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Biliary lipid output during three meals and an overnight fast

I Relationship to bile acid pool size and cholesterol saturation of bile in gallstone and control subjects

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SUMMARY Using a duodenal perfusion technique, the biliary output of bile acids, phospholipid, and cholesterol was measured hourly during three meals and an overnight fast in seven Caucasians with radiolucent gallstones in a functioning gallbladder, and in seven healthy controls without gallstones, closely matched for age, sex, and weight. Before the perfusion, bile acid kinetics were defined by an isotope dilution procedure, and the biliary lipid composition of fasting gallbladder bile was determined. Total daily biliary lipid output was similar in gallstone and control subjects, and was unrelated to cholesterol saturation of fasting gallbladder bile and to bile acid pool size. There was an inverse relationship between the size and recycling frequency of the bile acid pool, so that secretion rate and hepatic return of bile acids remained constant, despite a wide range of pool sizes. The finding of a normal bile acid synthesis rate in subjects with a small pool size therefore indicated normal feedback regulation of bile acid synthesis. Hourly measurements of biliary lipid output showed a linear relationship between bile acid and cholesterol output, with a similar regression line for gallstone and control subjects, but a non-linear relationship between bile acid and phospholipid output. Consequently, samples from all subjects were consistently supersaturated with cholesterol at low bile acid outputs, especially during overnight fasting, but not at high bile acid outputs.

These findings indicate that hepatic secretion of bile supersaturated with cholesterol is physiological in man at low bile acid outputs, that bile acid pool size is probably determined in part by its recycling frequency, and that cholesterol cholelithiasis in some Caucasians may be due to an underlying extrahepatic abnormality.

It is currently believed that the primary defect in cholesterol gallstone formation is a hepatic abnormality (Swell, Bell, and Vlahcevic, 1971; Schoenfield, 1972; Small, 1972; Grundy, Metzger, and Adler, 1972). Two independent lines of evidence have led to this conclusion. The first was the demonstration by Small and Rapo (1970) and independently by Vlahcevic, Bell, and Swell (1970a) that fasting hepatic bile obtained at surgery from gallstone patients is saturated or supersaturated with cholesterol. It had been demonstrated previously that fasting gallbladder bile from gallstone patients, but not from normal subjects, was supersaturated with cholesterol (Admirand and Small, 1968). It was therefore assumed that healthy subjects would have unsaturated hepatic bile, and such samples from normal controls were not obtained.

The second line of evidence was the demonstration by Vlahcevic, Bell, Buhac, Farrar, and Swell (1970b) that gallstone subjects have a reduced bile acid pool size, yet a normal synthesis rate. The absence of a compensatory increase in bile acid synthesis in response to an inferred decreased return of bile acids to the liver was thus attributed to inappropriate repression of bile acid synthesis, probably due to a hepatic enzyme defect (Schoenfield, 1972).

Indirect estimates of biliary lipid secretion in Caucasians with gallstones have been made on the

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basis of bile acid pool size measurements and biliary lipid composition of fasting gallbladder bile. These computations predicted a reduced secretion rate of bile acids and phospholipid, without an equivalent reduction in cholesterol secretion (Swell *et al*, 1971) and appeared to provide an explanation for hepatic production of supersaturated bile. But their validity was based on two critical assumptions: (1) that a small bile acid pool in gallstone subjects recycles at the normal rate; and (2) that the lipid composition of secreted bile remains constant and identical to that present in fasting gallbladder bile.

In order to test this current concept of pathogenesis, we have carried out direct measurements of biliary lipid output by means of a duodenal perfusion technique in a group of Caucasians with radiolucent gallstones and in a group of control subjects with a normal cholecystogram. Our findings, admittedly based on small study groups, indicate that the above assumptions appear to be incorrect. Our findings also challenge the current concept of pathogenesis, and provide the basis for alternative explanations for the presence of a small bile acid pool, and for the hepatic production of bile supersaturated with cholesterol.

Some of our provisional results were presented at the annual meeting of the American Association for the Study of Liver Disease, 1972, and have also been published in the form of a brief preliminary communication (Northfield and Hofmann, 1973).

Materials and Methods

SUBJECTS

Seven Caucasians with radiolucent gallstones in a functioning gallbladder were compared with seven healthy volunteers with a recent normal cholecystogram (table I). Subjects in the two groups were carefully matched in pairs for sex and age (to the nearest five years), as well as for weight and body build (based on calculated ideal weight and body surface area). Four subjects in both groups were female (postmenopausal). Informed consent was given before the study.

PROTOCOL

Gallstone and control subjects were admitted to a metabolic unit for six days. Each received a standard diet of 30 Calories/kg body weight/day, containing 40% of calories as fat, 40% as carbohydrate, and 20% as protein. Before breakfast on the second day, ^{3}H-chenodeoxycholic acid (30 μCi) was administered intravenously. Before breakfast on the third day, a triple-lumen tube (total OD approximately 4 mm) was passed via the nasal route and positioned under fluoroscopic control so that the aspiration site was situated in the second part of the duodenum. Three ml of duodenal bile was then collected after gallbladder contraction stimulated by the intravenous infusion of 40 Ivy dog units of cholecystokinin (CCK, supplied by Karolinska Institutet, Stockholm, Sweden). A further sample of 1 ml was collected in in the same way before the evening meal on that day, and a further four samples of 1 ml each before breakfast and before the evening meal on the next two successive days for estimation of bile acid kinetics. A final sample of 3 to 10 ml bile was obtained fasting on the morning of the seventh day, after completion of the perfusion study, for determination of fasting-state biliary lipid composition as well as biliary bile acid specific activity. One ml of each bile sample was added to 20 ml 95% ethanol for extraction of bile acids and cholesterol. In the case of the first and last samples, 1 ml was

Gallstone Subjects							*Control Subjects*						
Subject No.	*Age*	*Sex*	*Weight (kg)*	*Height (m)*	*Ideal Weight*[1] *(kg)*	*Body Surface Area*[1] *(sq m)*	*Subject No.*	*Age*	*Sex*	*Weight (kg)*	*Height (m)*	*Ideal Weight*[1] *(kg)*	*Body Surface Area*[2] *(sq m)*
1	64	F	59·1	1·56	47-53	1·60	1	63	F	57·3	1·52	46-51	1·55
2	69	M	88·6	1·73	63-69	2·00	2	69	M	85·9	1·83	70-77	2·10
3	42	M	76·4	1·69	59-65	1·85	3	46	M	73·2	1·70	61-67	1·85
4	53	F	59·5	1·57	49-54	1·60	4	48	F	56·8	1·73	58-63	1·65
5	47	M	78·2	1·83	70-77	2·00	5	49	M	63·6	1·70	61-67	1·75
6	55	F	70·0	1·78	62-69	1·85	6	53	F	70·0	1·63	51-57	1·75
7	55	F	46·8	1·56	47-53	1·45	7	55	F	59·0	1·59	49-54	1·60
Mean ± SD	55·0 ± 9·3		68·4 ± 14·2	1·67 ± 0·11	59·5 ± 9·2	1·76 ± 0·22	Mean ± SD	55·4 ± 9·4		66·5 ± 10·6	1·67 ± 0·10	59·6 ± 8·7	1·71 ± 0·21

Table I *General details*

[1]Metropolitan Life Insurance Company Statistical Bulletin 40. New Weight Standards for Men and Women. (November-December, 1959). New York.
[2]According to Gehan and George (1970).

also added to 20 ml of chloroform-methanol (2/1, v/v) for extraction of phospholipids. All samples were kept at −5°C till completion of the study.

Before breakfast on the sixth day, the position of the nasoduodenal tube was adjusted under fluoroscopic control, so that the perfusion site was now situated in the second part of the duodenum and the aspiration site 25 cm distally at the ligament of Treitz. The third (reinfusion) port was situated 30 cm beyond the ligament of Treitz. A gastric tube was also passed perorally and positioned fluoroscopically for the introduction of liquid meals and for checking samples for duodenal reflux. In order to attain steady state conditions, duodenal perfusion was started at 7 am, 60 minutes before the first meal. Jejunal samples were obtained by siphonage, collected over ice, and pooled at 60-minute intervals. Ten ml of each hourly sample of jejunal aspirate was frozen at −10°C and kept for analysis; the remainder was rewarmed to 37°C and slowly reinjected into the distal port, in order to maintain the integrity of the enterohepatic circulation of bile acids. In case of reflux, the aspiration port was closed during this and the subsequent 10 minutes.

Three equicaloric liquid meals were administered by gastric tube at 8 am, 1 pm, and 6 pm. These contained the same number of calories and the same relative composition of fat, protein, and carbohydrate as the diet given during the previous five days. In order to improve the accuracy of the biliary lipid output measurements, dietary intakes of cholesterol and phospholipid were reduced to a minimum. Fat was given in the form of distilled butter oil, which contained 0·02% sterol by weight (generously supplied by Dr E. H. Ahrens Jr, of the Rockefeller University), the protein as dry, non-fat, skimmed milk powder (0·01% sterol by weight, and 0·2% phospholipid) and the carbohydrate as dextrose and skimmed milk powder. The volume was adjusted to 2 calories per ml. $^{51}CrCl_3$ (5 μCi, obtained from Cambridge Nuclear Corp, Cambridge, MA) was added to the meal as a non-absorbable marker.

BILIARY LIPID OUTPUTS

The hourly output of biliary lipids into the duodenum (representing the sum of hepatic and gallbladder bile) was measured by means of a duodenal perfusion technique. For measurement of bile acid output, it was assumed that no significant absorption of conjugated biliary bile acids occurred in the 20 cm mixing segment, and a non-absorbable recovery marker (polyethylene glycol) was used. For measurement of phospholipid and cholesterol outputs, absorbable markers were used (^{14}C-lecithin, 10 μC uniformly labelled, and ^{3}H-cholesterol, 50 μCi, both having a radiochemical purity of > 98% by zonal scanning). As non-radioactive carrier, chromatographically pure, unlabelled lecithin (30 mg) and cholesterol (6 mg) were also added. Labelled and unlabelled lecithin and cholesterol were dissolved together in 2-3 ml ether and dispersed in 5 ml water. The ether was evaporated under a stream of nitrogen and the volume made up to 10 ml with water. The opalescent suspension was then irradiated ultrasonically for 20 minutes in an atmosphere of nitrogen at 0°C (Saunders, Perrin, and Gammack, 1962). The resulting solution was optically clear, and was diluted to 4 litres with polyethylene glycol (PEG) solution (5 g PEG per litre of normal saline), and the solution thoroughly shaken. There was no detectable precipitation of labelled lecithin or cholesterol over the following 48 hours.

The solution was at pH 6, warmed to 37°C and perfused at 2 ml/minute. It was assumed that exogenous, labelled phospholipid and cholesterol would rapidly equilibrate with endogenous biliary phospholipid and cholesterol in the mixing segment, and that both forms of phospholipid and cholesterol would be absorbed at the same rate. No relationship was observed between duodenal output of phospholipid and its absorption in the mixing segment (which averaged approximately 50%) or that of cholesterol (which averaged 25%), suggesting that marker phospholipid and cholesterol did in fact equilibrate with biliary phospholipid and cholesterol. Total interruption of the enterohepatic circulation of bile acids due to sampling of duodenal content averaged 5·8 ± 0·5% (mean ± SEM) for all studies, and did not exceed 10% in any subject. There was no significant difference in the degree of cholesterol saturation of fasting gallbladder bile obtained on the second day of the study and that obtained on the seventh day, following the perfusion, and there was no alteration in the fractional turnover rate of the chenodeoxycholic acid pool following the perfusion. Replicate studies on three normal controls gave a mean coefficient of variation of 14·3% for bile acid output and 12·5% for cholesterol output (Brunner, Northfield, Hofmann, and Summerskill, 1975).

The output of phospholipid and cholesterol into the duodenum was due mainly to biliary secretion, but in part to gastric emptying of dietary phospholipid and cholesterol. The latter contribution was kept to a minimum by using a low sterol-low phospholipid test meal, as already described, but the dietary contribution was subtracted from the total output in order to obtain the biliary output of phospholipid and cholesterol. For this purpose, $^{51}CrCl_3$ was used as a non-absorbable meal marker. From this, and the measured concentration of phospholipid and cholesterol in the meal, the gastric

output of phospholipid and cholesterol could be calculated. During the 24-hour period, this correction factor never exceeded 10% of the total phospholipid and cholesterol output into the duodenum in any subject.

Significant reflux of duodenal content into the stomach did not occur at the low duodenal perfusion rate used (2 ml/minute), as judged by measuring the concentration of bile acids and polyethylene glycol in random samples of gastric contents from each subject.

BILE ACID KINETICS

An isotope procedure was used, based on that of Lindstedt (1957). 2,4-^{3}H-chenodeoxycholic acid was synthesized (Hofmann, Szczepanik, and Klein, 1968) and purified by preparative thin-layer chromatography. Radiochemical purity was more than 98% by zonal scanning (Snyder, 1965), and this label has been shown to be valid for measurement of bile acid kinetics by isotope dilution in man (LaRusso, Hoffman, and Hofmann, 1974). The specific activity of chenodeoxycholic acid was determined for each bile sample, and the natural logarithms were plotted (ordinate) against time (abscissa) to permit calculation of pool size, turnover rate, and synthesis rate, as described previously (Hepner, Hofmann, and Thomas, 1972a). The mean (± SD) correlation coefficient for fractional turnover rate in the 14 subjects was 0·98 (± 0·02). The pool sizes of cholic acid and deoxycholic acid were calculated from the measured chenodeoxycholic acid pool size and from the relative bile acid composition of bile, determined by gas/liquid chromatography: the validity of this calculation has been previously established (Hepner, Hofmann, and Thomas, 1972b).

Because total daily bile acid output was also measured, it was possible to calculate the recycling frequency of the bile acid pool and intestinal absorption efficiency and hepatic return for chenodeoxycholic acid (see below).

LIPID COMPOSITION OF BILE

This has been expressed as the molar percentage of bile acids, lecithin, and cholesterol; as the ratio bile acids + phospholipids: cholesterol (Isaksson, 1952); and also as percentage saturation with cholesterol. Percentage saturation was calculated from polynomial equations (Thomas and Hofmann, 1973) describing the cholesterol solubility line proposed by Admirand and Small (1968), as well as the more recent line described independently by Hegardt and Dam (1971) and by Holzbach, March, Olszewski, and Holan (1973).

ANALYTICAL METHODS

For analysis of gallbladder bile, phospholipid concentration was measured as lipid-soluble phosphorus in the chloroform phase, following extraction into Folch solution (chloroform : methanol 2:1 v/v) using the method of Fiske and Sabbarow (1925). Cholesterol concentration was measured by the method of Abell, Levy, Brodie, and Kendall (1952), using Harleco reagent (Harleco Division of American Hospital Supply Corp, Philadelphia, PA), following petroleum ether extraction of the ethanol fraction. Total bile acid concentration was measured by an automated modification of the steroid dehydrogenase method of Iwata and Yamasaki (1964), using the ethanol fraction following cholesterol extraction. Relative biliary bile acid composition was determined in the ethanol fraction by gas/liquid chromatography (F & M 402 with flame ionization; 1·2 mm U columns, 3 mm inside diameter; packed with 3% QF1-coated Gas Chrom S), following alkaline saponification in nickel bombs at 115°C for four hours. The free bile acids were dissolved in methanol, methylated with freshly distilled diazomethane in ether, prepared as described by Schlenk and Gellerman (1960), and then acetylated (Roovers, Evrard, and Vanderhaeghe, 1968). To obtain a standard curve, pure reference bile acids were included in each run. ^{3}H radioactivity due to cholesterol was determined on a measured portion of the petroleum ether extract. It was found that some of the ^{14}C radioactivity due to lecithin was lost into the upper (methanol) phase during Folch extraction. Zonal scanning showed that this was present solely in the form of lysolecithin, resulting from the action of pancreatic enzymes, and that there was approximately an equimolar amount of labelled lysolecithin and fatty acid present in the sample as a whole. This is in keeping with the observation of Arnesjö, Nilsson, Barrowman, and Borgström (1969) that lecithin is hydrolysed to L-lysolecithin and free fatty acid, which are absorbed in parallel from the upper small intestine. The measured specific activity of lecithin in the lower phase was therefore corrected in accordance with the loss of radioactivity as lysolecithin into the upper phase, which was determined by subtracting the measured radioactivity in the lower phase from that present in an equivalent aliquot of the whole sample. Because of this correction, the phospholipid outputs in the present publication are higher than those reported in our preliminary commiuncation (Northfield and Hofmann, 1973).

Polyethylene glycol was determined by the method of Hyden (1956). ^{51}Cr radioactivity was determined by gamma spectrometer. ^{14}C and ^{3}H radioactivity were measured by liquid scintillation counting, using external standardization to compensate for quenching (Beckman L-S 250).

CALCULATION OF DATA

a Bile acid output = $BA_a \frac{PEG_p \times vol_p \times time}{PEG_a}$

b Cholesterol (and phospholipid) output = $Chol_a$

$(Phos_a) \frac{DPM_p \times vol_p \times time}{DPM_a}$

c Meal output = $\frac{Cr_a}{Cr_m} \times \frac{PEG_p}{PEG_a} \times vol_p \times time$

Where BA = bile acid concentration, PEG = PEG concentration, Cr = $^{51}CrCl_3$ concentration, chol = cholesterol concentration, phos = phospholipid concentration, a = duodenal aspirate, p = duodenal perfusate, m = meal.

d Daily recycling frequency = $\frac{\text{24-hour bile acid output}}{\text{pool size}}$

e Absorption efficiency (percentage of pool absorbed during each cycle) = $\frac{(\text{recycling frequency} - \text{fractional turnover}) \times 100}{\text{recycling frequency}}$

f Total amount of bile acids returned to the liver per day = amount secreted daily − amount synthesized daily.

Results

BILIARY LIPID OUTPUTS

There was no significant difference between gallstone and control subjects for any of the three biliary lipids (table II). Mean bile acid output (± SD) was 472 ± 119 μmoles/kg/day in the gallstone subjects and 405 ± 123 in the control subjects. The corresponding figures for cholesterol output were 56 ± 11 and 48 ± 18 μmoles/kg/day, and for phospholipid output 213 ± 125 and 217 ± 84 μmoles/kg/day.

When results for the hourly rate of bile acid output were plotted against percentage saturation of bile with cholesterol for both groups of subjects (fig 1), a curvilinear relationship was found, so that samples were consistently more saturated with cholesterol at low bile acid outputs than at high bile acid outputs. This relationship did not, however, hold for the first sample of the day, which in several cases was

Case No.	*Total Daily Outputs*				*First Postprandial Hourly Output*				*Mean Hourly Bile Acid Output*	
	Bile Acids (μmoles/kg)	*Cholesterol (μmoles/kg)*	*Phospholipid (μmoles/kg)*	*BA+PL C (ratio)*	*Bile Acids (μmoles/kg)*	*Cholesterol (μmoles/kg)*	*Phospholipid (μmoles/kg)*	*BA+PL C (ratio)*	*Day (8 am-8 pm) (μmoles/kg)*	*Night (8 pm-8 am) (μmoles/kg)*
Gallstone subjects										
1	255·2	36·5	45·5	8·1	15·7	4·2	10·6	6·3	12·0	9·2
2	455·4	55·2	192·9	11·7	44·8	6·0	13·6	9·7	27·9	13·9
3	419·6	51·2	161·2	11·2	27·8	4·3	18·8	10·8	23·0	12·5
4	441·6	72·6	333·2	10·6	49·6	11·1	56·1	9·5	21·7	15·1
5	575·0	65·8	417·8	15·0	46·6	5·5	25·1	13·0	31·9	16·0
6	562·9	54·9	139·7	12·7	36·5	4·9	11·6	9·8	27·6	19·3
7	595·7	53·5	197·7	14·7	38·1	4·9	14·8	10·8	34·6	15·1
Mean ± SD	472·2 ± 119·1	55·7 ± 11·4	212·6 ± 124·6	12·0 ± 2·4	37·0 ± 11·9	5·9 ± 2·4	21·5 ± 16·0	10·0 ± 2·0	25·5 ± 7·5	14·4 ± 3·1
									P < 0·0005	
Control subjects										
1	309·8	33·3	237·9	16·4	21·8	3·1	7·6	9·5	16·3	11·0
2	319·4	35·2	124·2	12·6	24·9	2·6	14·6	15·2	16·0	10·6
3	507·9	71·8	271·4	11·2	39·2	7·1	9·6	6·9	28·2	16·3
4	408·4	51·2	145·1	10·8	54·9	6·7	15·7	10·5	25·2	8·8
5	630·7	72·3	362·6	13·7	48·3	5·9	42·2	15·3	33·4	19·2
6	359·9	42·6	221·6	13·6	20·8	5·3	48·2	13·0	19·0	11·0
7	299·6	31·3	156·6	14·4	35·2	3·9	25·0	15·4	16·4	8·5
Mean ± SD	405·0 ± 123·1	48·2 ± 17·6	217·0 ± 83·6	13·4 ± 1·8	35·0 ± 13·3	4·9 ± 1·8	23·3 ± 16·1	12·3 ± 3·4	22·1 ± 6·9	12·2 ± 4·0
									P < 0·0005	

Table II *Biliary lipid outputs*

P values are calculated using t test for paired samples.

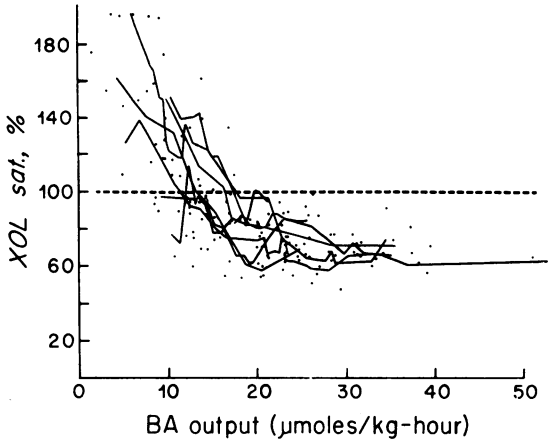

Fig 1a

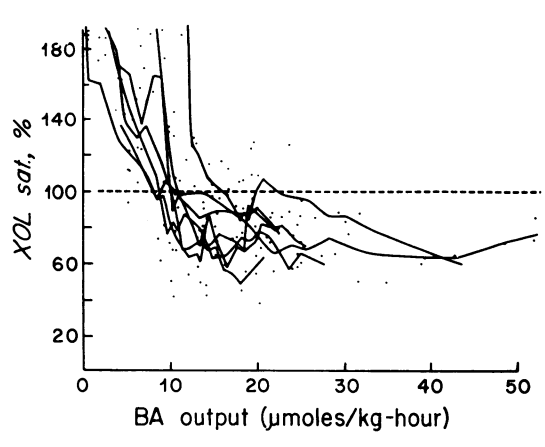

Fig 1b

Fig 1 *Relationship between the hourly rate of bile acid output and percentage saturation of samples with cholesterol (XOL) (according to the criterion of Admirand and Small, 1968) in (a) gallstone subjects and (b) control subjects. Individual data points are shown for all subjects. The lines represent values for each individual subject, plotted in order of magnitude as a moving average.*

supersaturated with cholesterol despite a high bile acid output. This sample was obtained immediately after the first liquid meal, and therefore probably represented bile from gallbladder emptying almost unaffected by hepatic secretion. This interpretation is supported by the fact that bile acid output during this first postprandial hour in all 14 subjects exceeded the mean output for both night time and day time samples (table II); and by the fact that there was a significant correlation between the biliary lipid composition of this sample and that of fasting gallbladder bile obtained following CCK infusion, whereas this correlation was absent for the relative biliary lipid composition of the total daily output (see below). Therefore, the first sample for each subject has been excluded from all figures.

Since bile acid outputs were significantly lower during overnight fasting than during the day time in both groups of subjects (table II), samples were more frequently supersaturated with cholesterol during the night time (table III). All 14 subjects intermittently secreted bile that was supersaturated with cholesterol, according to both sets of criteria, and there was no significant difference between normal and gallstone subjects in the frequency with which this occurred.

There was a linear relationship between bile acid and cholesterol output in both groups of subjects (fig 2). The regression equation for all 168 data points for the seven gallstone subjects was y =

Case No.	*Hours of Supersaturated Bile*			
	Day (A&S)	*Night (A&S)*	*Total (A&S)*[1]	*Total (HDH)*
Gallstone subjects				
1	11	10	21	24
2	0	2	2	16
3	1	3	4	14
4	6	7	13	20
5	1	3	4	5
6	0	3	3	18
7	0	5	5	9
Mean	2·7	4·7	7·4	15·1
± SD	± 4·2	± 2·9	± 7·0	± 6·5
	$P < 0.01$[2]			
Control subjects				
1	6	3	9	10
2	1	8	9	13
3	3	3	6	9
4	5	6	11	16
5	2	7	9	9
6	2	9	11	21
7	1	7	8	12
Mean	2·9	6·1	9·0	12·9
± SD	± 2·0	± 2·3	± 1·7	± 4·4
	$P < 0.025$			

Table III *Hourly frequency of secretion of supersaturated bile into the duodenum*

[1]Percentage saturation with cholesterol was calculated according to the data of Admirand and Small, 1968 (A&S) and according to the data of Hegardt and Dam (1971), and of Holzbach *et al* (1973) (HDH) as described (Thomas and Hofmann, 1973), and bile having cholesterol saturation ≥ 100% is considered supersaturated.

[2]values are calculated using t test for paired samples.

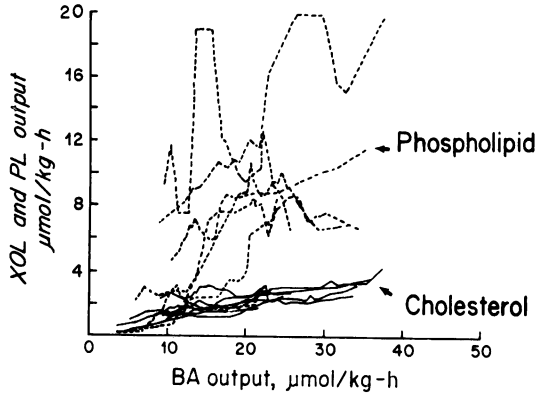

Fig 2a

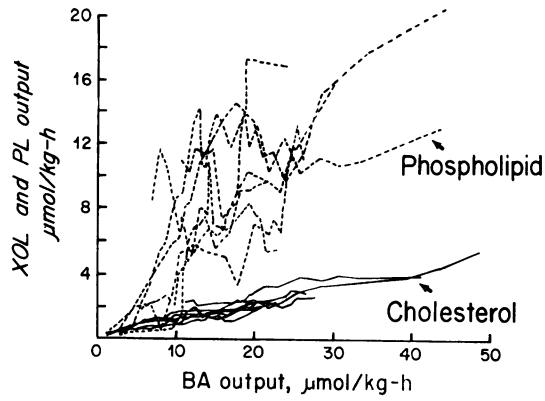

Fig 2b

Fig 2 *Relationship between the hourly rate of bile acid output and the hourly rates of phospholipid and cholesterol (XOL) output in (a) gallstone subjects and (b) control subjects.*
The lines represent values for each individual subject, plotted in order of magnitude as a moving average.

0·4743 + 0·0944x (r = 0·78), and for the seven control subjects y = 0·3191 + 0·0997x (r = 0·89). There was no significant difference between the slopes of these two regression lines, and in both cases the intercept was not significantly different from zero. A curvilinear regression did not fit the data better than a linear regression for either group of subjects. By contrast, a non-linear relationship was found between bile acid and phospholipid output (fig 2). Phospholipid output markedly exceeded cholesterol output at high bile acid outputs, but not at outputs below 7-10 μmoles/kg/hour.

BILE ACID KINETICS

There was no significant difference in total bile acid pool size between the two groups of subjects (table IV). This was not, however, the explanation for the absence of any difference in biliary lipid output between the two groups, since there was no significant correlation between total bile acid pool

Case No.	*For Chenodeoxycholic Acid*					*For Other Bile Acids*			
	Pool Size (μmoles/kg)	*Fractional Turnover ($days^{-1}$)*	*Daily Synthesis (μmoles/kg)*	*Absorption Efficiency (%)*	*Daily Hepatic Return (μmoles/kg)*	*Cholic Acid Pool Size (μmoles/kg)*	*Deoxycholic Acid Pool Size (μmoles/kg)*	*Total Bile Acid Pool Size (μmoles/kg)*	*Recycling Frequency ($days^{-1}$)*
Gallstone subjects									
1	25·8	0·128	3·3	96·7	97·1	13·0	12·8	66·2	3·9
2	28·9	0·241	7·0	96·2	176·5	19·9	19·6	71·7	6·4
3	13·0	0·500	6·5	93·8	98·9	12·5	25·9	52·3	8·1
4	13·9	0·384	5·3	93·0	71·5	10·7	53·0	80·0	5·5
5	26·0	0·540	14·0	93·4	197·6	21·9	21·6	70·7	8·1
6	14·7	0·344	5·1	96·9	133·4	12·0	20·0	51·0	11·0
7	15·8	0·232	3·7	96·5	102·5	10·5	57·5	84·6	6·7
Mean	19·7	0·338	6·4	95·2	125·4	14·4	30·1	68·1	7·1
± SD	± 6·8	± 0·149	± 3·6	± 1·7	± 46·2	± 4·6	± 17·7	± 12·8	± 2·3
Control subjects									
1	29·1	0·141	4·1	96·2	104·2	21·7	28·5	80·1	3·7
2	27·0	0·251	6·8	94·6	125·6	15·5	25·3	68·5	4·6
3	16·3	0·466	7·6	95·4	156·6	6·5	23·9	50·4	10·1
4	14·7	0·468	6·9	94·4	116·8	16·6	7·5	48·2	8·4
5	52·0	0·230	12·0	95·0	229·6	41·0	31·0	136·1	4·6
6	26·9	0·415	11·2	95·2	227·1	8·0	4·3	40·6	8·7
7	29·4	0·291	6·6	93·4	121·9	27·0	10·6	68·5	4·4
Mean	27·9	0·323	7·9	94·9	154·5	19·5	18·7	70·3	6·4
± SD	± 12·2	± 0·127	± 2·8	± 0·9	± 52·9	± 11·9	± 10·9	± 32·1	± 2·6

Table IV *Bile acid kinetics*

size and total daily bile acid output (r = 0·39). Recycling frequency showed a significant correlation with fractional turnover rate for chenodeoxycholic acid (r = 0·73; P < 0·01), and with the percentage of the bile acid pool secreted in the first hour of the study (r = 0·70; P < 0·01). Pool size for chenodeoxycholic acid showed no significant correlation with synthesis rate (r = 0·48) or with intestinal absorption efficiency (r = 0·05).

BILIARY LIPID COMPOSITION OF FASTING GALLBLADDER BILE

The mean lithogenic index (Metzger, Heymisfield, and Grundy, 1972) was more than 1 in the gallstone subjects (1·16 ± 0·53) and less than 1 in the control subjects (0·80 ± 0·22), according to the criteria of Admirand and Small (1968), but this difference was not statistically significant (table V).

The lithogenic potential of total daily biliary lipid outputs has been expressed in the form of Isaksson's (1952) ratio (bile acid + phospholipid: cholesterol, table II). The lithogenic potential of the total daily biliary lipid output, when expressed by this ratio, did not correlate with that of fasting gallbladder bile (r = 0·28). By contrast, output for the first postprandial hour correlated fairly well with that of fasting gallbladder bile (r = 0·59; P < 0·05).

Discussion

TOTAL DAILY BILIARY LIPID OUTPUTS

Our Caucasian subjects with radiolucent gallstones had the same total daily outputs of all three biliary lipids as a group of closely matched control subjects without gallstones. These unexpected findings contrast with those of Grundy *et al* (1972) who reported a reduced bile acid secretion rate and an increased cholesterol secretion rate in a group of obese American Indian women with gallstones, by comparison with a group of thin Caucasian controls without gallstones. This discrepancy in results is likely to be due to differences in subjects studied or to differences in methodology or both: (1) American Indian women have a very high prevalence of cholesterol gallstones (70% by the age of 30, according to the survey of Sampliner, Bennett, Comess, Rose, and Bunch, 1970), so that pathogenesis may be different. (2) Our subjects differed from those of Grundy *et al* (1972) in being closely matched for weight and race as well as for age and sex; obesity is associated with increased cholesterol synthesis (Nestel, Whyte, and Goodman, 1969) and increased biliary secretion of cholesterol (Grundy, Duane, Adler, Aron, and Metzger, 1974). (3) Their methodology (Grundy and Metzger, 1972) is different from ours, and involves continuous intra-

Case No.	*Molar Percentage*			*BA+PL / C*	*Cholesterol Saturation (%)*	
	Bile Acids	*Cholesterol*	*Lecithin*		*Admirand and Small (1968)*	*Hegardt and Dam (1971); Holzbach et al (1973)*
Gallstone subjects						
1	73·5	16·0	10·5	5·3	186	363
2	74·4	7·5	18·1	12·3	77	128
3	75·2	6·9	17·9	13·4	71	119
4	77·9	10·9	11·2	8·2	126	246
5	77·8	6·9	15·2	13·5	74	133
6	75·5	7·8	15·3	11·7	84	150
7	84·2	12·2	3·6	7·2	192	377
Mean	76·9	9·7	13·1	10·2	116	217
± SD	3·6	± 3·5	± 5·1	± 3·3	± 53	± 113
Control subjects						
1	73·2	8·2	18·5	11·2	84	137
2	71·7	6·7	22·2	13·9	67	99
3	70·5	9·1	20·4	10·0	92	140
4	80·0	7 3	12·7	12·6	83	157
5	83·5	3·8	12·8	25·3	43	83
6	67·7	11·3	21·0	7·8	113	168
7	71·2	8·0	20·8	11·5	80	123
Mean	74·0	7·8	18·3	13·2	80	130
± SD	5·7	± 2·3	± 4·0	± 5·7	± 23	± 30

Table V *Biliary lipid composition of fasting gallbladder bile*

duodenal infusion of a liquid formula diet. They have pointed out that, after initial contraction, the gallbladder appears to be functionally inactive in their system during the subsequent hours of constant formula infusion. If the differences in recycling frequency found in our study are due to differences in gallbladder emptying, as suggested by the correlation (r = 0·70) between recycling frequency and the percentage of the pool secreted in the first postprandial hour, their system might be expected to produce a constant recycling frequency as an artefact, with bile acid secretion rate mirroring bile acid pool size. Bile acid pool size was not measured in their study, so that recycling frequency could not be calculated. More recently, Grundy *et al* (1974) have carried out the same studies in a group of obese Caucasians with gallstones. They found a higher cholesterol output than in thin Caucasian controls without gallstones, but this appeared to be related to obesity. Bile acid output tended to be lower than in the controls, but there was considerable overlap, and no definite abnormality was demonstrated.

A limitation of our study was that, because of the large number of measurements carried out, only a relatively small number of subjects could be studied. Thus, our failure to find a significant difference in total daily biliary lipid output does not by any means exclude its presence in a larger population of gallstone and control subjects. Indeed, the small number of subjects studied is probably the explanation for the finding that, although fasting gallbladder bile tended to be supersaturated with cholesterol in our gallstone patients, and unsaturated in our control subjects, this difference was not statistically significant, nor was the difference in total bile acid pool size. Most of our subjects were female, and these appear to have a larger overlap in bile acid pool size (Danzinger, Hofmann, Thistle, and Schoenfield, 1973; Pomare and Heaton, 1973) than do males (Vlahcevic *et al*, 1970b). Since 10-20% of the population will eventually develop gallstones, any control group will contain patients at high risk for cholelithiasis. In addition, any patient group with radiolucent gallstones may contain patients with radiolucent pigment stones. Fortunately, the large number of measurements carried out in our study can be used to buttress the weakness due to the small number of subjects by analysing the relationship between the different measurements in all 14 subjects. Thus, total daily bile acid output was unrelated to bile acid pool size (r = 0·38), because of differences in recycling frequency, indicating that it is quite unjustifiable to predict biliary lipid secretion from bile acid pool size. There was no correlation between the lithogenic potential of total daily biliary lipid output, expressed as the ratio bile acid + phospholipid : cholesterol outputs, and that of fasting gallbladder bile (r = 0·28), indicating that the presence of gallbladder bile supersaturated in cholesterol cannot be explained in our subjects on the basis of an abnormality in total daily biliary lipid output. The very low correlation coefficients found for these two relationships makes it unlikely that a good correlation would have been found if more than 14 subjects had been studied. Strong evidence that our gallstone subjects did not have an abnormality in bile acid/cholesterol coupling, as reported by Grundy *et al* (1972) in obese American Indian women with gallstones, is provided by the dose/response curves based on 312 hourly output measurements. These showed no tendency for the slope of the regression line in the gallstone subjects (0·0944) to be steeper than that in the control subjects (0·0997). This finding throws some doubt on the physiological significance of the recent biochemical finding that gallstone patients have increased hepatic concentrations of cholesterol and of HMG CoA reductase compared with non-gallstone patients (Nicolau, Shefer, Salen, and Mosbach, 1974).

BILE ACID OUTPUTS AND DEGREE OF CHOLESTEROL SATURATION OF HEPATIC BILE

Although hepatic production of bile supersaturated with cholesterol could not be explained in our subjects by an abnormality of total daily biliary lipid output, it could be explained by variations in the hourly rate of bile acid output. Samples were consistently supersaturated with cholesterol at low bile acid outputs in all 14 subjects, but not at high bile acid outputs. This finding suggests that biliary lipid composition in man is related to hepatic bile acid flux, and that this process is physiological. The bile acid output was lowest during overnight fasting, so that this mechanism accounts for the diurnal variation in biliary lipid composition described previously (Metzger, Adler, Meymsfield, and Grundy, 1973; Northfield and Hofmann, 1973). The reduction in bile acid output during overnight fasting presumably results from a physiological interruption of the enterohepatic circulation, due to sequestration of the bile acid pool in the gallbladder. Surgical interruption of the enterohepatic circulation is known to result in hepatic production of bile supersaturated with cholesterol in man (Thureborn, 1962) and other primates (Dowling, Mack, and Small 1971; McSherry, Glenn, and Javitt, 1971).

This effect of bile acid output on biliary lipid composition could be explained in our subjects by the finding of a linear relationship between the bile acid and cholesterol outputs, but a non-linear relationship between bile acid and phospholipid outputs. When cholesterol and phospholipid outputs

were plotted against bile acid outputs, phospholipid outputs decreased more rapidly than cholesterol outputs at low bile acid outputs, giving a sigmoid shape in those subjects in whom bile acid outputs fell below 7-10 μmoles/kg/hour. Any duodenal perfusion technique gives no information on either the composition of canalicular bile or the modification of hepatic bile during passage through the biliary tree, and mechanistic interpretations of our data are speculative. Scherstén, Nilsson, Cahlin, Filipson, and Brodin-Persson (1971) measured lipid outputs in five gallstone patients with controlled (T-tube) interruption of the enterohepatic circulation. Like us, they found a linear relationship between bile acid and cholesterol output in three subjects, but they obtained a positive Y-intercept corresponding to a cholesterol secretion rate of 1·5 μmoles/minute, independent of bile acid and phospholipid secretion, which is very difficult to explain on a physicochemical basis. By contrast, the intercepts obtained in our study were not significantly different from zero. Like us, they also observed a non-linear relationship between phospholipid and bile acid output, but this was not sigmoid in shape. It is conceivable that transit rates along the biliary tract in patients with a T-tube who have recently had biliary tract surgery are more rapid than in our patients with an intact sphincter of Oddi. This difference, if present, might be important at low bile flow rates since supersaturated bile would occur if lecithin were absorbed to a greater extent than cholesterol. In intestinal perfusion studies with a micellar solution containing cholesterol solubilized in monoglyceride-bile acid micelles, the monoglyceride was more rapidly absorbed than the cholesterol, whereupon cholesterol precipitated from solution (Simmonds, Hofmann, and Theodore, 1967). These observations suggest a possible model for the formation of supersaturated bile associated with low bile acid output rates. Schersten *et al* have proposed that bile acid flow through the liver induces biliary lecithin synthesis. If this induction requires a certain bile acid flow rate to be initiated, then bile would be supersaturated at low bile acid flow rates.

BILE ACID OUTPUTS AND BILE ACID POOL SIZE

Our finding of similar bile acid outputs in subjects with small and large bile acid pools was explained by the inverse relationship between pool size and recycling frequency reported previously (Northfield and Hofmann, 1973). This curvilinear regression is unlikely to be due to chance ($P < 0·01$). Since secretion was not decreased in patients with a small bile acid pool, the bile acid return to the liver was similar in all subjects. These observations provide an explanation for the similar rates of bile acid synthesis in gallstone patients with small bile acid pools when compared to healthy subjects with larger (normal) pools. Previously, it had been proposed that gallstone patients had 'inappropriate repression' of bile acid synthesis (Schoenfield, 1972), but our data indicate that synthesis is appropriately similar to that of healthy subjects. It had also been suggested that the increased fractional turnover rate observed in gallstone patients indicated decreased efficiency of intestinal absorption. Our data indicate that the efficiency of intestinal absorption of bile acids was similar in both groups of patients. If efficiency of intestinal absorption is similar and unrelated to the size of the bile acid pool, then the fractional turnover rate (or half life) should be directly correlated with recycling frequency, as was observed ($r = 0·73$). As suggested previously (Northfield and Hofmann, 1973; Low-Beer and Pomare, 1973), increased recycling frequency could be the cause of the decreased bile acid pool size reported to be present in most Caucasians with gallstones, as a result of normal feedback regulation of bile acid synthesis. Our finding of a significant correlation between recycling frequency and the percentage of the total bile acid pool secreted in the first postprandial hour ($r = 0·70$) may indicate that the increase in recycling frequency found in those with a small bile acid pool is due to enhanced gallbladder emptying in response to food. Intestinal factors such as increased transit rate seem less likely. At any rate, the correlation suggests that increased recycling frequency cannot be attributed to decreased gallbladder contractility.

BILE ACID POOL SIZE AND DEGREE OF CHOLESTEROL SATURATION OF FASTING GALLBLADDER BILE

In Caucasian men with radiolucent gallstones, the presence of supersaturated fasting gallbladder bile is associated with a small bile acid pool size (Swell *et al*, 1971). Since this supersaturated gallbladder bile does not appear to be due to an abnormality in total daily biliary lipid output, it is necessary to look for some alternative explanation. One possible explanation is as follows: the bile acid pool enters the gallbladder after digestion of the evening meal, accompanied by hepatic bile that is unsaturated with cholesterol. We speculate that the volume of this unsaturated bile is smaller in a subject with a small bile acid pool than in a subject with a large bile acid pool. During overnight fasting, the consequent interruption of the enterohepatic circulation of bile acids leads to secretion of supersaturated hepatic bile, some of which also enters the gallbladder. If this fasting supersaturated bile is diluted in a smaller volume of unsaturated bile in a gallstone patient

with a small pool, then the net effect would be to make the fasting gallbladder bile more saturated than in the normal subject with the large bile acid pool. The full sequence of events according to this hypothesis is that enhanced gallbladder emptying causes an increased recycling frequency of the bile acid pool, and thus a decreased bile acid pool size; and that this renders fasting gallbladder bile supersaturated with cholesterol. Evidence is accumulating that gallstone formation cannot be explained solely by the presence of supersaturated bile in the gallbladder, since supersaturated bile is observed not infrequently in healthy subjects. Thus, supersaturated bile in the gallbladder is probably best categorized as a necessary but not sufficient event for cholesterol gallstone formation.

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