rate and viability were maintained at a high level. This report presents results which may explain the need for periodic addition of L-arginine for sustained growth of the L cells.

Methods. Spinner cultures of L strain cells were set up as previously described(1), using Eagle's basal medium. The bidaily additions of L-arginine-HCl, L-citrulline and L-aspartic acid were such that final concentration of the supplement was 0.2 micromole. Initial cell concentrations were approximately 2×10^5 cells per ml. Samples were removed daily for estimations of cellular proliferation and viability and of ammonia content of the growth medium. Free ammonia in the medium was determined as follows: The cells were removed by a short centrifugation; 1.0 ml of the supernatant fluid was placed in a 16 x 150 mm test tube fitted with an air inlet; 2.0 ml of a saturated solution of potassium carbonate and 1 drop of octyl alcohol were added; the ammonia was driven off by vigorous aeration for 30 minutes into 4 ml of 1 N sulfuric acid(2). Amount of ammonia trapped in the acid was determined by nesslerization(3). Chromatographic analysis of the reaction products was carried out with a newly available sulfonic acid type cation exchange resin paper,† which, according to Tuckerman

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