Diurnal serum levels of primary conjugated bile acids¹

Assessment by specific radioimmunoassays for conjugates of cholic and chenodeoxycholic acid

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SUMMARY The serum levels of conjugates of chenodeoxycholic acid (chenyl conjugates) and of cholic acid (cholyl conjugates) were determined by specific radioimmunoassays during a 24-hour period, which included three liquid meals and an overnight fast, in five healthy volunteers, five patients with previous cholecystectomy, five patients with documented bile acid malabsorption because of ileal resection, and four pregnant women. In healthy subjects, fasting-state levels of chenyl conjugates, when compared with those of cholyl conjugates, were higher; postprandially, levels of chenyl conjugates rose to a peak sooner (30 minutes vs 60 minutes) and to higher levels ($5.2 \pm 1.3 \mu\text{M}$ vs $2.0 \pm 0.5 \mu\text{M}$, $M \pm \text{SE}$). In cholecystectomised patients, the integrated areas under the curve for both bile acids were similar to those of the healthy controls, but postprandial peaks were less marked. In patients with bile acid malabsorption, postprandial rises of chenyl conjugates were lower but remained relatively constant throughout the day, whereas cholyl conjugate levels diminished progressively with each successive meal, consistent with depletion of the cholyl, but not the chenyl, pool. In three of four pregnant women, the postprandial rise of chenyl conjugates was disproportionately less compared with that of healthy controls. These results confirm the dynamic complexity of serum bile acid levels in man and indicate that the major circulating primary bile acids are chenyl conjugates. They support previous proposals that jejunal absorption of chenyl conjugates is important in the normal enterohepatic circulation of bile acids; and they suggest an abnormality in the enterohepatic circulation in pregnancy.

¹Supported in part by a research grant (AM 16770) from the National Institutes of Health and by grants-in-aid from the Share Foundation, Mead Johnson and Company, and Eli Lilly and Company.

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Received for publication 26 June 1978

We have recently developed sensitive and accurate radioimmunoassays for conjugates of cholic acid (cholyl conjugates) (Simmonds *et al.*, 1973) and chenodeoxycholic acid (chenyl conjugates) (Schalm *et al.*, 1977). Other research groups have also developed successful radioimmunoassays for cholyl conjugates (Murphy *et al.*, 1974; van den Berg *et al.*, 1976; Demers and Hepner, 1976; Roda *et al.*, in press; Spenney *et al.*, 1977) and for chenyl conjugates (Roda *et al.*, in press; Murphy *et al.*, 1976; Spenney *et al.*, 1977). The radioimmunoassay for cholyl conjugates was subsequently used to measure the response of this class of bile acids to meals and overnight fasting in healthy subjects, as well as patients with bile acid malabsorption due to ileal resection and patients with cholecystectomy (LaRusso *et al.*, 1974). We observed that the level of cholyl conjugates rose after each meal and fell during overnight fasting. In patients with bile acid malabsorption, the postprandial increases were smaller and decreased with each successive meal.

These results suggested that the level of serum bile acids was largely determined by intestinal absorption.

To prove this, we carried out a series of experiments which supported the hypothesis that the serum level of any bile acid reflected spillover of that bile acid returning from the intestine, and, further, that hepatic (first-pass) clearance of any bile acid was relatively constant during fasting and digestion (LaRusso *et al.*, 1978). As, based on animal studies, the hepatic clearance of chenyl conjugates is less efficient than that of cholyl conjugates (Hoffman *et al.*, 1975a, b; Reichen and Paumgartner, 1976), fasting serum bile acids should be enriched in chenyl acid, and this was found to be the case by radioimmunoassay (Schalm *et al.*, 1977).

In this paper, we report values for chenyl conjugates in the samples obtained from the healthy volunteers, the cholecystectomised patients, and the patients with bile acid malabsorption because of ileal resection for whom we had previously reported levels of cholyl conjugates (LaRusso *et al.*, 1974). The aim of these studies was to determine whether there were differences in enterohepatic circulatory dynamics of the two primary bile acids; in addition, we thought that such experiments might provide new, albeit indirect, information on the importance of jejunal absorption of chenyl conjugates. We also defined enterohepatic circulatory dynamics in four pregnant women, since little information is available on bile acid metabolism in pregnancy, and measurement of blood levels is simple and safe.

Methods

Nineteen adult volunteers were studied after informed consent was obtained: five healthy volunteers; five patients who had undergone cholecystectomy at least six months before the study; five patients with ileal resection and documented bile acid malabsorption; and four pregnant women, one six months pregnant, one seven months, one eight months, and one nine months. The groups were not well matched for age, sex, and weight, but all had unremarkable physical examinations and normal results on conventional liver tests (serum bilirubin, alkaline phosphatase, and glutamic oxalacetic transaminase values). In addition, a one-hour sulphobromophthalein retention test was carried out and was normal in the non-pregnant subjects. No subject had received any medication for at least one week before the study. Details about the first three groups of patients have been published earlier (LaRusso *et al.*, 1974).

On the morning of the study, a 21-gauge scalp vein needle was inserted into a large antecubital vein; the needle remained in place for the duration

of the 24-hour study and was kept patent by periodic irrigation with a dilute solution of heparin. Two fasting-state venous blood samples were taken. Equicaloric (30 kcal/kg body weight) liquid meals (40% protein, 20% fat, 40% carbohydrates) were given at 0800, 1230, and 1730. Venous blood was taken at 0830 and at regular intervals (15 or 20 minutes during the day and one or two hours during the night).

Venous blood samples were allowed to clot, and, after centrifugation, the separated serum was frozen for subsequent analysis. The concentration of conjugates of chenyl acid was determined by radioimmunoassay as previously described (Schalm *et al.*, 1977). Our assay has a coefficient of variation of less than 10% for values of 1 mol/l or more and of less than 16% for values below 1 mol/l. The assay measures predominantly conjugates of chenyl acid; unconjugated chenyl acid and ursodeoxycholyglycine show that 10% cross-reactivity; the contribution of these bile acids to the chenodeoxycholic values should be negligible, as their serum concentration in man is considered to be considerably below that of chenodeoxycholic acid.

Significance was tested by Student's *t* test. The integrated 'area under the curve' was calculated by a simple computer programme, which calculated the area under the curve of the time course of the serum levels (we acknowledge the advice of Dr Paul J. Thomas). The protocol was approved by the Mayo Clinic Human Studies Committee in July 1974.

Results

HEALTHY SUBJECTS (Tables 1 and 2, Fig. 1)

The concentration of chenyl conjugates had risen sharply by 30 minutes after each test meal; the maximal postprandial increase for each patient was between 250 and 1400% (mean 600%) above fasting-state values (mean $0.87 \mu\text{mol/l}$, range $0.5-1.43$). After the maximum, serum chenyl conjugate concentrations decreased rapidly for one hour, then levelled off or showed a small rise, followed by another decrease to approximately twice baseline values four hours after the meal (Fig. 1). Serum concentrations, fasting-state levels, and maximal postprandial peaks of chenyl conjugates were about twice as high as those of cholyl conjugates; the integrated area under the serum concentration curve was 3.5 times that of cholyl conjugates. The maximal postprandial peak of chenyl conjugates occurred about 30 minutes earlier than that of cholyl conjugates (Fig. 1).

Chenyl conjugates comprised 76% of the primary bile acids in serum over the 24-hour period; their proportion varied slightly during digestion, in-

Table 1 Fasting-state serum levels and 'area under curve' (AUC) of primary bile acid conjugates ($M \pm SD$) during 24-hour study period

Group	Fasting-state levels ($\mu\text{mol/l}$)		Daily AUC ($\text{h} \cdot \mu\text{mol/l}$)		Postprandial AUC * ($\text{h} \cdot \mu\text{mol/l}$)					
	chn-conj	chl-conj	chn-conj	chl-conj	Meal 1		Meal 2		Meal 3	
Normals (n = 5)	0.87 ± 0.42	0.45 ± 0.24	54.0 ± 16.8	15.0 ± 4.3	16.9 ± 4.9	5.64 %1.61	15.0 ± 4.48	4.24 ± 1.68	13.3 ± 4.53	4.80 ± 1.52
Cholecystectomy (n = 5)	1.30† ± 0.34	0.33 ± 0.12	49.2 ± 13.0	14.8 ± 4.2	15.0 ± 3.41	3.95 ± 1.03	11.5 ± 2.89	3.14 ± 0.83	11.4 ± 3.41	3.15 ± 0.92
Ileal resection (n = 5)	1.30 ± 0.66	0.22 ± 0.02	41.1 ± 3.5	5.4† ± 0.5	10.0† ± 3.12	2.01† ± 0.24	10.2 ± 1.64	0.98† ± 0.08	8.30† ± 1.75	0.71† ± 0.12
Pregnancy (n = 4)	0.60 ± 0.14	0.45 ± 0.25	29.4† ± 16.9	16.2 ± 5.0	8.9 ± 6.0	5.95 ± 1.30	8.50 ± 5.47	4.66 ± 1.23	7.42 ± 5.25	4.35 ± 1.17

Figures in parentheses represent number of subjects.

*AUC for the three hours after each meal.

†Different from normal, $P < 0.05$, by Wilcoxon rank sum test for unpaired replicates.

Table 2 Maximum postprandial level of primary bile acid conjugates ($M \pm SD$)

	Meal 1		Meal 2		Meal 3	
	chn-conj	chl-conj	chn-conj	chl-conj	chn-conj	chl-conj
Normals (n = 5)	4.98 ± 1.27	2.10 ± 0.48	5.25 ± 1.98	1.56 ± 0.41	4.28 ± 1.07	1.87 ± 0.52
Cholecystectomy (n = 5)	4.79 ± 0.71	1.12 ± 0.22	4.46 ± 1.28	0.85 ± 0.23	4.17 ± 1.79	0.83 ± 0.28
Ileal resection (n = 5)	2.56* ± 0.72	0.72* ± 0.13	2.91* ± 0.86	0.39† ± 0.04	2.46* ± 0.44	0.23† ± 0.05
Pregnancy (n = 4)	2.41 ± 0.96	1.94 ± 0.17	3.23 ± 1.48	1.96 ± 0.08	3.02 ± 1.31	1.86 ± 0.39

* $P < 0.01$ by Wilcoxon rank sum test for unpaired replicates.

† $P < 0.05$.

Fig. 1 Profile of chenyl conjugates and cholyl conjugates in serum during 24 hours, including ingestion of three liquid test meals, five representative healthy subjects.

Fig. 2 Profile of chenyl conjugates and cholyl conjugates in serum during 24 hours, including ingestion of three liquid test meals, in five subjects at least six months after cholecystectomy.

creasing when, for example, the postprandial rise of chenyl conjugates preceded that of cholyl conjugates. They tended to fall during the latter hours of the overnight fast.

CHOLECYSTECTOMY (Tables 1 and 2, Fig. 2)

In these patients, the serum concentration of chenyl conjugates also increased sharply after each

test meal; the maximal postprandial increase was similar in value to that of healthy subjects. The relative increase (mean 440%) was lower than that of healthy subjects mainly because of the higher fasting-state values in cholecystectomised patients (mean $1.3 \mu\text{mol/l}$, range 0.75 – 1.61). The area under the serum concentration curve did not differ from that in the healthy control subjects. The interval between meal

Fig. 3 Profile of chenyl conjugates and cholyl conjugates in serum during 24 hours, including ingestion of three liquid test meals, in five patients with ileal resection and documented bile acid malabsorption.

ingestion and the maximum was not longer than that observed in healthy subjects. After the maximum, serum chenyl conjugate concentrations decreased more rapidly than normal; a small secondary peak was observed in the majority of cases. Levels then returned to approximately baseline ($1.5 \mu\text{mol/l}$, range $0.9\text{--}2.5$) four hours after the meal (Fig. 2).

Fasting-state levels and maximal postprandial peaks of chenyl conjugates were about four times as high as those of cholyl conjugates. The ratio of integrated areas (chenyl) under the serum concentration curve (3.3) was comparable with that of healthy subjects. The maximal postprandial peak of chenyl conjugates occurred about one hour earlier than that of cholyl conjugates (Fig. 2).

Chenyl conjugates comprised 75% of the total serum primary bile acid conjugates over the 24-hour period; the proportion was similar to that in health during the digestive period and during the fasting period.

ILEAL RESECTION (Tables 1 and 2, Fig. 3)

In this group, chenyl conjugate concentrations peaked after a meal in only four out of 15 instances. The maximal postprandial increase was small ($110\text{--}420$; mean 240%) compared to healthy subjects due both to higher fasting-state values (mean $1.3 \mu\text{mol/l}$,

range $0.81\text{--}2.20$) and to the lower absolute increase. The interval between meal ingestion and the maximum was much longer (2.9 hours) than in health. After the maximum, little decline was observed, except during the fasting period ($15\text{--}24$ hours) (Fig. 3).

The $0\text{--}3$ hour postprandial level was significantly lower for each meal. However, the area under the serum concentration curve for the entire 24-hour period was not statistically smaller ($0.05 < P < 0.10$), probably because of the absence of troughs.

Fasting-state levels and integrated area under the serum concentration curve were about seven times those of cholyl conjugates; as in healthy subjects, the maximal postprandial peak of chenyl conjugates occurred earlier than that of cholyl conjugates (Fig. 3).

Cholyl conjugates comprised only 13% of the total serum primary bile acid conjugates over the 24-hour period; this proportion was lower than in health both during the digestive and fasting period (Fig. 3).

PREGNANCY (Tables 1 and 2, Fig. 4)

The postprandial increase in the level of chenyl conjugates was less in three of the four subjects than any of the normal controls. The only subject having a normal increase in the level of chenyl

Fig. 4 Profile of chenyl conjugates in serum during 24 hours, including ingestion of three liquid test meals, in four pregnant women.

Fig. 5 Profile of cholesteryl conjugates in serum during 24 hours, including ingestion of three liquid test meals, in four pregnant women.

conjugates was in her ninth month of pregnancy. The maximal postprandial increase for each patient was between 220 and 450% (mean 320%); this was lower than in nonpregnant healthy subjects. After the maximum, serum chenyl conjugate levels declined more rapidly than normal to baseline, followed by a small secondary peak. During the night, chenyl conjugate values were lower than normal (Fig. 4). Fasting-state levels, maximal postprandial peaks, and area under the serum concentration curve were less than twice those of cholesteryl conjugates (Fig. 5). As in normal subjects, the postprandial peaks occurred earlier than those of cholesteryl conjugates (Fig. 5).

The mean percentage of chenyl conjugates of the total serum concentration of primary bile acid conjugates was lower (61%) than that of the healthy controls (76%) over the 24-hour period, both during the digestive and fasting period.

Discussion

These results indicate that the two major classes of bile acids, cholesteryl conjugates and chenyl conjugates, have different enterohepatic circulatory dynamics. The rhythm of the enterohepatic circulation is determined by eating, but the response to a standard meal of the individual bile acid classes differs; chenyl conjugates rise sooner, achieve higher levels, and descend more slowly. The data suggest that the pool of chenyl conjugates has a daily recycling frequency significantly greater than that of the pool of cholesteryl conjugates, but the recycling frequency

cannot be calculated from the present studies or our previous studies as the relative first-pass clearances of cholesteryl conjugates and chenyl conjugates are not known.

The more rapid postprandial increase is probably attributable to preferential jejunal absorption of chenyl conjugates. However, even though preferential jejunal absorption might cause earlier increases of chenyl conjugates, it would not explain the much greater postprandial rise of chenyl conjugates when compared with cholesteryl conjugates. This is most reasonably explained by a lower fractional hepatic clearance of chenyl conjugates, causing a greater spillover into the plasma compartment.

The prolonged rise of chenyl conjugates is to be explained by continuing jejunal absorption at the end of the digestive period. It is well established that nearly half of the hepatic bile secreted during fasting bypasses the gallbladder in man (von Bergmann *et al.*, 1976; van Berge Henegouwen and Hofmann, 1976), and, as soon as this bile enters the small intestine, some of it chenyl conjugates will be absorbed. Cholesteryl conjugates will not be absorbed to a similar extent because of the slowing of intestinal transit, although the decrease in the proportion of chenyl conjugates occurring late in overnight fasting might indicate that cholesteryl conjugates require several hours to reach the distal ileum where they are then actively absorbed.

After this work had been completed, similar studies were reported by Hepner and Demers (1977) and by Barbara *et al.* (1976). The results of

Fig. 6 Time course of proportion of chenyl conjugates in primary bile acid conjugates in serum during 24 hours, including ingestion of three liquid test meals, in five representative healthy subjects and five patients with ileal resection and documented bile acid malabsorption.

these groups are in good agreement with our own work, although the postprandial increase in chenyl conjugates observed by us was somewhat greater than that observed by Hepner and Demers (1977), whose assay measured only glycine conjugates. Angelin *et al.* (1976) have recently published studies showing evidence for jejunal absorption of chenyl conjugates in man.

CHOLECYSTECTOMY

We noted previously for cholyl conjugates that the major change in the diurnal pattern was smoothing of the postprandial response (LaRusso *et al.*, 1974). As the area under the curve was similar to that of the healthy controls, we inferred that bile acid secretion during meals was essentially normal, despite a report to the contrary (Malagelada *et al.*, 1973); however, normal or near normal bile acid output after cholecystectomy has recently been documented in a careful study of Shaffer and Small (1977). The results for chenyl conjugates lend additional support to our view. The postprandial increase in chenyl conjugates did not differ from that of the healthy subjects. Detailed interpretation of the diurnal pattern in cholecystectomised patients is impossible because the anatomical location of the bile acid pool is not known, nor has the pattern of bile acid secretion into the intestine been measured under physiological conditions in cholecystectomised patients.

BILE ACID MALABSORPTION AFTER ILEAL RESECTION

Loss of the site of active absorption of bile acids

causes a striking change in their circulation with a progressive depletion of the cholyl acid pool throughout the day, so that it is nearly dissipated by the evening meal (LaRusso *et al.*, 1974). It is restored to a considerable extent during overnight fasting.

In Fig. 6 we have plotted the time course of the mole fraction (proportion) of chenyl conjugates in the total primary bile acid conjugates for the healthy controls and the patients with ileal resection. Ileal absorption causes a postprandial enrichment in cholyl conjugates. Loss of the ileum leads to progressive depletion of cholyl conjugates signalled by a progressive enrichment in chenyl conjugates.

These data suggest the importance of the jejunum in conserving the lipophilic chenyl conjugates which can be absorbed by passive nonionic diffusion based on perfusion studies (Hislop *et al.*, 1967) or analysis of fasting-state jejunal contents (Angelin *et al.*, 1976), as well as *in vitro* studies (Schiff *et al.*, 1972). Since chenylglycine should be absorbed far better than chenyltaurine, an explanation is also provided for the marked increase in the glycine/taurine ratio of bile acids in patients with ileal resection (McLeod and Wiggins, 1968; Garbutt *et al.*, 1969). What is remarkable is the constancy of levels of chenyl conjugates throughout the day. This would suggest that little gallbladder filling occurred. We speculate that the loss of enteroglucagon or another hormone from the ileum might be responsible for failure of the sphincter of Oddi to contract at the end of the digestive period. This could explain why most of the hepatic bile which is secreted bypasses the gallbladder.

PREGNANCY

The pattern of cholyl conjugates, not reported previously, did not differ from that of the healthy controls, consistent with normal gallbladder function and normal intestinal absorption and hepatic uptake. The strikingly smaller postprandial increase in chenyl conjugates in the three women at six, seven, and eight months of pregnancy is noteworthy. A possible explanation is that pregnancy causes a striking inhibition of chenilic acid synthesis, as has been observed in the baboon (McSherry *et al.*, 1977), causing a selective shrinkage of the chenilic acid pool with a consequent decrease in secretion. Other explanations are also possible, and, clearly, detailed studies of chenilic acid metabolism in pregnancy are warranted. These are quite feasible as ^2H - and ^{13}C -labelled chenodeoxycholic acid are available (Cowen *et al.*, 1976; Tserng and Klein, 1977) and both labels should give valid results when used for isotope dilution studies of bile acid kinetics in man (Balistreri *et al.*, 1975).

SERUM BILE ACID PROFILES

The data presented here indicate the enormous complexity of serum bile acids. The pattern at any moment represents the instantaneous spillover of all the bile acids returning to the liver. Each bile acid has its characteristic spillover rate, which remains relatively constant throughout the day. Thus, the pattern of serum bile acids should reflect the pattern of biliary bile acids but with a systematic distortion. Total serum bile acids reflect, not only hepatic spillover of the major conjugated bile acids in bile, but in addition the variety of secondary bile acids which are absorbed from the ileum and colon. If any of these are poorly cleared by the liver, they will be proportionately increased in the peripheral plasma compartment. When values for hepatic clearance are known for each bile acid, it should be possible to use serum bile acid measurements to monitor intestinal events. Such measurements might prove useful for characterising intestinal absorptive function.

Our work is incomplete in that we have not characterised the enterohepatic circulatory dynamics of the other four major bile acid species in man—deoxycholic, ursodeoxycholic, lithocholic, and sulpholithocholic. Demers and Hepner (1977) have recently characterised the behaviour of the glycine conjugates of deoxycholic and sulpholithocholic acid during a 24-hour period.

Our work confirms our hypothesis that both the level and the pattern of serum bile acids are determined by both intestinal and hepatic factors. There is increasing evidence that measurement of serum bile acid levels does provide clinically useful informa-

tion (Hofmann *et al.*, 1974; Korman *et al.*, 1974; Fausa and Gjone, 1976; Osuga *et al.*, 1977), but mechanistic interpretation of raised levels in patients with liver disease will require even more detailed studies of the enterohepatic circulation.

References

- Angelin, B., Einarsson, K., and Hellström, K. (1976). Evidence for the absorption of bile acids in the proximal small intestine of normo- and hyperlipidaemic subjects. *Gut*, **17**, 420-425.
- Balistreri, W. F., Cowen, A. E., Hofmann, A. F., Szczepanik, P. A., and Klein, P. D. (1975). Validation of use of $^{11,12}\text{-}^3\text{H}$ -labeled chenodeoxycholic acid in isotope dilution measurements of bile acid kinetics in man. *Pediatric Research*, **9**, 757-760.
- Barbara, L., Roda, A., Roda, E., Aldini, R., Mazzella, G., Festi, D., and Sama, C. (1976). Diurnal variations of serum primary bile acids in healthy subjects and hepatobiliary disease patients. *Rendiconti di Gastro-enterologia*, **8**, 194-198.
- van den Berg, J. W. O., van Blankenstein, M., Bosman-Jacobs, E. P., Frenkel, M., Höchner, P., Oost-Harwig, O. I., and Wilson, J. H. P. (1976). Solid phase radioimmunoassay for determination of conjugated cholic acid in serum. *Clinica Chimica Acta*, **73**, 277-283.
- van Berge Henegouwen, G. P., and Hofmann, A. F. (1976). Measurement of gallbladder storage and emptying in man: Method and preliminary application. *Gastroenterology*, **71**, 932. (Abstract).
- von Bergmann, K., Mok, H. Y. I., and Grundy, S. M. (1976). Distribution of the bile acid pool in the fasting state in man. *Gastroenterology*, **71**, 934. (Abstract).
- Cowen, A. E., Hofmann, A. F., Hachey, D. L., Thomas, P. J., Belobaba, D. T. E., Klein, P. D., and Tokes, L. (1976). Synthesis of $^{11,12}\text{-}^3\text{H}_2$ - and $^{11,12}\text{-}^3\text{H}_2$ -labeled chenodeoxycholic and lithocholic acids. *Journal of Lipid Research*, **17**, 231-238.
- Demers, L. M., and Hepner, G. (1976). Radioimmunoassay of bile acids in serum. *Clinical Chemistry*, **22**, 602-606.
- Fausa, O., and Gjone, E. (1976). Serum bile acid concentrations in patients with liver disease. *Scandinavian Journal of Gastroenterology*, **11**, 537-543.
- Garbutt, J. T., Heaton, K. W., Lack, L., and Tyor, M. P. (1969). Increased ratio of glycine to taurine-conjugated bile salts in patients with ileal disorders. *Gastroenterology*, **56**, 711-720.
- Hepner, G. W., and Demers, L. M. (1977). Dynamics of the enterohepatic circulation of the glycine conjugates of cholic, chenodeoxycholic, deoxycholic and sulfolithocholic acid in man. *Gastroenterology*, **72**, 499-501.
- Hislop, I. G., Hofmann, A. F., and Schoenfield, L. J. (1967). Determinants of the rate and site of bile acid absorption in man. *Journal of Clinical Investigation*, **46**, 1070. (Abstract).
- Hoffman, N. E., Donald, D. E., and Hofmann, A. F. (1975a). Effect of primary bile acids on bile lipid secretion from perfused dog liver. *American Journal of Physiology*, **229**, 714-720.
- Hoffman, N. E., Iser, J. H., and Smallwood, R. A. (1975b). Hepatic bile acid transport: Effect of conjugation and position of hydroxyl groups. *American Journal of Physiology*, **229**, 298-302.

- Hofmann, A. F., Korman, M. G., and Krugman, S. (1974). Sensitivity of serum bile acid assay for detection of liver damage in viral hepatitis type B. Prospective study in five patients. *American Journal of Digestive Diseases*, **19**, 908-910.
- Korman, M. G., Hofmann, A. F., and Summerskill, W. H. J. (1974). Assessment of activity in chronic active liver disease: Serum bile acids compared with conventional tests and histology. *New England Journal of Medicine*, **290**, 1399-1402.
- LaRusso, N. F., Hoffman, N. E., Korman, M. G., Hofmann, A. F., and Cowen, A. E. (1978). Determinants of fasting and postprandial serum bile acid levels in healthy man. *American Journal of Digestive Diseases*, **23**, 385-391.
- LaRusso, N. F., Korman, M. G., Hoffman, N. E., and Hofmann, A. F. (1974). Dynamics of the enterohepatic circulation of bile acids. *New England Journal of Medicine*, **291**, 689-692.
- McLeod, G. M., and Wiggins, H. S. (1968). Bile-salts in small intestinal contents after ileal resection and in other malabsorption syndromes. *Lancet*, **1**, 873-876.
- McSherry, C. K., Dietrick, J. E., May, P. S., Niemann, W., Morrissey, K. P., Palmer, R. H., and Glenn, F. (1977). Biliary lipid metabolism in the pregnant baboon. *Surgery, Gynecology, and Obstetrics*, **144**, 727-733.
- Malagelada, J. R., Go, V. L. W., Summerskill, W. H. J., and Gamble, W. S. (1973). Bile acid secretion and biliary bile acid composition altered by cholecystectomy. *American Journal of Digestive Diseases*, **18**, 455-459.
- Murphy, G. M., Edkins, S. M., Williams, J. W., and Catty, D. (1974). The preparation and properties of an antiserum for the radioimmunoassay of serum conjugated cholic acid. *Clinica Chimica Acta*, **54**, 81-89.
- Murphy, G. M., Sampson, D. G., Cross, L. M., and Catty, D. (1976). The methodology of bile acid radioimmunoassay. *Clinical Science and Molecular Medicine*, **50**, 25p. (Abstract).
- Osuga, T., Mitamura, K., Mashige, F., and Imai, K. (1977). Evaluation of fluorimetrically estimated serum bile acid in liver disease. *Clinica Chimica Acta*, **75**, 81-90.
- Reichen, J., and Paumgartner, G. (1976). Uptake of bile acids by perfused rat liver. *American Journal of Physiology*, **231**, 734-742.
- Roda, A., Roda, E., Sama, C., Festi, D., Mazzella, G., Aldini, R., and Barbara, L. (1978). Radioimmunoassay of serum bile acids in man. Methodological and diagnostic consideration. *Journal of Nuclear Biology and Medicine*. (In press).
- Schalm, S. W., van Berge Henegouwen, G. P., Hofmann, A. F., Cowen, A. E., and Turcotte, J. (1977). Radioimmunoassay of bile acids: development, validation, and preliminary application of an assay for conjugates of chenodeoxycholic acid. *Gastroenterology*, **73**, 285-290.
- Schiff, E. R., Small, N. C., and Dietschy, J. M. (1972). Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. *Journal of Clinical Investigation*, **51**, 1351-1362.
- Shaffer, E. A., and Small, D. M. (1977). Biliary lipid secretion in cholesterol gallstone disease. *Journal of Clinical Investigation*, **59**, 828-840.
- Simmonds, W. J., Korman, M. G., Go, V. L. W., and Hofmann, A. F. (1973). Radioimmunoassay of conjugated cholyl bile acids in serum. *Gastroenterology*, **65**, 705-711.
- Spenny, J. G., Johnson, B. J., Hirschowitz, B. I., Mihos, A. A., and Gibson, R. (1977). An ¹²⁵I radioimmunoassay for primary conjugated bile salts. *Gastroenterology*, **72**, 305-311.
- Tserng, K.-Y., and Klein, P. D. (1977). An improved synthesis of 24-¹³C-labeled bile acids using formyl esters and a modified lead tetraacetate procedure. *Journal of Lipid Research*, **18**, 400-403.