The enterohepatic circulation of bile acids during continuous liquid formula perfusion of the duodenum

P. Rutgeerts, Y. Ghoos, and G. Vantrappen

Department of Medical Research, University of Leuven, University Hospital St. Rafaël, B-3000 Leuven, Belgium

Abstract The enterohepatic circulation of bile acids was studied in 16 normal volunteers and 8 cholecystectomy patients during continuous liquid formula perfusion of the duodenum. The fasting bile acid pool size in the 16 normals (5.534 ± 1.472 mmol) exceeded the circulating pool size (3.643 ± 1.126 mmol) by 33.4% ± 13.4. The difference between fasting and circulating pool sizes was significantly and inversely related to the percentage of the fasting pool that was emptied in the duodenum during the first perfusion hour ($r = -0.76$; $P < 0.001$). No relation was found between the circulating bile acid pool size and the calculated rate of recycling of the pool, but a strong relation was found between the cycling pool and the basal hourly bile acid outputs (2.08 ± 0.77 mmol/hr) in the duodenum ($r = 0.82$; $P < 0.001$). In cholecystectomy patients the circulating pool size (3.30 ± 1.39 mmol) and the basal bile acid output (1.65 ± 0.98 mmol/hr) were slightly lower than in the normal volunteers. The bile acid pool size was not related to the cycling rate and there was a strong relation between pool size and bile acid secretion ($r = 0.85$; $P < 0.001$). As the fasting pool, measured using the method of Duane et al. (1975. *J. Lipid Res.* **16:** 155–158), is probably overestimated by ±14%, the remaining difference of ±19% with the pool circulating during formula perfusion might be caused by incomplete gallbladder emptying. In these experimental conditions basal bile acid outputs reflect closely the size of the circulating bile acid pool in normals as well as in cholecystectomy patients.—**Rutgeerts, P., Y. Ghoos, and G. Vantrappen.** The enterohepatic circulation of bile acids during continuous liquid formula perfusion of the duodenum. *J. Lipid Res.* **24:** 614–619.

Supplementary key words cholecystectomy • gallbladder emptying

The study of the regulation of the metabolism and the enterohepatic circulation of the bile acids is of great clinical importance. In steady state conditions, the pool size of the primary bile acids is determined by the synthesis rate in the liver and by the daily losses. Hepatic bile acid synthesis by conversion of cholesterol to cholic acid and chenodeoxycholic acid is regulated by a feedback inhibition (1). The size of the pool of the primary bile acids is inversely related to the turnover in health (2) and disease (3, 4), and in turn is controlled by the bile acid reabsorption during enterohepatic circulation. It has been suggested that the main factors determining the turnover and the synthesis of primary bile acids are gallbladder emptying and intestinal transit time. Indirect evidence is abundant. Delayed gallbladder emptying in celiac disease (5, 6) and with reduced intake of protein and fat (7) is associated with expansion of the bile acid pool. Cholecystectomy in contrast reduces the pool size (8). Bile diversion experiments in the rhesus monkey (9) designed to mimic differences in frequency of gallbladder emptying showed the importance of intermittent partial interruption of the enterohepatic circulation on the regulation of the bile acid pool size. Increase or decrease of the intestinal transit time by pharmacological agents influences the size of the bile acid pool and bile acid secretion rates (10–12).

Duane and Hanson (2) offered direct evidence of the role of gallbladder emptying and intestinal transit in the regulation of the bile acid pool size. Their studies suggested that gallbladder emptying was the main regulating factor of the pool size of the primary bile acids, while the intestinal transit time seemed to be related to the synthesis of the primary bile acids. The enterohepatic circulation of bile acids can be studied directly by measuring bile acid secretion into the duodenum by means of duodenal perfusion and marker dilution techniques. The Mayo Clinic Procedure (13) is based on duodenal marker perfusion and administration of three separate liquid meals into the stomach. The method of Grundy and Metzger (14, 15, 18) used continuous infusion of a liquid test meal and a nonabsorbable marker into the duodenum. These methods have two main disadvantages. The recycling frequency is not determined directly but is calculated from measurements of the total bile acid pool size and the daily bile acid secretion ($Q = P \times n$, where Q = daily secretion, n = recycling frequency, and P = pool size), assuming that the total bile acid mass (= pool) takes part in the enterohepatic circulation at every moment of the perfusion study. The

present investigations were designed to study some of the mechanisms that regulate the bile acid pool size and the enterohepatic circulation; more specifically, *1*) the relation between the fasting bile acid pool size and the size of the bile acid pool circulating during continuous liquid formula perfusion, and *2*) the relation between the circulating bile acid pool, secretion rate, and cycling frequency in normals and in patients with cholecystectomy.

METHODS

Subjects

Sixteen normal volunteers were studied (11 males and 5 females) with a mean age of 33 yr (21–51 yr). None of the volunteers had evidence of hepatobiliary, intestinal, or metabolic disease. All subjects were on a diet of 80 g of fat for at least 1 week prior to the study. Eight patients, 5 females and 3 males, mean age 46 (32–58 yr) who had been submitted to a cholecystectomy 1–15 yr (mean 3 yr) previously were also studied. Six patients had been operated upon for cholesterol gallstones, two for pigment stones. The study was approved by the ethical committee and informed consent was obtained from all subjects studied.

Radioactive material

[Carboxyl-^{14}C]cholic acid (sodium salt in 2% ethanol solution) was purchased from the Radiochemical Centre (Amersham, Buckinghamshire, England). [^{3}H(G)]Glycocholic acid (2% ethanol solution) was obtained from New England Nuclear (Boston, MA). The products were checked for chemical purity by thin-layer chromatography. The radiopurity as determined by radioscanning (Berthold, model LB 2723, Wildbad, Germany) was found to be greater than 99.5%. The radioactivity measurements were carried out with a liquid scintillation counter (Packard Instruments 2450 Downers Grove, IL). Quenching was corrected by external standardization.

Measurement of the total fasting bile acid pool size

At 9 PM, 4 hr after a light evening meal, 5 μCi of [^{14}C]cholic acid was administered intravenously, according to the method of Duane et al. (16). The bile acid pool size was calculated using the mean ^{14}C specific activity of the duodenal outputs obtained during the first perfusion hour the next morning.

Infusion and aspiration procedures

Duodenal liquid meal perfusion and marker dilution techniques of Grundy and Metzger (14) were used with a slight modification. After an overnight fast a triple-lumen tube was positioned under fluoroscopic control in the duodenum with the infusion outlet near the ampulla of Vater and the aspiration outlet in the third part of the duodenum. A reinfusion outlet was positioned beyond the angle of Treitz. The liquid formula with marker solution was infused at a constant rate of 2 ml/min with an infusion pump (model Infuso-mat, B. Braun, Melsungen). With these infusion rates reflux of duodenal formula back into the stomach is minimal (4). Aspiration of duodenal contents was carried out by siphonage. After mixing, 5 ml of each sample was kept for analysis; the remainder was returned to the proximal jejunum through the reinfusion outlet in order to keep the interruption of the enterohepatic circulation to a minimum. The quantity kept for analysis represented less than 5% of the duodenal output so that the enterohepatic circulation was not significantly interrupted. The rate of formula input was determined in such a way that each volunteer received 30 Kcal/kg body weight over a 12-hr period (17). The infusion solution contained 40% of the caloric intake as fat (corn oil), 40% as carbohydrate (sucrose), and 20% as protein hydrolyzate in emulsion. Phenol red was added as nonabsorbable marker. Dilution techniques using phenol red have been shown to allow accurate measurements of bile acid excretion (18). Bile acid output was calculated using the formula:

$$\text{bile acid output} = (\text{BA})a \times \frac{(\text{phenol red})p}{(\text{phenol red})a} \times V_p \times t$$

in which (BA)a = bile acid concentration of the aspirate, V_p = volume of the perfusate, t = time in minutes and p and a = perfusate and aspirate.

Measurement of the circulating bile acid mass

The circulating bile acid mass was measured by the method of Grundy (19). After 4 hr of liquid perfusion, when basal bile acid outputs had been reached (14), 5 μCi of [^{3}H]glycocholic acid, mixed with 10 ml of reinfusion liquid, was infused through the distal duodenal outlet. Duodenal contents were collected in 10-min fractions to monitor the appearance of this labeled conjugated bile acid in the duodenum. At the end of the study 5 mg/ml patent blue (V-color index 42051) was mixed with the infusion solution and the time interval was measured between the appearance of the dye in the aspiration and the reinfusion tubes.

Bile acid analysis

To measure phenol red and bile acids, protein-free solutions of intestinal contents were prepared by adding 0.8 ml of water, 0.6 ml of methanol, 0.2 ml of a 0.75 M NaOH solution, and 0.2 ml of a solution of zinc sulfate ($ZnSO_4 \cdot 7\ H_2O$, 10 g per 100 ml) to 0.2 ml of intestinal

contents. For the determination of the phenol red concentration, this mixture (pH 9) was spun in the Beckman Spinco model centrifuge at 10,000 rpm for 10 min. One ml of the supernatant was diluted with 2 ml of 0.1 M sodium pyrophosphate (pH 10.0). The absorbance of the supernatant was measured at 560 nm in a Pye Unicam SP 800 spectrophotometer, and the concentration of phenol red was read from a standard curve. The bile acid concentration was determined by the 3α-hydroxysteroid dehydrogenase method. (Worthington Biochemical Corp., Freehold, NY).

Statistics

All results were expressed as mean ± SD. Linear regressions were calculated by the method of least squares, the correlation coefficient r was calculated and significances were tested by the t-test. Values between subgroups were compared for significance by Student's t-test (20).

RESULTS

Data on the enterohepatic circulation of bile acids in normal subjects during continuous liquid formula perfusion of the duodenum are summarized in **Table 1.** The fasting bile acid pool size in normal subjects measured by means of the Duane et al. method (16) averaged 5.534 ± 1.472 mmol. The output of bile acids was high during the first hour of perfusion but basal outputs were reached within 4 hr of perfusion. The mean hourly output during the subsequent 8 hr was taken as the basal value. Fluctuations in hourly basal secretion rate were relatively small. These basal bile acid outputs averaged 2.08 ± 0.77 mmol/hr. Eight-hr bile acid outputs were calculated by multiplying the basal output by 8 as proposed by Mok, Von Bergmann, and Grundy (15) and averaged 16.6 ± 6.2 mmol/8 hr. When [^{3}H]glycocholic acid mixed with reinfusion liquid was infused into the distal duodenum, the first ^{3}H activity appeared in the duodenum after 10–20 min in seven subjects, after 20–30 min in six subjects, and after 30–40 min in three subjects. The time interval for the duodenal contents to progress from the aspiration site to the reinfusion outlet, measured with the dye technique, averaged 2.8 ± 0.7 min. The peak of activity occurred after 40–50 min in one subject, 50–60 min in four subjects, 60–70 min in eight subjects, 70–80 min in two subjects, and 80–90 min in one subject. The specific activity of ^{3}H then gradually decreased to steady state levels, which were always reached within 4 hr after the injection of [^{3}H]glycocholic acid into the duodenum.

The circulating bile acid pool, calculated on the basis of the ^{3}H mean specific activity (19) during the steady state perfusion hours, averaged 3.643 ± 1.126 mmol. The fasting pool size exceeded the circulating pool size by an average of 33.4% ± 13.4 ($P < 0.05$). The enterohepatic recycling rate calculated on the basis of the circulating bile acid pool averaged 4.5 ± 1.0 cycles/8 hr. There was no correlation between the size of the circulating bile acid pool and the cycling rate ($r = 0.09$). When the pool size was calculated in μmol/kg, the correlation between the size of the circulating pool and the cycling rate remained poor ($r = -0.30$). The difference between the fasting and circulating bile acid pool was inversely related to the fraction of the fasting pool size

TABLE 1. Data on the enterohepatic circulation of bile acids in normal subjects during continuous meal perfusion of the duodenum

Subjects	Weight	Bile Acid Pool (Duane)	Bile Acid Pool (Grundy)	% Difference between Pools	% of Fasting Pool Emptied during First Hour	Basal Bile Acid Output	Cycling Rate of the Circulating Pool
	kg	*mmol*	*mmol*			*mmol SD*	*cycles/8 hr*
W.D.	68	4.28	3.53	17.5	114	2.24 (0.31)	5.1
S.K.	62	5.68	3.30	42.0	69	1.87 (0.29)	4.5
V.P.	75	4.85	1.95	59.8	56	1.58 (0.18)	6.5
S.M.	75	6.34	4.86	23.3	92	2.18 (0.19)	3.6
V.R.	55	5.95	3.01	49.4	84	1.99 (0.29)	5.3
S.L.	61	3.20	1.99	37.9	86	0.97 (0.10)	3.9
V.Ra.	54	6.83	6.11	10.5	92	3.69 (0.27)	4.8
T.L.	75	7.08	4.72	33.3	84	3.27 (0.43)	5.5
T.D.	73	4.10	3.29	19.8	86	1.97 (0.22)	4.8
D.G.	61	7.49	4.52	39.7	69	2.46 (0.26)	4.3
V.H.	69	6.75	4.17	38.1	52	1.38 (0.18)	2.6
T.Lo.	77	6.51	4.30	34.0	76	2.46 (0.31)	3.0
W.A.	65	3.21	2.77	14.0	101	1.05 (0.08)	3.0
V.L.	61	7.40	4.15	44.0	57	2.99 (0.19)	5.8
M.K.	63	4.96	3.12	37.1	78	1.52 (0.12)	3.9
V.E.	76	3.92	2.52	35.7	70	1.60 (0.21)	5.1
Mean ± SD		5.53 ± 1.47	3.64 ± 1.13	33.4 ± 13.4	79 ± 17	2.08 ± 0.77	4.5 ± 1.0

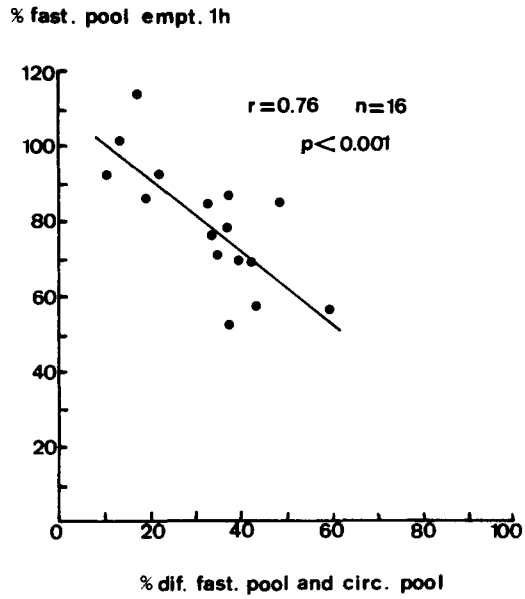

Fig. 1. Relation between the differences (%) between the size of the pool on fasting and of the pool circulating enterohepatically and the portion (%) of the fasting pool size emptied in the duodenum during the first hour of continuous liquid formula perfusion in normal subjects.

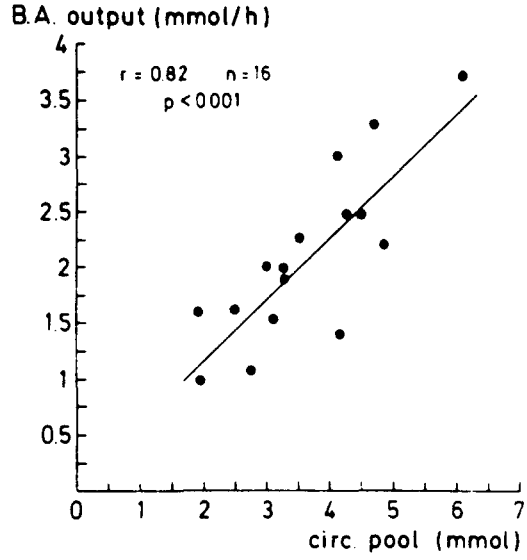

Fig. 2. Relation between the pool (mmol) circulating enterohepatically and the basal bile acid output (mmol/hr) in normal subjects.

that was excreted during the first perfusion hour ($r = -0.76$; $P < 0.001$) (**Fig. 1**). There was no significant inverse relation between the difference between the fasting and the circulating bile acid pool sizes and the circulating pool size ($r = 0.45$; $P < 0.1$). The size of the circulating pool correlated well with the basal bile acid outputs and the 8-hr bile acid outputs ($r = 0.82$; $P < 0.001$) (**Fig. 2**). There existed no significant relation between the basal outputs of bile acids and the cycling rate of the circulating bile acid pool ($r = 0.44$; $P < 0.1$).

Data on the enterohepatic circulation of bile acid in patients with cholecystectomy were obtained by continuous liquid formula perfusion of the duodenum and are summarized in **Table 2.** The bile acid pool measured by the Duane method (16) averaged 4.12 ± 1.74 mmol, exceeding the pool measured by the method of Grundy (19) (3.30 ± 1.39 mmol) by 18.6 ± 8.8% in average. The circulating pool in cholecystectomy patients (3.30 mmol ± 1.39) was not significantly lower than the circulating pool in normal volunteers. The pool cycling in patients with cholecystectomy was however significantly lower than the pool on fasting in normal volunteers (5.534 ± 1.472; $P < 0.05$).

In cholecystectomy patients no relation was found between the difference in the pools measured with different methods and the portion of the pool emptied in the first perfusion hour ($r = 0.09$). The basal outputs of bile acids averaged 1.65 ± 0.98 mmol/hr, which was not significantly lower than the basal outputs of normals. The calculated rate of enterohepatic cycling of the circulating pool averaged 3.9 ± 1.0 cycles/8 hr. [^{3}H]Glycocholic acid appeared in the duodenum after

TABLE 2. Data on the enterohepatic circulation of bile acids in cholecystectomy patients during continuous meal perfusion of the duodenum

Subjects	Weight	Bile Acid Pool (Duane)	Bile Acid Pool (Grundy)	% Difference between Pools	% of Fasting Pool Emptied during First Hour	Basal Bile Acid Output	Cycling Rate of the Circulating Pool
	kg	*mmol*	*mmol*			*mmol SD*	*cycles/8 hr*
H.D.	51	2.07	1.83	11.7	62	1.03 (0.06)	4.5
C.A.	75	5.14	3.93	23.4	50	1.27 (0.12)	2.6
H.M.	48	3.41	2.32	31.9	43	1.11 (0.22)	3.8
C.G.	56	6.08	5.25	13.6	37	3.60 (0.31)	5.5
V.J.	56	1.58	1.50	5.1	37	0.65 (0.12)	3.5
B.J.	85	5.78	4.78	17.3	100	2.63 (0.19)	4.4
V.K.	78	5.53	3.99	28.0	69	1.34 (0.17)	2.7
V.D.	76	3.45	2.83	18.0	54	1.55 (0.14)	4.4
Mean ± SD		4.12 ± 1.74	3.30 ± 1.39	18.6 ± 8.8	56 ± 21	1.65 ± 0.98	3.9 ± 1.0

10–20 min in three patients, after 20–30 min in another three patients, and after 30–60 min in two patients. The peak activity occurred after 40–50 min in one patient, 50–60 min in four patients, 60–70 min in two patients, and 70–80 min in one patient. In cholecystectomy patients there was a very good inverse relation between the circulating bile acid pool size and the secretion rate ($r = -0.85$; $P < 0.001$).

No relation was found between pool size and recycling rate ($r = 0.19$). In the combined groups of normal volunteers and cholecystectomy patients there was a linear relation between the circulating pool size and the basal bile acid output under conditions of liquid formula perfusion of the duodenum ($r = 0.83$; $P < 0.001$). However, no relation was found between the cycling rate of the circulating pool and its size ($r = 0.03$), even if the pool size is calculated in μmols/kg body weight ($r = -0.11$).

DISCUSSION

Two major points about the enterohepatic circulation of bile acids resulted from the present investigations. In normal subjects the bile acid pool size (fasting) measured using the Duane method exceeded the circulating pool during continuous formula perfusion (Grundy) by a mean of 33.4%. Second, a high correlation was found between the circulating pool size and the basal bile acid secretion rate in normal subjects as well as in cholecystectomy patients.

The differences found between the total fasting pool and the pool circulating during formula perfusion were important. Several factors are probably involved in the origin of these differences. As a large quantity of bile secreted during the night bypasses the gallbladder (21, 22), losses of isotope overnight with the method of Duane et al. (16) may result in an overestimation of the fasting pool. In his original studies Duane et al. (16) found that the pool measured with his method overestimated the pool obtained by the Lindstedt disappearance plot (23) by 13.7%. In our patients with functioning gallbladders, loss of isotope probably accounted for an overestimation of about 14% of the fasting pool size. In the cholecystectomy patients studied, the mean difference between the pool sizes measured by both methods amounted to 18.6%. In these patients the loss of isotope overnight should be greater than in patients with normal functioning gallbladders, and the 18.6% difference is probably entirely due to isotope loss.

On the other hand, the circulating pool size could also have been overestimated by a disproportionate loss of labeled bile acid compared to the unlabeled endogenous bile acids. About 5% could have been absorbed with the first cycle and another 5% aspirated on the first few passes when specific activity peaked. Lack of complete sequestration of isotope in the gallbladder overnight and incomplete mixing of the isotope with the bile acid pool would also result in different values for the pool measured by different methods. Still the fairly steady state specific activities for ^{14}C and ^{3}H suggest that good mixing had occurred at the moment of sampling for both methods. If an overestimation of ±14% is accepted for the size of the fasting pool, a difference of more than 19% still remains between the fasting pool size and the mass of bile acids circulating during formula perfusion. The significant inverse correlation of the differences between the fasting and the circulating pool sizes with the portion of the fasting pool that is excreted during the first perfusion hour suggests that the differences between the two pool sizes might be due to incomplete gallbladder emptying, i.e., the more the gallbladder emptied the less the discrepancy between the total fasting and circulating pool. These data are in agreement with the 20% sequestration of bile acids in the gallbladder calculated by Shaffer (24) for patients receiving liquid infusion meals to stimulate gallbladder contractions.

Mok et al. (15) found a good inverse relation between the bile acid pool size and the calculated rate of enterohepatic cycling during continuous liquid formula perfusion of the duodenum, a situation in which the gallbladder is thought to be excluded from the enterohepatic circulation. In this study we did not find any relation between the circulating bile acid pool size and the cycling rate in normal volunteers, but a very good relation ($r = 0.82$; $P < 0.001$) between the pool size and the basal bile acid outputs.

The present data suggest that during liquid formula perfusion of the duodenum the rate of bile secretion reflects the size of the circulating pool. The apparent contradiction with the data of Mok et al. (15) may be due to the fact that the group of subjects they studied was composed of a few normal subjects and a majority of patients with obesity and hyperlipoproteinemia, who are known to have large bile acid pools (8). Mok et al. (15) found a good inverse hyperbolic relation between the bile acid pool size and the cycling rate in the whole group of 31 patients studied ($r = -0.61$; $P < 0.01$) and a less good linear relation between the bile acid pool size and the secretion rate ($r = 0.50$; $P < 0.01$). Recalculations of their data for groups I and II (who had bile acid pool sizes of 1–3 g) indicate that the bile acid pool is not related to the cycling rate ($r = -0.12$) but to the bile acid secretion rates ($r = 0.74$). In contrast, the bile acid pool size of their groups III and IV (who had large pools of 3–6 g) was not related to the secretion rate ($r = 0.11$), but was inversely related to the cycling rate

($r = -0.64$). The normal volunteers we studied were not obese and most of these had pool sizes within the range of groups I and II of Mok et al. (15). Our results are in agreement with some data of Shaffer and Small (8) who also found a close relation between the circulating bile acid pool size and the bile acid secretion rate in patients with functioning gallbladders.

It may be concluded that the bile acid pool circulating during continuous liquid formula perfusion of the duodenum is lower than the fasting bile acid pool possibly due to incomplete gallbladder emptying. Under these conditions the basal bile acid output reflects closely the size of the circulating bile acid pool in both normal subjects and cholecystectomy patients.

We acknowledge the skillful technical assistance of Mrs. S. Rutten, Miss L. Swinnen, Mr. L. Verhaegen, and Mr. A. Degreef. This research was sponsored by grants from the Fonds voor Wetenschappelijk Geneeskundig Onderzoek Van Belgie, Brussels, Belgium.

Manuscript received 11 May 1982 and in revised form 11 January 1983.

REFERENCES

1. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646–655.
2. Duane, W. C., and K. C. Hanson. 1978. Role of gallbladder emptying and small bowel transit in regulation of bile acid pool size in man. *J. Lab. Clin. Med.* **92:** 858–872.
3. Low-Beer, T. S., and E. W. Pomare. 1973. Regulation of bile salt pool size in man. *Br. Med. J.* **i:** 338–340.
4. Northfield, T. C., and A. F. Hofmann. 1975. 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. *Gut.* **16:** 1–17.
5. Low-Beer, T. S., K. W. Heaton, and A. E. Read. 1971. Gallbladder inertia and sluggish enterohepatic circulation of bile salts. *Lancet.* **I:** 991–994.
6. Low-Beer, T. S., K. W. Heaton, E. W. Pomare, and A. E. Read. 1973. The effect of coeliac disease upon bile salts. *Gut.* **14:** 204–208.
7. Hepner, G. W. 1975. Effect of decreased gallbladder stimulation on enterohepatic cycling and kinetics of bile acid. *Gastroenterology.* **68:** 1574–1581.
8. Shaffer, E. A., and D. M. Small. 1977. Biliary lipid secretion in cholesterol gallstone disease: the effect of cholecystectomy and obesity. *J. Clin. Invest.* **59:** 828–840.
9. Motson, R. W., K. J. Hammerman, W. H. Admirand, and L. W. Way. 1981. Effect of chronic changes in rate of enterohepatic cycling on bile acid kinetics and biliary lipid composition in the rhesus monkey. *Gastroenterology.* **80:** 655–660.
10. Duane, W. C. 1978. Simulation of the defect of bile acid metabolism associated with cholesterol cholelithiasis by sorbitol ingestion in man. *J. Lab. Clin. Med.* **91:** 969–978.
11. Duane, W. C., and J. R. Bond. 1980. Prolongation of intestinal transit and expansion of bile acid pools by propantheline bromide. *Gastroenterology.* **78:** 226–230.
12. Hardison, W. G. M., N. Tomaszewski, and S. M. Grundy. 1979. Effect of acute alterations in small bowel transit time upon the biliary excretion rate of bile acids. *Gastroenterology.* **76:** 568–574.
13. Go, V. L. W., A. F. Hofmann, and W. M. J. Summerskill. 1970. Simultaneous measurement of total pancreatic, biliary, and gastric outputs in man using a perfusion technique. *Gastroenterology.* **58:** 321–328.
14. Grundy, S. M., and A. L. Metzger. 1970. A physiological method for estimation of hepatic secretion of biliary lipid in man. *Gastroenterology.* **62:** 1200–1217.
15. Mok, H., K. Von Bergmann, and S. M. Grundy. 1977. Regulation of pool size of bile acids in man. *Gastroenterology.* **73:** 648–690.
16. Duane, W., R. D. Adler, L. J. Bennion, and R. L. Ginsberg. 1975. Determination of bile acid pool size in man: a simplified method with advantages of increased precision, shortened analysis time, and decreased isotope exposure. *J. Lipid Res.* **16:** 155–158.
17. Brunner, H., T. C. Northfield, A. F. Hofmann, V. L. W. Go, and W. M. J. Summerskill. 1974. Gastric emptying and secretion of bile acids, cholesterol, and pancreatic enzymes during digestion. *Mayo Clin. Proc.* **49:** 851–860.
18. Porter, H. P., and D. R. Saunders. 1971. Isolation of the aqueous phase of human intestinal contents during the digestion of a fatty meal. *Gastroenterology.* **60:** 997–1007.
19. Grundy, S. M. 1975. Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. *J. Clin. Invest.* **55:** 269–282.
20. Downie, N. M., and R. W. Heath. 1970. Basic Statistical Methods. Third edition. Harper and Row, New York. 256.
21. Van Berge Henegouwen, G. P., and A. F. Hofmann. 1978. Nocturnal gallbladder storage and emptying in gallstone patients and healthy subjects. *Gastroenterology.* **75:** 879–885.
22. Mok, H. Y. I., K. Von Bergmann, and S. M. Grundy. 1980. Kinetics of the enterohepatic circulation during fasting: biliary lipid secretion and gallbladder storage. *Gastroenterology.* **78:** 1023–1033.
23. Lindstedt, S. 1957. The turnover of cholic acid in man. *Acta Phys. Scand.* **40:** 1–9.
24. Shaffer, E. A. 1979. The role of the gallbladder in gallstone formation. *In* Gallstones. M. M. Fisher, C. A. Goresky, E. A. Shaffer, and S. M. Strasberg, editors. Plenum Press, New York and London. 223–249.