# Feedback regulation of bile acid biosynthesis in the rat

SARAH SHEFER, SUSAN HAUSER, IHOR BEKERSKY, and ERWIN H. MOSBACH*

Department of Laboratory Diagnosis, Public Health Research Institute of the City of New York, Inc., and the Bureau of Laboratories, New York City Department of Health, New York 10016

ABSTRACT The hepatic biosynthesis of bile salts in the rat has been shown to be controlled homeostatically by the quantity of bile salt returning to the liver via the portal circulation. The feedback mechanism was demonstrated in two kinds of experiments. In the first, rats with bile fistulas were infused intraduodenally with sodium taurocholate 12 hr after surgery. If the rate of infusion was greater than 10 mg per 100 g rat per hr, the increase in bile acid output normally observed in bile fistula rats was prevented. In the second type of experiment, the rats were infused with taurocholate 48–72 hr after biliary diversion, when bile acid output had reached a maximal value. Provided the rate of infusion exceeded 10 mg per 100 g rat per hr, bile acid secretion returned to the low levels observed in intact rats. Previous attempts to demonstrate the feedback control have been unsuccessful because too little bile salt was infused.

The taurocholate pool of the experimental animals was measured as approximately 15 mg per 100 g rat; it was calculated from this and the above results that this pool circulated 10–13 times daily.

SUPPLEMENTARY KEY WORDS bile fistula rat · biliary diversion · in vivo bile acid biosynthesis · bile acid pool · regulation · inhibition · feedback mechanism · enterohepatic circulation · enterohepatic circulation rate

EVIDENCE that the production of bile salts by the liver is regulated by a feedback mechanism similar to that of cholesterol biosynthesis is as follows. First, biliary diversion in experimental animals (1) and in man (2, 3) results in a several-fold increase in bile acid production. Second, the oral administration of bile acids to experimental animals (4) and to human subjects (5) produces a decrease in bile acid biosynthesis. Third, the intestinal infusion of taurochenodeoxycholate into rats with bile fistulas is reported to inhibit hepatic taurocholate production (6).

However, recent attempts to demonstrate the feedback inhibition, to define its site on the biochemical pathway between acetate and cholanoic acid, and to evaluate the role of particular bile acids in this process have led to equivocal or negative conclusions. For example, Cronholm and Sjövall (7) concluded from their study of bile acids in rat portal blood that "the data obtained do not support the theory that the production of bile acids from cholesterol is regulated through a direct feedback inhibition by the bile acids returned to the liver via the portal blood." These workers further pointed out that in the earlier studies (6) dealing with the inhibition of taurocholate synthesis by intraduodenal infusion of taurochenodeoxycholate, the infusion rate used (approximately 10 $\mu$moles/hr) was much greater than the portal flow of about 0.7 $\mu$moles of taurochenodeoxycholate per hr observed in their studies. In addition, unpublished experiments from this laboratory showed that infused taurochenodeoxycholate is mostly converted to $\alpha$- and $\beta$-muricholic acids, so that its inhibitory effect is difficult to assess.

A preliminary report by Lee, Parke, and Whitehouse (8) on the effect of taurocholate infusion on cholesterol catabolism and a careful study by J. D. Wilson, W. H. Bentley, and C. T. Crowley (personal communication) dealing with the effect of taurocholate infusion on taurocholate biosynthesis failed to disclose feedback control of

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; HMG, 3-hydroxy-3-methylglutaric acid.

Systematic names of the sterols and bile acids referred to in the text by their trivial names are as follows: cholesterol, cholest-5-*en*-3$\beta$-ol; cholic acid, 3$\alpha$,7$\alpha$,12$\alpha$-trihydroxy-5$\beta$-cholanoic acid; deoxycholic acid, 3$\alpha$,12$\alpha$-dihydroxy-5$\beta$-cholanoic acid; chenodeoxycholic acid, 3$\alpha$,7$\alpha$-dihydroxy-5$\beta$-cholanoic acid; $\alpha$-muricholic acid, 3$\alpha$,6$\beta$,7$\alpha$-trihydroxy-5$\beta$-cholanoic acid; $\beta$-muricholic acid, 3$\alpha$,6$\beta$,7$\beta$-trihydroxy-5$\beta$-cholanoic acid.

* Reprint requests should be sent to Dr. E. H. Mosbach, Public Health Research Institute, 455 First Avenue, New York, New York 10016.

bile acid biosynthesis by bile salt. It seems possible to account for some of these apparent contradictions by considering the magnitude of the bile acid pool of the rat and the rate of circulation of this pool.

The magnitude of the bile acid pool in the rat has previously been measured directly by (*a*) analysis of intestinal contents (9), (*b*) collection of fistula bile for several hours immediately after establishment of the fistula (1, 10), and (*c*) analysis of portal blood (7), and indirectly by isotopic means (11, 12). Estimates of this pool range from 5 to 10 mg of bile acid per 100 g rat (13, 14). The rate of circulation of this pool is not known with certainty. Estimates of 10–12 circulations per 24 hr have been made, but definite data are lacking (12, 13). If we assume a bile acid pool of 10 mg per 100 g rat, circulating 10 times per day, intraduodenal infusion of 100 mg of sodium taurocholate per 100 g rat per day (simulating the circulating pool) would be required to produce feedback inhibition. Thus a 300 g rat would require 300 mg (558 μmoles) per 24 hr (23 μmoles/hr) to produce inhibition of bile acid synthesis. Wilson's group has already shown that this quantity of taurocholate will not inhibit hepatic bile acid biosynthesis (Wilson, J. D., W. H. Bentley, and C. T. Crowley. Personal communication.).

Hofmann (15) has pointed out that the rate of circulation of the bile acid pool can be estimated if the daily synthesis and the efficiency of the intestinal bile acid absorption are known. If it is assumed that the size of the pool remains constant (i.e., the liver synthesizes and immediately replaces the bile salt lost with each circulation), the following equation is obtained:

Daily synthesis = circulations per day × (1 − efficiency of absorption) × pool. (Daily synthesis = fecal loss.)

For a 300 g rat, assuming 98% absorption efficiency, excretion of 7 mg of bile acid per day, and a pool of 30 mg, the number of circulations per day = 7/([1 − 0.98] × 30) = 11.7.

These calculations suggest that even small errors in measuring bile acid excretion, pool size, or absorption efficiency will lead to considerable error in the estimate of bile acid flux in the enterohepatic circulation. It seemed preferable, therefore, not to rely on estimates but to determine pool size, and then to measure circulation time by infusing bile fistula rats with bile salt at a rate just sufficient to inhibit hepatic bile acid synthesis, thus simulating the circulation of the bile acid pool in the intact rat. Presumably, if bile fistula rats are prepared and infused soon after surgery with taurocholate at a rate closely resembling that of the enterohepatic circulation, hepatic bile acid synthesis should remain at the minimal value existing in the intact ("inhibited") animal. It should further be possible to allow rats to attain the maximal rate of bile acid synthesis usually observed 24–48 hr after biliary diversion ("noninhibited state") and then to restore the normal (inhibited) synthetic rate by intraduodenal infusion of taurocholate. In order to distinguish between administered and endogenous bile salt, we labeled the latter by administering radioactive precursors during the experiment. Such an experiment should demonstrate the existence of the postulated feedback mechanism and provide information on the magnitude and rate of circulation of the rat's bile acid pool.

## METHODS

### *Experimental Animals*

Male Wistar strain rats (240–400 g) were fed a powdered diet consisting of glucose and a casein hydrolysate (Bacto Casamino Acids; Difco Laboratories, Inc., Detroit, Mich.) in the same proportions (3:2, w/w) as the liquid diet described below. After 3 days on this diet the rats were anesthetized with Diabutal (Diamond Laboratories, Inc., Des Moines, Iowa), and cannulas were inserted into the bile duct, duodenum, and femoral vein. The animals were placed in restraining cages and given unrestricted access to water. The duodenal cannula was connected to an infusion pump (Harvard Apparatus Co., Inc., Dover, Mass.) which delivered a liquid diet at a volume of 30 ml/day. The diet consisted of an aqueous solution of glucose (9.2%) and casein hydrolysate (6.4%) plus 0.1% KCl and 0.9% NaCl. The NaCl content of the casein hydrolysate (14%) was taken into account in adjusting the NaCl concentration. The casein hydrolysate contained less than 0.01% cholesterol. Therefore, the cholesterol intake of the experimental animals was less than 0.2 mg/day. The glucose and casein hydrolysate of the liquid diet supplied the rat with 6.5 kcal per 100 g rat per day.

The venous cannula was connected to a second infusion pump which delivered 5 μc (0.2 mg) of sodium acetate-1-$^{14}$C (New England Nuclear Corp., Boston, Mass.), dissolved in 0.624 ml of isotonic saline, per hr. Bile was collected by a fraction collector at 6-hr intervals. Bile volume ranged from 1 to 1.5 ml per rat per hr.

### *Sodium Taurocholate*

The bile salt was purchased from Calbiochem, Los Angeles, Calif., and was shown to be of sufficient purity for the infusion studies, as follows. The infrared spectrum was identical in every important respect with that of a highly purified reference sample kindly supplied by A. F. Hofmann. Both compounds exhibited no carbonyl absorption at 1720 cm$^{-1}$, which indicated that unconjugated carboxylic acids were absent.

The absence of free bile acids or fatty acids was confirmed by TLC (Silica Gel G, chloroform–methanol–

acetic acid 80:12:3) and detection of the spots by charring with 50% $H_2SO_4$. The sample contained less than 0.1% dihydroxy- or trihydroxycholanoic acids. In addition, when 100 mg of sodium taurocholate was dissolved in water, and the solution was then acidified to pH 1 and extracted with diethyl ether–methanol 10:1, the organic solvent contained less than 0.05% of solids.

TLC of increasing amounts of sodium taurocholate (100–2000 μg) on Silica Gel G with the system water–*n*-propanol–propionic acid–isoamyl acetate 5:10:15:20 indicated that it contained less than 0.1% taurodeoxycholate. No other free or conjugated bile acids were detected after charring with 50% $H_2SO_4$.

The sodium taurocholate was dissolved in the liquid diet and infused via the duodenal cannula at rates ranging from 0 to 14 mg per 100 g rat per hr. Infusion of the bile salt did not cause diarrhea.

### *Analytical Methods*

The volume of each 6 hr bile sample was measured, and 0.5 ml aliquots were deproteinized, hydrolyzed, and extracted as described by Evrard and Janssen (16). The free cholanoic acids were dissolved in 1.0 ml of methanol, one drop of concentrated $H_2SO_4$ was added, and esterification was allowed to proceed at room temperature overnight. The methyl esters of the bile acids were extracted (17), their radioactivity was determined, and aliquots were taken for GLC and TLC. Losses of bile acids during these manipulations were corrected for by analysis of a standard mixture of taurocholate and taurochenodeoxycholate with each series of bile samples. Recovery of cholate averaged 70–75%, and of chenodeoxycholate 80–85%.

*GLC.* GLC was used to determine the concentrations of biliary cholesterol, chenodeoxycholate, deoxycholate, and the combined trihydroxycholanoates (cholate plus α- and β-muricholates). The methyl ester mixture was injected onto a 180 cm × 4 mm glass column packed with 3% QF-1 on 80–100 mesh Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa.). The column temperature (isothermal operation) was between 255° and 270°C. The instrument used was a Barber-Colman Selecta 5000 gas chromatograph with hydrogen flame detectors and disc integrators. The bile acids were quantified by comparison with standards of known concentration, which were measured twice daily (estimated precision of measurement ±5%). Detector response was identical for the three trihydroxycholanoates. Retention times at 255°C relative to methyl deoxycholate = 1 (9.2 min) were as follows: cholesterol, 0.3; methyl chenodeoxycholate, 1.20; methyl cholate, 2.01; methyl α-muricholate (18), 2.05; methyl β-muricholate (19), 1.96. Since a mixture of cholate and α- and β-muricholates is not resolved, concentration of these acids was determined indirectly from radioactivity data as described below.

*TLC.* The individual bile acids (methyl esters) were separated by TLC and removed from the plate for radioactivity assay as follows. A suitable aliquot was combined with 20 μg of cholesterol, 20 μg of methyl chenodeoxycholate, 20 μg of methyl α-muricholate, 20 μg of methyl β-muricholate, and 20 μg of palmitic acid as carriers and was applied as a spot to a plate coated with a 250 μm thick layer of Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.). The plate was developed with acetone–benzene 2:3 by ascending TLC (17). The spots were made visible by spraying with 2′,7′-dichlorofluorescein and inspected under long-wave UV radiation. When care was taken not to overload the plate with methyl cholate (particularly in the analysis of bile from rats that had been infused with large amounts of sodium taurocholate), this solvent system provided satisfactory separation of the following biliary constituents: methyl cholate ($R_f$ 0.16), methyl α-muricholate ($R_f$ 0.23), methyl β-muricholate ($R_f$ 0.33), methyl chenodeoxycholate ($R_f$ 0.54), cholesterol ($R_f$ 0.87), and fatty acid ($R_f$ 0.98).

Appropriate spots were removed with a suction device (20) and transferred quantitatively to scintillation vials. Methyl cholanoates were eluted from the silica gel by adding 4 ml of ethanol and heating the closed vial at 60°C for 45 min. The radioactivity of all samples was determined after addition of 12 ml of 2,5-bis[2-(5-*tert*-butyl benzoxazolyl)]-thiophene solution (4 g/liter in toluene) in a liquid scintillation counter and corrected for background and quenching. On the average, recovery of labeled methyl cholanoates from the thin-layer plate was found to be better than 95% (range 90–100%).

## EXPERIMENTAL DESIGN

Two types of animal experiments were carried out. In the first, no venous cannula was inserted, and no labeled material was administered. Known concentrations of taurocholate incorporated into the nutrient solution were infused into the duodenum either 6–12 or 48–72 hr after the bile fistula had been established. We reasoned that inhibition of chenodeoxycholic acid synthesis would be observed when the rate of taurocholate infusion just exceeds the rate of normal circulation of the bile acid pool in an intact rat. The magnitude of the pool was estimated by measuring bile salt output from the fistula during the first 6–12 hr after surgery. Biliary cholesterol and chenodeoxycholate concentrations were measured to determine (*a*) whether hepatic synthesis and biliary excretion of these biliary constituents would be maintained at the low levels (taurochenodeoxycholate, 0.01–0.10 mg/hr; cholesterol, 0.01–0.05 mg/hr) observed in

inhibited animals; and (*b*) whether a noninhibited animal, i.e., one in which the biliary output of cholesterol and chenodeoxycholate had reached maximal levels at the end of 48–72 hr, could be inhibited by intraduodenal administration of bile salt. In such experiments the rate of biosynthesis of cholic acid and muricholic acids cannot be estimated since endogenous cholate production may amount to less than 5% of the quantity infused, and the muricholic acids are not distinguishable from cholic acid by the GLC method used. Nevertheless, the decrease of taurochenodeoxycholate production observed in some of these studies cannot be ascribed to an increased conversion of this bile salt to tauromuricholates. This is evident from the tracer studies described below (see also Table 2).

The second type of experiment was similar to the first, except that the bile fistula animals received in addition an intravenous infusion of a precursor (acetate-1-$^{14}$C) of cholesterol and bile acids. The formation and excretion of *labeled* bile acids made it possible to measure the rate of endogenous cholate production as well as the quantity of muricholic acids formed. Since the specific radioactivity of chenodeoxycholic acid was known (from combined TLC–GLC data), and since $\alpha$- and $\beta$-muricholic acids are derived solely from chenodeoxycholic acid (21), they must have the same specific radioactivity per mole as chenodeoxycholic acid. Thus, the radioactivity data could be used to quantify $\alpha$- and $\beta$-muricholic acids. This quantity was then subtracted from the mass of cholic acid plus muricholic acids obtained by GLC. Endogenous synthesis of taurocholate was estimated as follows: since cholic acid and chenodeoxycholic acid arise from the same precursor, namely cholesterol, they should have identical specific radioactivities after the bile fitsula animal has reached a steady state with respect to bile acid synthesis. It was found that at the end of 36–48 hr, biliary cholate and chenodeoxycholate had attained identical specific radioactivities, within the precision of measurement, estimated at ±7% (Table 1).

Assuming that the specific activities of *endogenous* cholanoic acids remain equal during the experimental manipulations, the amount of cholate produced from endogenous precursors can be calculated from the known specific radioactivity of biliary chenodeoxycholate and the total radioactivity of biliary cholate. The calculations involved in a typical experiment (rat No. 73) are summarized in Table 2.

TABLE 1* SPECIFIC RADIOACTIVITIES OF BILIARY CHOLANOIC ACIDS DURING INHIBITION EXPERIMENTS†

| Period | Rat No. 63 Specific Activity | | Rat No. 73 Specific Activity | |
|---|---|---|---|---|
| | TCD | TC | TCD | TC |
| *hr* | *dpm* × 10$^{-2}$/*mg* | | *dpm* × 10$^{-2}$/*mg* | |
| 12–24 | 182 | 81 | 68 | 38 |
| 24–36 | 330 | 267 | 192 | 106 |
| 36–48 | 640 | 591 | 201 | 209 |
| 48–60 | 652 | 581 | 262‡ | 15‡ |
| 60–72 | 588 | 562 | 275‡ | 13‡ |
| 72–84 | 1605‡ | 9‡ | 361‡ | 10‡ |
| 84–96 | 1170‡ | 7‡ | 803‡ | 2‡ |
| 96–108 | 1055‡ | 7‡ | 1191 | 1 |
| 108–120 | 419 | 37 | 951 | 25 |
| 120–132 | 379 | 345 | 897 | 55 |
| 132–144 | 487 | 443 | 612 | 80 |
| 144–156 | 627 | 631 | 458 | 166 |
| 156–168 | | | 384 | 214 |
| 168–180 | | | 371 | 338 |
| 180–192 | | | 359 | 347 |

* Abbreviations used in this table are as follows: TCD, sodium taurochenodeoxycholate; TC, sodium taurocholate.

† Bile fistula rats (Nos. 63 and 73) were infused intravenously with sodium acetate-1-$^{14}$C at a rate of 5 $\mu$c/hr. Specific radioactivities of biliary taurocholate and taurochenodeoxycholate were determined at 12-hr intervals. At the end of 72 hr, rat No. 63 was infused intraduodenally with sodium taurocholate at a rate of 11.3 mg per 100 g rat per hr. Intraduodenal infusion of rat No. 73 with taurocholate was begun 48 hr after biliary diversion. Duration of taurocholate administration was 36 hr for rat No. 63 and 48 hr for rat No. 73.

‡ Periods of taurocholate infusion.

## RESULTS

Fig. 1 illustrates one of a series of experiments in which we attempted to inhibit the biosynthesis of taurochenodeoxycholate by infusing sodium taurocholate intraduodenally at rates ranging from 4 to 14 mg per 100 g rat per hr. Control animals receiving identical treatment and nutrition but no taurocholate infusion were studied simultaneously. Data from both the experimental and control rats are shown in the same figure. With an infusion rate of 8.3 mg per 100 g rat per hr, the experimental rat showed the same pattern of taurochenodeoxycholate excretion as the control. After excretion of the pool (approximately 4 mg of taurochenodeoxycholate), production of taurochenodeoxycholate fell to the low level (0.07 mg/hr) usually observed in inhibited animals. Taurocholate infusion at the rate of 8.3 mg per 100 g rat per hr failed to prevent the subsequent rise of taurochenodeoxycholate synthesis toward the maximal rate (0.95 mg/hr).

The data from 38 rats receiving increasing amounts of taurocholate are summarized in Table 3. No decrease in taurochenodeoxycholate production was detected in rats receiving 4–9 mg of taurocholate by infusion per 100 g rat per hr, but in all experiments in which the infusion rate was greater than 10 mg per 100 g rat per hr inhibition was observed. When the infusion rate was exactly 10 mg per 100 g rat per hr, the inhibition was observed in some but not in all animals.

The data in this table make it evident that 60–80% of the *infused* taurocholate reached the liver. The remainder was presumably not absorbed, but fecal analyses to con-

TABLE 2* TYPICAL CALCULATION OF BILE ACID CONCENTRATIONS IN FISTULA BILE OF A RAT INFUSED WITH ACETATE-1-$^{14}$C†

| | Experimental Data from | | | | | | Calculated Data | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GLC | | | TLC | | | | | | | | |
| Time | A TCD | B TC + TαM + TβM | C TCD | D TC | E TαM | F TβM | G = C/A Spec. Act. TCD | H = E/G TαM | I = F/G TβM | J = B − [H + I] TC in Bile | K = D/G TC Synthesized | L = D/J Spec. Act. of Biliary TC |
| *hr* | *mg/hr* | *mg/hr* | *dpm/hr* | *dpm/hr* | *dpm/hr* | *dpm/hr* | *dpm/hr* | *mg/hr* | *mg/hr* | *mg/hr* | *mg/hr* | *dpm/mg* |
| 48–60‡ | 1.626 | 42.1 | 42,600 | 60,440 | 3,250 | 8,700 | 26,200 | 0.124 | 0.332 | 41.6 | 2.31 | 1,453 |
| 60–72‡ | 1.638 | 32.7 | 42,100 | 41,950 | 2,010 | 10,950 | 25,700 | 0.078 | 0.426 | 32.2 | 1.63 | 1,303 |
| 72–84‡ | 0.423 | 23.1 | 15,280 | 22,900 | 1,697 | 15,280 | 36,100 | 0.047 | 0.423 | 22.6 | 0.634 | 1,013 |
| 84–96‡ | 0.010 | 13.09 | 803 | 2,360 | 273 | 2,890 | 80,300 | 0.0034 | 0.036 | 13.1 | 0.029 | 180 |
| 96–108 | 0.009 | 15.03 | 1,072 | 1,890 | 238 | 3,570 | 119,100 | 0.002 | 0.030 | 15.0 | 0.016 | 126 |
| 108–120 | 0.056 | 1.082 | 5,330 | 2,560 | 571 | 4,380 | 95,100 | 0.006 | 0.046 | 1.0 | 0.027 | 2,560 |

* Abbreviations used in this table are as follows: TCD, sodium taurochenodeoxycholate; TC, sodium taurocholate; TαM, sodium tauro-α-muricholate; TβM, sodium tauro-β-muricholate.

† Rat No. 73. The left side of the table illustrates GLC and TLC data from six successive 12-hr bile collections. The concentrations of taurochenodeoxycholate (column A) and of combined taurocholate plus tauromuricholates (column B) were determined by GLC. Total radioactivities of taurochenodeoxycholate, tauro-α-muricholate, and tauro-β-muricholate for each 12 hr bile sample were measured by TLC (columns C through F). Calculations based on these experimental data are summarized on the right side of the table and are identified by the column heading.

The data shown in this table are representative of changes in bile salt composition observed in most of the experiments. The noninhibited bile fistula rat excreted nearly equal amounts of taurocholate, and of combined taurochenodeoxycholate plus muricholates. Tauro-α-muricholate accounted for 2–3% of total bile salt, and tauro-β-muricholate for 5–10%. In the inhibited rat, total endogenous bile salt production was greatly reduced, and the proportion of taurochenodeoxycholate plus tauromuricholates in total endogenous bile salts increased. β-Muricholic acid frequently became the predominating bile acid (40–50% of total endogenous bile acids). Compare, for example, the 48–60-hr and 84–96-hr periods.

‡ Periods of sodium taurocholate infusion.

TABLE 3* EFFECT OF SODIUM TAUROCHOLATE INFUSION ON CONCENTRATION OF BILIARY BILE ACIDS AND CHOLESTEROL

| No. of Experiments | TC Infused | Exogenous TC Reexcreted in Bile† | Average Biliary TCD after 48 hr of TC Infusion | Average Biliary Cholesterol after 48 hr of TC Infusion |
|---|---|---|---|---|
| | *mg/100 g rat/hr* | *mg/100 g rat/hr* | *mg/rat/hr* | *mg/rat/hr* |
| 10 | None‡ | 0.5§ (0.4–0.6)‖ | 1.05 (0.6–1.2)‖ | 0.09 (0.06–0.11)‖ |
| 4 | 4 | 3.3 (3.2–3.6) | 0.96 (0.6–1.1) | 0.07 (0.06–0.1) |
| 2 | 5 | 3.7 (3.3–4.1) | 0.75 (0.6–0.9) | 0.08 (0.07–0.09) |
| 3 | 6 | 4.4 (4.2–4.6) | 0.99 (0.8–0.9) | 0.11 (0.09–0.12) |
| 2 | 8 | 5.9 (5.8–6.0) | 1.0 (0.9–1.1) | 0.11 (0.1–0.12) |
| 1 | 9 | 6.3 | 1.1 | 0.07 |
| 4 | 10 | 6.7 (6.6–6.9) | 0.98 (0.8–1.1) | 0.08 (0.06–0.12) |
| 2 | 10 | 6.9 (6.8–7.0) | 0.03 (0.02–0.04) | 0.09 (0.08–0.1) |
| 3 | 11 | 7.5 (7.0–8.3) | 0.05 (0.03–0.08) | 0.06 (0.04–0.08) |
| 7 | 11¶ (12, 13, 14) | 7.9 (7.0–8.7) | 0.02 (0.01–0.04) | 0.08 (0.06–0.1) |

* Bile fistula rats were infused intraduodenally with solutions of sodium taurocholate (0–14 mg per 100 g rat per hr), and the concentrations of biliary taurocholate, taurochenodeoxycholate, and cholesterol were determined at the end of 48 hr. Inhibition of endogenous bile salt production was observed when taurocholate reached the liver at a rate of approximately 7 mg per 100 g rat per hr. (TC, sodium taurocholate; TCD, sodium taurochenodeoxycholate).

† Total biliary taurocholate minus endogenous taurocholate.

‡ Control animals receiving no intraduodenal infusion of taurocholate. Concentration of biliary constituents measured 48 hr after biliary diversion.

§ Control animals. This value represents endogenous taurocholate.

‖ Range.

¶ Three rats infused with 12 mg, three with 13 mg, and one with 14 mg per 100 g rat per hr.

firm this point were not carried out. We noted, however, that both the concentration and the rate of infusion of the taurocholate solution influenced the efficiency of intestinal absorption. For example, when a solution of 68 mg/ml was infused into a 300 g rat at the rate of 0.62 ml/hr, 98% of the bile salt was absorbed.

The data in the last column of Table 3 indicate that the control rats and those infused with taurocholate showed little difference in biliary cholesterol concentrations.

A typical experiment demonstrating inhibition of bile salt production is illustrated in Fig. 2. The control rat (No. 60) showed the usual pattern of taurochenodeoxy-

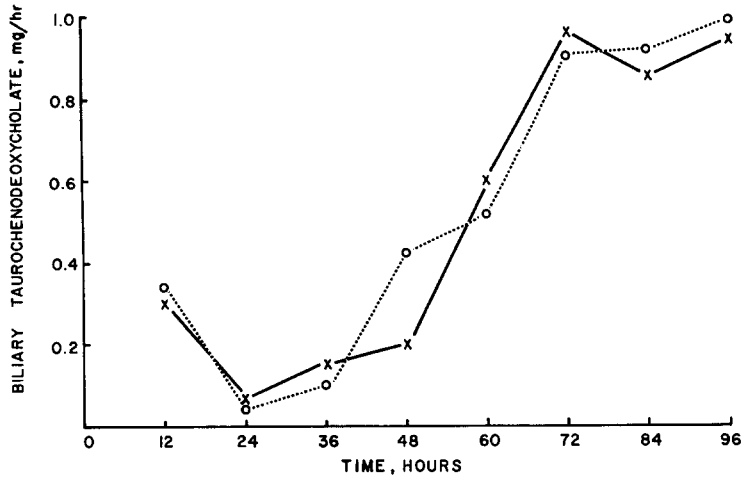


FIG. 1. Effect of intraduodenal infusion of sodium taurocholate (8.3 mg per 100 g rat per hr) on biliary secretion of sodium taurochenodeoxycholate. Infusion of sodium taurocholate begun 12 hr following establishment of bile fistula and continued for remainder of experiment.

×—×, Biliary taurochenodeoxycholate, mg/hr, rat No. 77, experimental, weight 264 g, taurocholate pool 16.1 mg per 100 g rat.

O---O, Biliary taurochenodeoxycholate, mg/hr, rat No. 75, control, weight 240 g, taurocholate pool 18.0 mg per 100 g rat.

This rate of taurocholate infusion produced no inhibition of taurochenodeoxycholate formation.

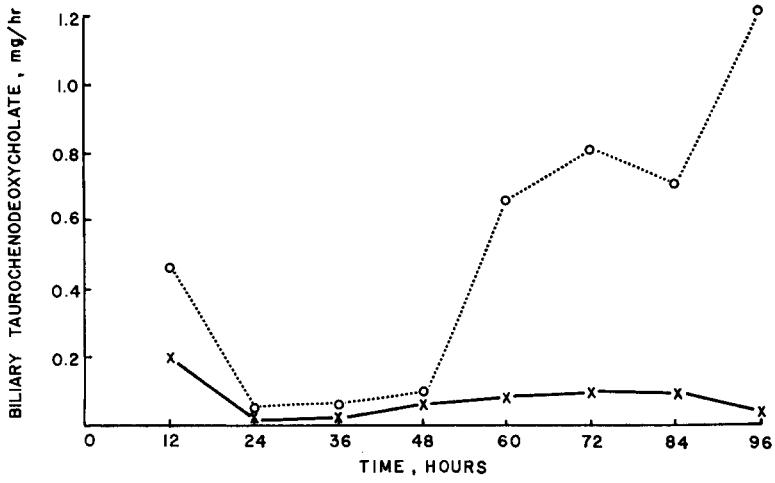


FIG. 2. Effect of intraduodenal infusion of sodium taurocholate (11.8 mg per 100 g rat per hr) on biliary secretion of sodium taurochenodeoxycholate. Infusion of sodium taurocholate begun 12 hr following establishment of bile fistula and continued for remainder of experiment.

×—×, Biliary taurochenodeoxycholate, mg/hr, rat No. 61, experimental, weight 255 g, taurocholate pool 15.0 mg per 100 g rat.

O---O, Biliary taurochenodeoxycholate, mg/hr, rat No. 60, control, weight 255 g, taurocholate pool 12.8 mg per 100 g rat.

This rate of taurocholate infusion inhibited the formation of taurochenodeoxycholate.

cholate production seen in bile fistula rats. After excretion of the pool, taurochenodeoxycholate secretion remained at a minimal value of less than 0.03 mg/hr for 24 hr, then rose to a maximum of 1.2 mg/hr at the end of 96 hr. Rat No. 61 which was infused with 11.8 mg of sodium taurocholate per 100 g rat per hr, starting 12 hr after surgery, excreted taurochenodeoxycholate at a rate never exceeding 0.08 mg/hr during the entire experiment (24–96 hr).

Fig. 3 illustrates the biliary excretion of taurocholate and taurochenodeoxycholate of the same experimental rat (No. 61) for which results were given in Fig. 2. Although taurocholate was infused at a rate of 30 mg/hr, biliary taurocholate excretion averaged only 17.7 mg/hr (33 μmoles/hr) indicating that approximately 59% of the infused bile salt actually reached the liver.

Fig. 4 illustrates an experiment in which a bile fistula animal (rat No. 63) was infused intravenously with

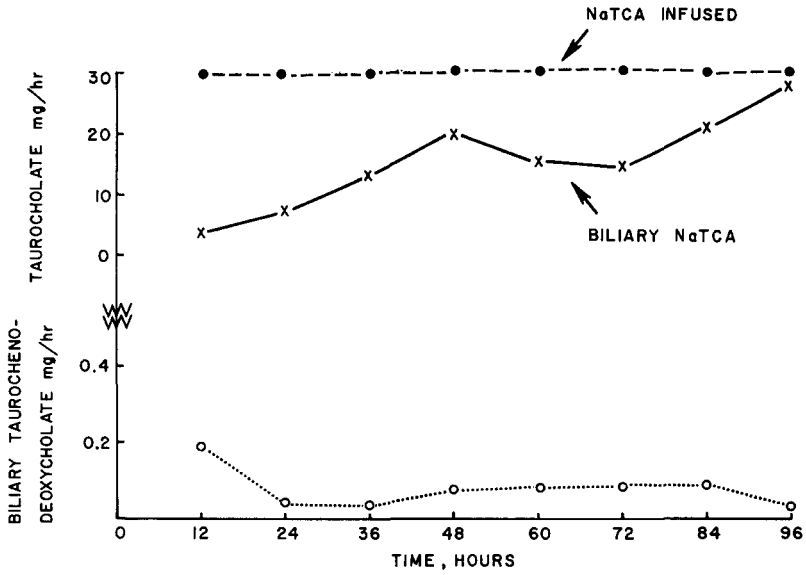


FIG. 3. Concentration of bile salts in bile of rat No. 61, receiving intraduodenal infusion of sodium taurocholate at a rate of 11.8 mg per 100 g rat per hr.
×—×, Biliary taurocholate, mg/hr.
O---O, Biliary taurochenodeoxycholate, mg/hr.
●---●, Taurocholate infused, mg/hr.

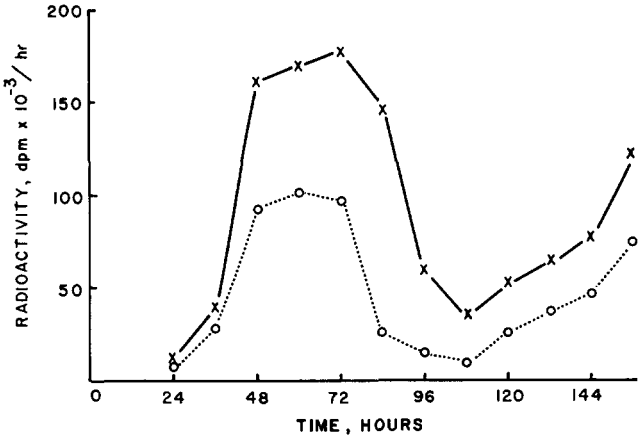


FIG. 4. Effect of taurocholate infusion on incorporation of acetate-1-$^{14}$C into biliary bile salts. Rat No. 63, weight 265 g, taurocholate pool 15.3 mg per 100 g rat. Intravenous infusion of acetate-1-$^{14}$C (5 μc [0.205 mg]/hr) begun 12 hr after biliary diversion.

NATCA = Intraduodenal infusion of sodium taurocholate (11.3 mg per 100 g rat per hr) for 36 hr, begun 72 hr after surgery.

Sodium taurocholate infusion produced approximately 80% inhibition in the incorporation of $^{14}$C into total bile salts (taurocholate, muricholates plus taurochenodeoxycholate) and 87% inhibition in the incorporation of $^{14}$C into taurocholate.

×—×, Radioactivity in total bile salts, dpm/hr.
O---O, Radioactivity in taurocholate, dpm/hr.

labeled acetate starting 12 hr after surgery. After 72 hr, when bile acid production had attained its maximal value and biliary excretion of $^{14}$C had reached a plateau of 170,000 dpm/hr, sodium taurocholate was infused intraduodenally at a rate of 11.3 mg per 100 g rat per hr (of which 8.4 mg per 100 g rat per hr, or 74%, actually passed through the liver, as determined by measurement of biliary taurocholate excretion). After 36 hr of taurocholate administration, incorporation of radioactivity from acetate-1-$^{14}$C into total bile salts fell approximately 80%, from 170,000 to 33,000 dpm/hr. This fall amounted to 87% (from 98,000 to 13,000 dpm/hr) for taurocholate and to 74% (from 77,000 to 20,000 dpm/hr) for taurochenodeoxycholate plus its metabolites, the tauromuricholates. When infusion of taurocholate was discontinued, radioactivity in total bile salts rose to 127,000 dpm/hr, and in taurocholate to 85,000 dpm/hr.

Fig. 5 summarizes the results of the same experiment in terms of the actual amounts of endogenous bile salt excreted per hour. Taurochenodeoxycholate was measured directly by GLC, while the quantities of taurocholate and of tauromuricholates were estimated from TLC and radioactivity data as described in the experimental section. This graph demonstrates that the infusion of taurocholate at a rate of 11.3 mg per 100 g rat per hr reduced production of taurocholate from a maximum of 1.74 to 0.006 mg/hr. Subtraction of this value from the output of total bile salt leads to the conclusion that the biliary excretion of taurochenodeoxycholate plus metabolites likewise decreased, from 1.3 to 0.19 mg/hr. This closely approximates the synthesis rates in inhibited rats, i.e., animals observed 12–24 hr after biliary diversion.

Fig. 6 illustrates a similar tracer experiment with intravenous infusion of acetate-1-$^{14}$C begun 12 hr after surgery, and taurocholate administration started 36 hr later. The animal (rat No. 73) chosen for this experiment

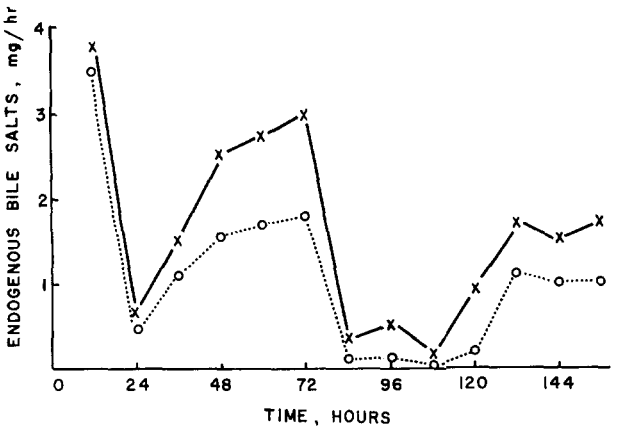


FIG. 5. Effect of taurocholate infusion in the quantity of endogenous bile salts excreted in bile. Rat No. 63 (for experimental details see Fig. 4).
×—×, Total endogenous bile salts, mg/hr.
O---O, Endogenous taurocholate, mg/hr.

was of a different strain (Charles River) and larger (345 g) than the rats used for most of the studies. Taurocholate was infused at a rate of 12 mg per 100 g rat per hr for a 48 hr period. Again, the inhibitory effect of the infused bile salt on endogenous bile acid synthesis is evident from the decrease in the radioactivity incorporated into total bile acids. This fell from a maximum of 115,000 dpm/hr to a minimum of 6,300 dpm/hr, a 98% decrease. This apparent difference between percentage inhibition obtained by radioactivity measurements and by a combination of radioactivity and GLC data can be ascribed to the fact that the concentration of biliary chenodeoxycholate in inhibited animals is so small that accurate quantification by GLC becomes difficult.

TABLE 4* CIRCULATION RATE OF BILE ACID POOL IN THE RAT†

| Exp. No. | A TC Pool | B Infused TC Reaching the Liver | N‡ No. of Enterohepatic Cycles per Day |
|---|---|---|---|
| | *mg/100 g rat* | *mg/100 g rat/hr* | |
| 60 | 12.8 | 6.95 | 13 |
| 61 | 14.9 | 6.96 | 11 |
| 63 | 15.3 | 8.4 | 13 |
| 73 | 17.3 | 8.0 | 11 |
| 74 | 15.1 | 6.9 | 11 |
| 75 | 18.0 | 7.5 | 10 |
| 80 | 12.6 | 6.8 | 13 |

* The abbreviation used in this table is as follows: TC, sodium taurocholate.

† The taurocholate pool of the bile fistula rats was determined by analysis of the bile collected during the 12 hr period immediately following biliary diversion. Endogenous bile acid synthesis was then inhibited by intraduodenal infusion of taurocholate at an average rate of 11.8 mg per 100 g rat per hr, and the amount of the infused taurocholate reaching the liver was then determined. The table was constructed with the assumption that the amount of taurocholate listed in column B is the minimal amount required to inhibit endogenous bile acid synthesis.

‡ N = (B/A) × 24; circulation rate of bile salt pool during 24 hr period estimated by dividing the quantity of taurocholate reaching the liver per hour (and causing inhibition of bile acid biosynthesis) by the magnitude of the taurocholate pool, multiplied by 24.

Table 4 gives estimated circulation times of the taurocholate pool in seven rats infused with taurocholate at rates sufficient to inhibit bile salt biosynthesis. Since the amount of taurocholate reaching the liver and the magnitude of the taurocholate pool were known, the circulation time of the pool could be calculated (Table 4). Unde-

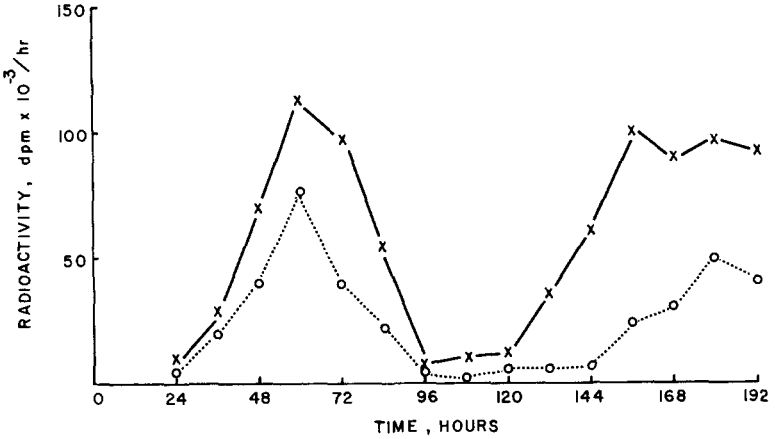


FIG. 6. Effect of taurocholate infusion on incorporation of acetate-1-$^{14}$C into biliary bile salts. Rat No. 73, Charles River strain, weight 345 g, taurocholate pool 17.3 mg per 100 g rat. Intravenous infusion of acetate-1-$^{14}$C (5 μc [0.205 mg]/hr) begun 12 hr following establishment of bile fistula.
NATCA = Intraduodenal infusion of sodium taurocholate, 12.0 mg per 100 g rat per hr for 48 hr starting 2 days after surgery. Sodium taurocholate administration produced a 91% decrease in the incorporation of acetate-1-$^{14}$C into total bile salts and a 94% decrease in the incorporation into taurocholate.
×—×, Radioactivity in total bile salts, dpm/hr.
O---O, Radioactivity in taurocholate, dpm/hr.

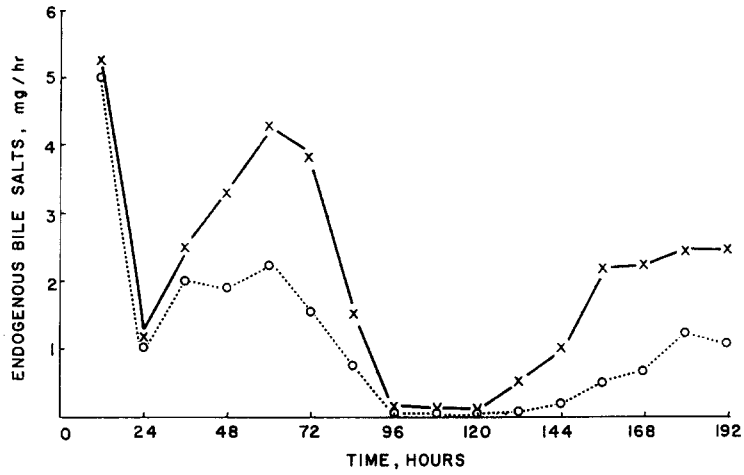


FIG. 7. Effect of taurocholate infusion on the quantity of endogenous bile salts excreted in bile. Rat No. 73 (for experimental details, see Fig. 6).
×—×, Total endogenous bile salts, mg/hr.
O---O, Endogenous taurocholate, mg/hr.

these experimental conditions the number of enterohepatic cycles ranged from 10 to 13 per day.

## DISCUSSION

These experiments prove that hepatic bile acid synthesis is regulated, at least in part, by the amount of taurocholate reaching the liver via the enterohepatic circulation. Sodium taurocholate, infused intraduodenally at a rate greater than 10 mg per 100 g rat per hr, inhibited the biosynthesis of the primary bile salts of the rat, namely taurocholate and taurochenodeoxycholate plus the tauromuricholates. Since the magnitude of the taurocholate pool of the rats was also determined (by measurement of the biliary taurocholate content for 6–12 hr after establishment of the fistula), the rate of circulation could be determined for the first time in a fairly direct manner. The pertinent data summarized in Table 4 indicate that under the conditions of the present study the taurocholate pool averaged 15 mg per 100 g rat. In order to simulate the enterohepatic circulation of the intact rat, approximately 7.5 mg of taurocholate per 100 g rat per hr had to reach the liver, i.e., this flux of taurocholate was required to reduce hepatic bile acid synthesis to that commonly observed in the normal animal. The rate of circulation of the taurocholate pool can then be estimated as $(7.5/15) \times 24 = 12$ times per day. This finding serves to explain, at least in part, why previous studies (7, 8; Wilson, J. D., W. H. Bentley, and C. T. Crowley. Personal communication.) failed to demonstrate a regulatory effect; estimates of the magnitude of the bile acid pool or of its rate of circulation may have been too low. Thus, Van Belle (14) cites estimates of pool size of 8 mg per 100 g rat (1, 22) and of 10–12 circulations per day (23, 24). This would correspond to a rate of taurocholate infusion of $(8/0.8) \times 12 = 120$ mg per 100 g rat per day, assuming that 80% of the infused bile salt actually reached the liver. The present experiments have shown, however, that at least 240 mg per 100 g rat per day must be administered to inhibit hepatic bile acid synthesis. These conclusions may apply only to the present study since the experimental conditions, particularly the nutritional state and diet of the rats, would be expected to play an important role in determining pool size and circulation time.

In the intact rat, the circulating bile acid pool contains not only the primary bile salts—taurocholate, taurochenodeoxycholate, and tauromuricholates—but also a complex mixture of secondary bile salts and unconjugated cholanoic acids. In the present experiments we examined solely the inhibitory action of taurocholate, but there is no reason to assume that other taurocholanoates would not exert similar effects. Therefore, it should be of interest to compare the action of taurocholate with that of other bile salts or of mixtures of bile salts, and to determine the influence of structural features of the steroid on feedback inhibition. In addition, a regulatory role of other biliary constituents, particularly of cholesterol, in hepatic bile acid synthesis has not been ruled out. The experiments of Myant and Eder (10) made it clear that increased bile acid production in bile fistula rats was associated with an increase in cholesterol biosynthesis. In retrospect, this was to be expected since cholesterol is the sole precursor of mammalian bile acids and since the cholesterol content of the tissues of bile fistula animals is not markedly depleted (10, 13). In this connection it is of interest that the concentration and radioactivity of biliary cholesterol did not vary during our experiments in

the same way as those of the bile salts. Apparently, synthesis of biliary cholesterol is controlled by a mechanism which is not directly related to the control of bile acid production.

We do not know which enzyme or enzymes participate in the feedback control of endogenous bile acid production. When the acetate pool of the experimental animals was labeled by intravenous infusion of acetate-1-$^{14}$C, the incorporation of $^{14}$C into biliary bile acids was reduced whenever inhibitory amounts of sodium taurocholate were administered. It has been suggested that bile acids inhibit cholesterol biosynthesis in liver and intestinal mucosa by inhibiting HMG-CoA reductase (25). If this postulate is accepted, it might be argued that bile acid production was inhibited in the present experiments because insufficient quantities of cholesterol were synthesized to serve as the precursor. Unpublished experiments from our laboratory indicate, however, that the regulation cannot be only at the HMG-CoA reduction step, since the incorporation of tracer amounts of mevalonate-2-$^{14}$C into bile acids was inhibited by sodium taurocholate in just the same way as that of acetate-1-$^{14}$C. Therefore, even if it is accepted that the activity of intestinal and hepatic HMG-CoA reductase is controlled by bile salt, an additional step of the biosynthetic pathway between mevalonate and bile salt must also be inhibited. Previous studies (26, 27) showed that biliary diversion or cholestyramine administration enhanced the 7$\alpha$-hydroxylation of cholesterol, the initial step in the conversion of cholesterol to bile acids. Four other steps on the pathway between 7$\alpha$-hydroxycholesterol and cholic acid were not greatly affected by biliary drainage (26). The in vitro studies (27) further indicated that the microsomal enzyme that catalyzes the 7$\alpha$-hydroxylation of cholesterol is inhibited by relatively large concentrations of sodium taurocholate. While this result was not considered conclusive, because of the possible nonspecific, disruptive effect of bile salt on microsomal membranes, a second regulatory site of bile acid biosynthesis might well be the 7$\alpha$-hydroxylation of cholesterol.

The authors are grateful to Dr. Norman B. Javitt for his advice on the surgical techniques and maintenance of the animals.

This work was supported in part by Research Grants AM-05222 and HE-10894 from the National Institute of Health.

*Manuscript received 2 April 1969; accepted 8 July 1969.*

## References

1. Eriksson, S. 1957. *Proc. Soc. Exp. Biol. Med.* **94:** 578.
2. Samuel, P., G. M. Saypol, E. Meilman, E. H. Mosbach, and M. Chafizadeh. 1968. *J. Clin. Invest.* **47:** 2070.
3. Tennent, D. M., S. A. Hashim, and T. B. Van Itallie. 1962. *Fed. Proc.* **21:** 77.
4. Beher, W. T., G. D. Baker, W. L. Anthony, and M. E. Beher. 1961. *Henry Ford Hosp. Med. Bull.* **9:** 201.
5. Grundy, S. M., A. F. Hofmann, J. Davignon, and E. H. Ahrens, Jr. 1966. *J. Clin. Invest.* **45:** 1018 (Abstr.)
6. Bergström, S., and H. Danielsson. 1958. *Acta Physiol. Scand.* **43:** 1.
7. Cronholm, T., and J. Sjövall. 1967. *Eur. J. Biochem.* **2:** 375.
8. Lee, M. J., D. V. Parke, and M. W. Whitehouse. 1965. *Proc. Soc. Exp. Biol. Med.* **120:** 6.
9. Norman, A., and J. Sjövall. 1958. *J. Biol. Chem.* **233:** 872.
10. Myant, N. B., and H. A. Eder. 1961. *J. Lipid Res.* **2:** 363.
11. Lindstedt, S., and A. Norman. 1957. *Acta Physiol. Scand.* **38:** 121.
12. Eriksson, S. 1960. *Acta Physiol. Scand.* **48:** 439.
13. Weiner, I. M., and L. Lack. 1967. *In* Handbook of Physiology.Alimentary Canal. C. F. Code, editor. American Physiological Society, Washington, D. C. 1439.
14. Van Belle, H. 1965. Cholesterol, Bile Acids and Atherosclerosis. Noord-Hollandsche Uitgevers-Mij., Amsterdam, The Netherlands. 98–99.
15. Hofmann, A. F. 1965. *Gastroenterology.* **48:** 484.
16. Evrard, E., and G. Janssen. 1968. *J. Lipid Res.* **9:** 226.
17. Hofmann, A. F., and E. H. Mosbach. 1964. *J. Biol. Chem.* **239:** 2813.
18. Hsia, S. L., J. T. Matschiner, T. A. Mahowald, W. H. Elliott, E. A. Doisy, Jr., S. A. Thayer, and E. A. Doisy. 1957. *J. Biol. Chem.* **226:** 667.
19. Kagan, H. B., and J. Jacques. 1960. *Bull. Soc. Chim. Fr.* **26:** 871.
20. Chattopadhyay, D. P., and E. H. Mosbach. 1965. *Anal. Biochem.* **10:** 435.
21. Voigt, W., P. J. Thomas, and S. L. Hsia. 1968. *J. Biol. Chem.* **243:** 3493.
22. Eriksson, S. 1960. *Acta Physiol. Scand.* **48:** 439.
23. Bergström, S., and H. Danielsson. 1958. *Acta Physiol. Scand.* **43:** 1.
24. Olivecrona, T., and J. Sjövall. 1959. *Acta Physiol. Scand.* **46:** 284.
25. Dietschy, J. M. 1968. *J. Clin. Invest.* **47:** 286.
26. Danielsson, H., K. Einarsson, and G. Johansson. 1967. *Eur. J. Biochem.* **2:** 44.
27. Shefer, S., S. Hauser, and E. H. Mosbach. 1968. *J. Lipid Res.* **9:** 328.