The economy of the enterohepatic circulation of bile acids in the baboon. 2. Regulation of bile acid synthesis by enterohepatic circulation of bile acids[1]

Richard N. Redinger

Departments of Medicine, University of Louisville School of Medicine, Louisville, KY and Boston University School of Medicine, Boston, MA

Abstract Isotope dilution within bile acid pools and radiochemical assessment of cholesterol oxidation to bile acids were methods used to measure short-term feedback regulation of bile acid synthesis in baboons with controlled enterohepatic circulations. Intraduodenal infusion of labeled endogenous bile acid pools into bile acid-depleted animals with enhanced bile acid synthesis showed that the rate of bile acid returned to the liver affected the degree of inhibition of bile acid synthesis. Infusion of prepared bile acid pools of varying composition resulted in a specific pattern of feedback inhibition of bile salt synthesis related to pool composition and mass. Individual bile salts inhibited their own synthesis more than that of other bile salts, and chenodeoxycholic and deoxycholic acids were found to have greater inhibitory effects than cholic acid. Glycine-conjugated cholic and chenodeoxycholic acids had greater inhibitory effects than did the respective free bile salts. Infusion of mixed bile acid pools showed that dihydroxy bile acids (chenodeoxycholic or deoxycholic) enhanced feedback inhibition of cholic acid. In all studies, inhibition of bile acid synthesis occurred twice as fast as its derepression.—**Redinger, R. N.** The economy of the enterohepatic circulation of bile acids in the baboon. 2. Regulation of bile acid synthesis by enterohepatic circulation of bile acids. *J. Lipid Res.* 1984. **25:** 437–447.

Supplementary key words feedback regulation • cholic acid • chenodeoxycholic acid • deoxycholic acid • glycine-conjugated bile acids

Bile salts returning to the liver from the intestine via the portal circulation have been shown to inhibit bile acid synthesis in both subprimate and primate species (2, 3). Feedback regulation of bile acid synthesis therefore provides a homeostatic mechanism whereby bile salt pool size can be maintained within narrow limits in spite of varying fecal losses (4, 5). Cholic (CA) and cheodeoxycholic (CDC) acids, the primary bile acids synthesized by the liver, have different turnover rates but synthesis of each bile acid remains constant during steady-state conditions (6). When feedback regulation is nonoperative as it is with total bile fistula, the ratio of CA to CDC changes (7). Therefore the profile of bile acids returning to the liver, including their composition and mass, may dictate the nature of bile salt inhibition. We have observed that derepression of bile acid synthesis takes 12 or more hours to become effective after pool drainage in the baboon (8). Feedback inhibition may be much shorter since we have previously shown in this species that cholesterol oxidation to bile acids was decreased within 6 hours of pool return to the liver (9).

The development of a primate model with exteriorized but functionally intact enterohepatic circulation (EHC) has been used to assess biliary lipid secretion, but because of recycling of bile salts in this system, bile acid synthesis has been difficult to assess in animals with intact EHC (4, 9), and thus required fecal bile acid analysis (9, 10). Isotope dilution of radiolabeled bile acid pools may be used to study acute changes in bile acid synthesis in animals with intact EHC and constant pool sizes (6). Cholesterol oxidation to bile acids may also be assessed by isotopic CO_2 breath analysis in animals prelabeled with [26-^{14}C]-cholesterol (9, 11) and we have shown this method to be sensitive in detecting early inhibition of bile acid synthesis in the non-steady-state baboon (9). We therefore used both radiochemical methods to evaluate early inhibition of bile salt synthesis in animals that had their EHC reinstituted with intraduodenal infusions of their own, i.e., endogenous, bile acid pools or selected, i.e., exogenous, bile acid pools.

We found that feedback inhibition of bile acid synthesis in the baboon required less than half the time needed for derepression (i.e., enhancement of synthesis with total interruption of EHC) and was directly proportional to rate of bile acid return. Furthermore, there was specificity of feedback inhibition of bile acid synthesis which was

Abbreviations: BA, bile acid; CA, cholic acid; CDC, chenodeoxycholic acid; DC, deoxycholic acid; SA, specific activity; EHC, enterohepatic circulation; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

[1] This work was presented at the National Meeting of the Canadian Society for Clinical Investigation, September 19, 1983, Calgary, Canada, and was published in abstract form (1).

dependent upon bile acid composition in the EHC. The dihydroxy bile acids, chenodeoxycholic and deoxycholic acids, when infused individually or with cholic acid, had a greater inhibitory effect on bile acid synthesis than did infusion of the trihydroxy cholic acid. Glycine-conjugated CA was also more effective than unconjugated CA in inhibiting bile salt synthesis.

MATERIALS AND METHODS

Animal model

Six healthy adult female baboons (*Papio anubis*) were cared for according to the Guides for the Care and Use of Laboratory Animals of the National Research Council, U.S. Department of Health, Education and Welfare (12), and the Canadian Council for Animal Care.[2] Animals were housed in ventilated rooms with 6:00 AM to 6:00 PM light cycles and fed approximately 300 g of Purina Monkey Chow (Ralston Purina, St. Louis, MO) at 6:00–8:00 AM which was largely consumed within 4 hr. Following initial quarantine, animals were trained to adapt to specially designed primate chairs for the duration of the experiments, which allowed sampling of biological specimens, daily grooming and cleaning, and weekly weighing. Cholecystectomy and surgical implantation of biliary and duodenal T-tubes were performed to exteriorize the EHC as previously described (13). Three weeks postoperative recovery was allowed. We had previously established that the baboon, like the rhesus monkey, recovered normal biliary lipid secretion and BA pool size within 2 to 3 weeks following surgery (9, 14).

Experimental design

Endogenous bile acid pool infusion studies (see **Fig. 1** for protocol). Once normal bile acid secretion, composition, and pool size were established, the bile acid pool was recovered by pool washout at the start of each experiment and mixed with 5–10 μCi of [2,4-^{3}H]-CA and [COOH-^{14}C]-CDC or just [COOH-^{14}C]-labeled CA or CDC. Animals were continued on 100% bile collection after pool washout for 18 hr to allow enhancement of BA synthesis. The labeled pool was then returned to the EHC via the duodenal T-tube at 600, 200, or 100 μmol/hr (i.e., over 2, 6, or 12 hr) to assess the effect of altering rate of BA pool return to liver on BA synthesis. Bile salt pool composition and CA and/or CDC specific activities in bile (dpm/mg) were then followed for 12 or more hours during and following infusion of the labeled pool. During this time the EHC was maintained as it had been in the pre-experimental control period. Bile acid pool size was determined by the washout technique (8, 15) before each experiment as well as 12 hr following the end of pool infusion.

Exogenous bile acid pool infusion studies (see **Fig. 2** for protocol outline). For evaluation of the effects of individual bile acids on bile acid synthesis, bile acid pools containing either 1 g of cholic or chenodeoxycholic acid, or their respective glycine conjugates, were made up to 50–100 ml with 0.15 M saline at appropriate pH and mixed with 6 μCi of [2,4-^{3}H]-CA and/or [COOH-^{14}C]-labeled CA or CDC. These pools were infused intraduodenally at rates of 200 μmol/hr into animals maintained on 100% bile interruption for 48 hr prior to infusion, during the period of infusion, and for 12 hr in the post-pool infusion period. One gram of deoxycholic acid was also similarly infused in other experiments. Bile composition of drained pool was measured at the start of EHC interruption. BA composition and individual primary BA specific activities (CA and/or CDC) were followed during and for 12 hr beyond pool infusion in these bile-diverted animals. Changes in BA synthesis during BA infusions of exogenous pools were also monitored by assessing cholesterol oxidation to bile acids by isotopic breath analysis 6 hr following the end of infusion. Since we had previously shown that this technique can detect diurnal variation of cholesterol oxidation (9), infusions were started at the same time, i.e., 8 AM, so that post-infusion $^{14}CO_2$ breath analysis was carried out at 8 PM for 200 μmol/hr pool infusions. In additional experiments, 500-mg and 2-g doses of cholic acid were infused at 600 μmol/hr beginning at 8 AM to note the effect of pool size mass on feedback inhibition of bile salt synthesis and $^{14}CO_2$ breath analysis carried out at 4 PM.

For evaluation of combined bile acid pools, experiments were carried out with 1 g of total bile acid infusion at 600 μmol/hr in similar bile acid-depleted animals, but pools were prepared to contain different BA combinations, i.e., 500 mg of CA + 500 mg of CDC or similar amounts of CA + DCA or DCA + CDC, respectively. In one set of experiments a total dose of 500 mg of CA + CDC (i.e., 250 mg of each) or 2 g (1 g of CA + 1 g of CDC) was administered. Bile acid specific activities and composition were followed as described for single pool infusion studies.

Experiments were also carried out with combined pools of 1 g of CA + 1 g of CDC infused at 200 μmol/hr in animals similarly prepared with 48 hr interrupted EHC but whose EHC were maintained intact during and following pool infusion.

[2] Canadian Council for Animal Care. Personal communication.

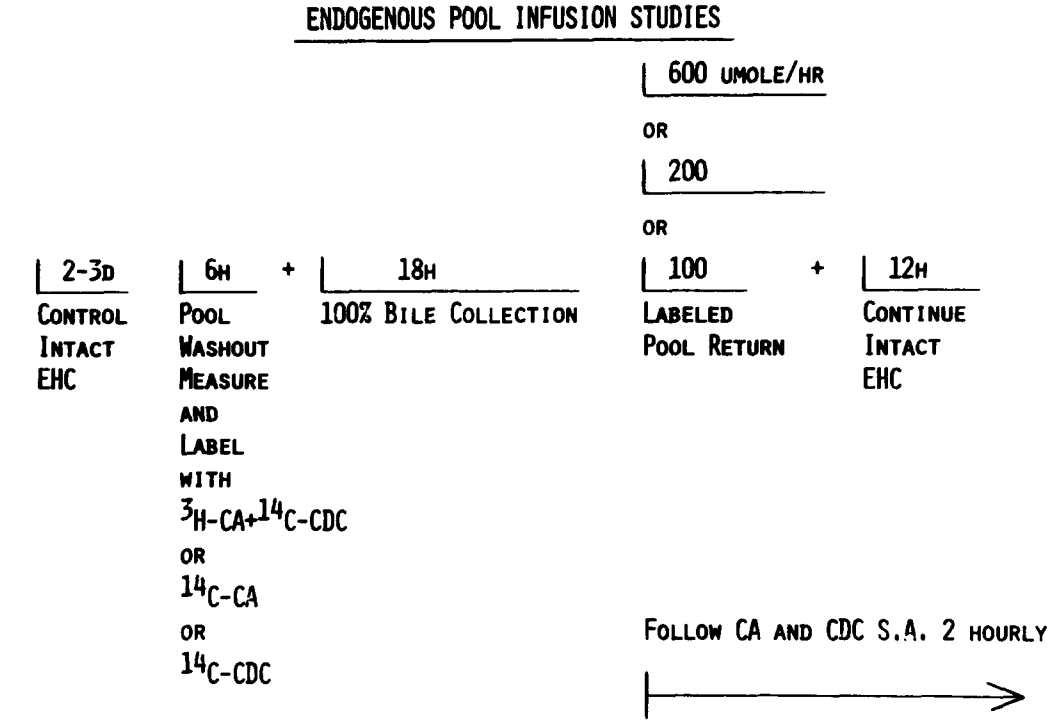

Fig. 1. Protocol for endogenous pool infusions.

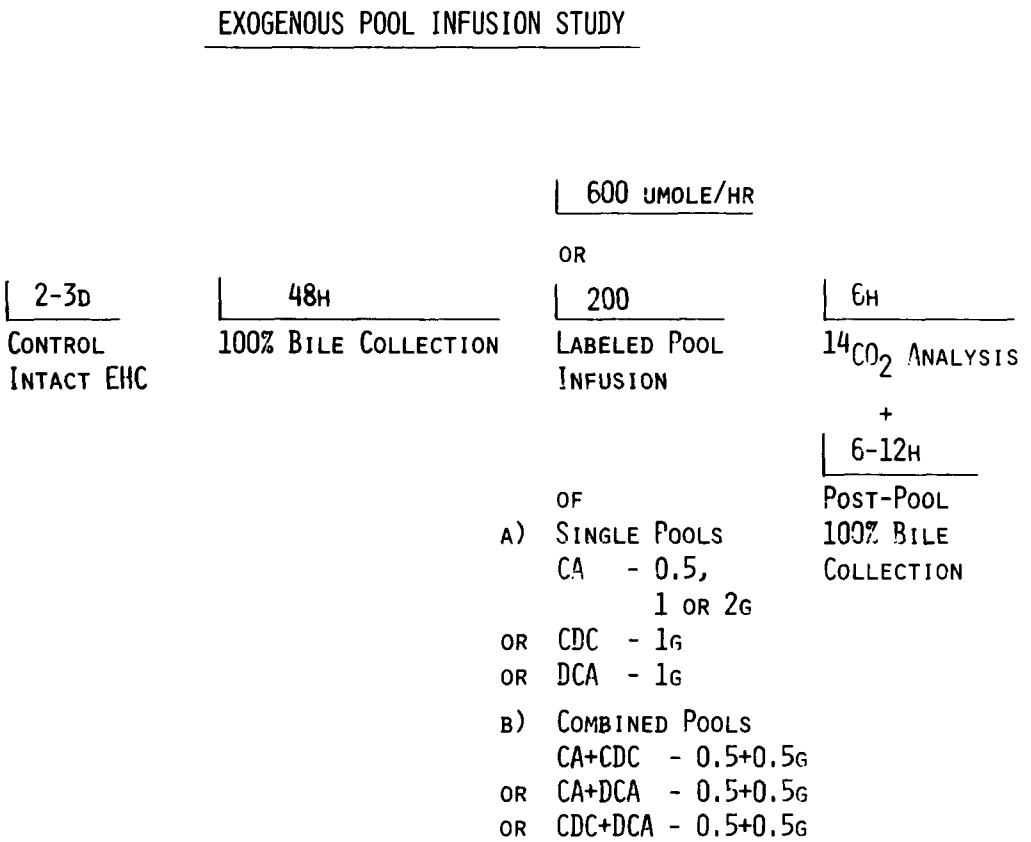

Fig. 2. Protocol for exogenous pool infusions.

Radiochemicals, reagents, and procedures

[2,4-^{3}H]CA (14 Ci/mmol), [COOH-^{14}C]-labeled CA (45 mCi/mmol) and [COOH-^{14}C]-labeled CDC (50 mCi/mmol) were purchased from New England Nuclear Corp. Radiochemical purity of 98% was determined by radiochromatography of the respective bile acid spots developed by preparative TLC. Isotopic assessment of extracted bile acids from bile was carried out in a Nuclear Chicago Isocap 300 counter with the external standard method used to correct for quenching as described previously (11). [26-^{14}C]Cholesterol was purchased from New England Nuclear and was 97% pure by preparative TLC and radioscan analysis. CA, CDC, and GCDC were purchased from Sigma Chemical Company, St. Louis, MO, and were found to be 97–98% pure by TLC. DCA and GCA, 96 and 98% pure by TLC, respectively, were purchased from Calbiochem-Behring Corp., San Diego, CA.

Plasma cholesterol was assessed by a modification of the method of Sperry and Webb (16). Total bile acid analyses were performed by the enzymatic method of Talalay (17), while individual bile acids in bile were isolated and quantitated by TLC and GLC as described previously by Vlahcevic et al. (18) and Redinger and Small (3).

The assessment of cholesterol oxidation to bile acids by $^{14}CO_2$ breath analysis involved simultaneous assessment of plasma cholesterol SA and exhaled $^{14}CO_2$. Animals were labeled intravenously with 15 μCi [26-^{14}C]cholesterol in Tween-20 several weeks before $^{14}CO_2$ breath analysis to allow equilibration of isotope within the metabolic cholesterol pool. During the metabolism of a molecule of cholesterol to a molecule of bile acid, the labeled carbon at position 26 is not retained following conversion to the C_{24} bile acid and is rapidly metabolized and exhaled as one molecule of $^{14}CO_2$ (19). Thus, the amount of newly synthesized BA can be calculated from the equation:

$$\text{mg}\ \frac{\text{cholesterol oxidized}}{\text{to BA per unit time}} = \frac{\text{dpm } ^{14}CO_2 \text{ exhaled per unit of time}}{\text{specific activity plasma cholesterol}}.$$

The experimental apparatus and method of collecting exhaled $^{14}CO_2$ in conscious, unanesthetized baboons have been described elsewhere (9). It had previously been shown that quantitative changes in bile acid synthesis were accurately reflected by the $^{14}CO_2$ method under steady-state conditions of cholesterol and bile acid turnover in baboons (9). This method was also sensitive in detecting inhibition of bile salt synthesis within 6 hr of cholic acid pool return, but underestimated enhanced synthesis consequent to total bile depletion. Since the [26-^{14}C]-label within plasma cholesterol is not transferred when oxidized to bile acids, there was no interference from this label with radiochemically labeled bile acid pools.

RESULTS

Endogenous pool infusion experiments

The attainment of constant levels of both CA and CDC specific activities (**Table 1**) for 4 or more consecutive hours following pool infusion in all experiments, regardless of rate of pool infusion, indicated complete mixing of the labeled pool in EHC after absorption and suggested inhibition of bile acid synthesis during these times. The higher level of CDC specific activity in all studies suggested that CDC synthesis was inhibited more than CA during and following pool infusion. The magnitude of the bile acid specific activity plateau and time taken to achieve it were related to rate of bile acid infusion. This relationship (**Fig. 3**) indicates that the degree of inhibition of bile acid synthesis was proportional to mass of total bile salts re-

TABLE 1. Bile acid specific activity with endogenous pool return[a]

	600 μmol/hr (n = 7)[b]		200 μmol/hr (n = 6)		100 μmol/hr (n = 4)	
Time	CA	CDC	CA	CDC	CA	CDC
hr			*dpm/mg*			
2	2056 ± 212[c]	4907 ± 2574	1187 ± 182	1123 ± 264	974 ± 127	1176 ± 313
4	4396 ± 1560	8384 ± 2196	5376 ± 1491	4665 ± 234	1948 ± 203	2454 ± 1381
6	11108 ± 1084	20020 ± 3071*	7915 ± 1452	10476 ± 2436	3876 ± 65	6141 ± 54
8	18598 ± 1977*[d]	21304 ± 3259*	9210 ± 3206	13050 ± 3619*	4243 ± 716	7448 ± 1530
10	18143 ± 1768*	21580 ± 2935*	12153 ± 8162*	13843 ± 1569*	5531 ± 642	6868 ± 1089
12	18276 ± 2157*	20793 ± 3073*	12126 ± 3437*	14103 ± 363*	6232 ± 510*	8579 ± 527*
18	NA	NA	11902 ± 2900*	12083 ± 4142*	6386 ± 970*	9561 ± 1280*
24	NA	NA	NA	NA	5189 ± 841	9224 ± 471*

[a] Both CA and CDC specific activities are shown for each rate of pool return.
[b] n, Number of experiments.
[c] Value ± SEM; NA, not available.
[d] Values with an asterisk indicate attainment of constant bile acid specific activities.

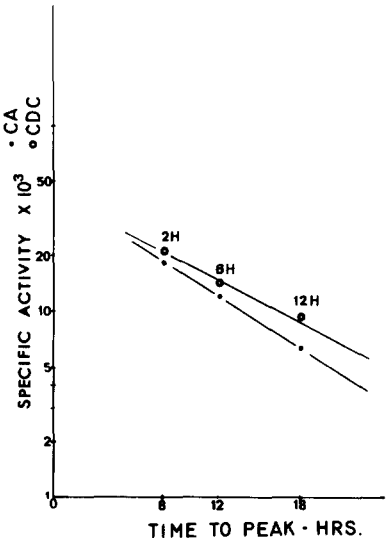

Fig. 3. The peak values of CA (●) and CDC (○) specific activities during 600 (2 hr), 200 (6 hr), and 100 μmol/hr (12 hr) endogenous pool infusions have been plotted against the time for the respective specific activity peaks to be achieved.

turned to the liver. Actual pool size changes in these experiments (**Table 2**) as measured by pool washout or as calculated from isotope dilution were less than half that anticipated had bile acid synthesis remained at preinfusion rates. These changes were also greater for CA than for CDC when size of pool changes was followed as a function of pool infusion rate, i.e., 35, 59, and 105% for CA vs 28, 38, and 75% for CDC ($P < 0.001$) for 600, 200, and 100 μmol/hr infusions, respectively. In all of these studies, cholic acid composition changed from 46.3 ± 1.3% with intact EHC to 56.8 ± 3.5% ($P < 0.05$) after institution of 100% bile collection, while that for CDC remained unchanged with this manipulation of EHC, i.e., 34.3 ± 2.1% vs 38.9 ± 2.2% (not significant). While it took 12 or more hours for this change to take place after pool drainage, bile acid composition returned to preinfusion levels within 6–8 hr following pool infusion.

Exogenous pool infusion studies with 100% bile collections

Single pool infusions. Bile salt secretion and cholesterol oxidation. Preinfusion secretion rates represented bile acid synthesis inasmuch as animals were on 100% bile collections for 48 hr, and synthesis rates were enhanced threefold over that observed with intact EHC (i.e., low point of BA secretion following pool drainage). Since labeled bile acids were still recovered 6 hr postinfusion, incomplete recovery of the pool prohibited equating secretion with synthesis at this time. However, since total secretion rates 6 hr post-infusion were less than preinfusion rates and included both newly synthesized bile acids plus bile acids that had been infused, bile acid synthesis at 6 hr postinfusion had to be decreased consequent to 200 μmol/hr pool infusion. This conclusion was verified by finding similarly decreased cholesterol oxidation to bile acids by $^{14}CO_2$ breath analysis at 6 hr postinfusion compared to control $^{14}CO_2$ breath analysis preinfusion levels (**Table 3A**).

Bile acid synthesis (i.e., secretion) was still inhibited at least twofold 12 hr after the completion of all bile acid pool infusions at a time when labeled bile salts were no longer excreted.

Both unconjugated dihydroxy bile acid infusions (i.e., CDC and DCA) consistently resulted in greater bile acid inhibition than did CA as determined by either method of assessing bile acid synthesis (i.e., BA secretion or cholesterol oxidation). Glycine-conjugated CA infused at 200 μmol/hr also inhibited bile acid synthesis more than did infusion of its unconjugated counterpart when measured 12 hr post-pool infusion. A similar trend was noted in three animals after infusion of glycine-conjugated CDC compared to infusion of unconjugated CDC.

TABLE 2. Bile acid pool size change (μmol) during endogenous pool infusion

Infusion Rate	No.[a]	Anticipated Change[b]		Pool Size by Washout Technique						Change Calculated from Isotope Dilution	
				Infused		Final		Δ[c]			
		CA	CDC	CA	CDC	CA	CDC	CA	CDC	CA	CDC
μmol/hr						μmol					
600	7	762	301	703 ± 53[d]	451 ± 102	946 ± 118	578 ± 78	243	127	209	66
200	6	895	430	659 ± 75	542 ± 62	1046 ± 107	747 ± 77	387	205	305	215
100	4	1121	753	546 ± 28	429 ± 22	1119 ± 107	751 ± 72	573	322	410	283

[a] Number of experiments.
[b] Anticipated change in bile acid synthesis was calculated using preinfusion bile acid synthesis rate in animals at 100% bile collections.
[c] Represents measured difference between infused and final (end of experiment) pool washout.
[d] Value ± SEM.

TABLE 3. Effect of exogenous pool infusion on feedback inhibition of bile acid synthesis

Bile Acids	No.[a]	Bile Salt Secretion Rate			Cholesterol Oxidation to Bile Salts	
		Control	Post 6 hr	Post 12 hr	Control	Post 6 hr
				μmol/hr		
A. Single pool infusion						
CA	8	282 ± 30[b]	194 ± 30	126 ± 20	235 ± 37	198 ± 34[c]
GCA	3		180 ± 26	88 ± 10[d]		NA
CDC	5		132 ± 43[d]	98 ± 22		175 ± 31
GCDC	3		147 ± 32	80 ± 20[d]		NA
DCA	5		76 ± 7[e]	103 ± 14[d]		145 ± 17[d]
B. Combined pool infusion						
CA + CDC	7	282 ± 30	118 ± 43[f]	97 ± 33[g]	235 ± 37	142 ± 33[h]
CA + DCA	5		101 ± 23[g]	125 ± 15[f]		166 ± 31[h]
CDC + DCA	6		145 ± 20[h]	116 ± 7[g]		138 ± 23[f]

[a] Number of experiments.
[b] Mean ± SEM; NA, not available.
[c] Not significant compared to control value. All other values in A are significantly different from respective control value.
[d] $P < 0.05$ compared to respective CA value.
[e] $P < 0.01$ compared to respective CA value.
[f] $P < 0.01$ compared to respective control value.
[g] $P < 0.001$ compared to respective control value.
[h] $P < 0.05$ compared to respective control value.

Bile acid composition. Infusion of unconjugated CA or CDC resulted in a bile acid pool comprising more than 80% of the respective infused bile acid (**Fig. 4**). CA content fell to below control values while CDC content remained unchanged, reflecting greater inhibition of CA than CDC synthesis following CA infusion. Following CDC infusion, CDC content fell while that of CA rose in the early postinfusion period indicating relatively greater inhibition of CDC synthesis. This suggestion was supported by a twofold greater relative change in CA vs CDC specific activity. The specific activity of CDC changed 15% (i.e., from $17.6 \pm 3 \times 10^3$ dpm/mg at the end of pool infusion to $15.0 \pm 2 \times 10^3$ at 6 hr postinfusion) while that of CA changed 32% (i.e., from $14.7 \pm 3 \times 10^3$ to $10.0 \pm 3 \times 10^3$ dpm/mg) during this same time. However, the relatively constant BA content and specific activity decay between 6 and 12 hr following CDC infusion suggested almost similar inhibition of these bile acids later in the postinfusion study period. DCA infusion resulted in a pool containing 66% DCA and an apparent greater inhibition of CDC compared to CA, since CDC content changed less than did CA throughout the postinfusion study period.

Analysis of bile acid composition 12 hr following infusion of glycine-conjugated bile acid indicated that glycocholate had depressed BA synthesis more than had CA, since the proportion of CA in bile was less after glycocholate than after CA infusion (see **Fig. 5A**). Similar but less marked observations were made with glycochenodeoxycholate compared to CDC infusion (see Fig. 5B).

Combined pool infusions. All combinations of bile acids infused at equal doses decreased bile acid synthesis as determined from observations of postinfusion secretion rates or cholesterol oxidation to bile salts. CA + CDC pool infusion was more effective in inhibiting synthesis than an equivalent mass of CA (Table 3A and 3B), i.e., 40 vs 16% ($P < 0.01$), respectively, as determined by oxidation to BA at 6 hr. The dihydroxy combination (i.e., CDC + DCA) also appeared as effective as CA + CDC in inhibiting bile acid synthesis.

Bile composition in these experiments showed apparent equal CA and CDC bile salt inhibition during combined CA + CDC pool infusions inasmuch as BA composition remained relatively constant following pool infusion (see **Fig. 6**). An apparent greater inhibition of CDC over CA synthesis occurred during CA + DCA infusion since CDC composition remained depressed while CA increased in the postinfusion period. Although CA content was depressed more than CDC during CDC + DCA infusion, equal inhibition of synthesis was likely in the postinfusion period since BA composition remained constant at this time.

Studies of bile salt pool mass. There was a definite relation between degree of inhibition of bile acid synthesis as determined by $^{14}CO_2$ analysis and mass of bile acid returned to liver for either selective or combined bile acid infusions

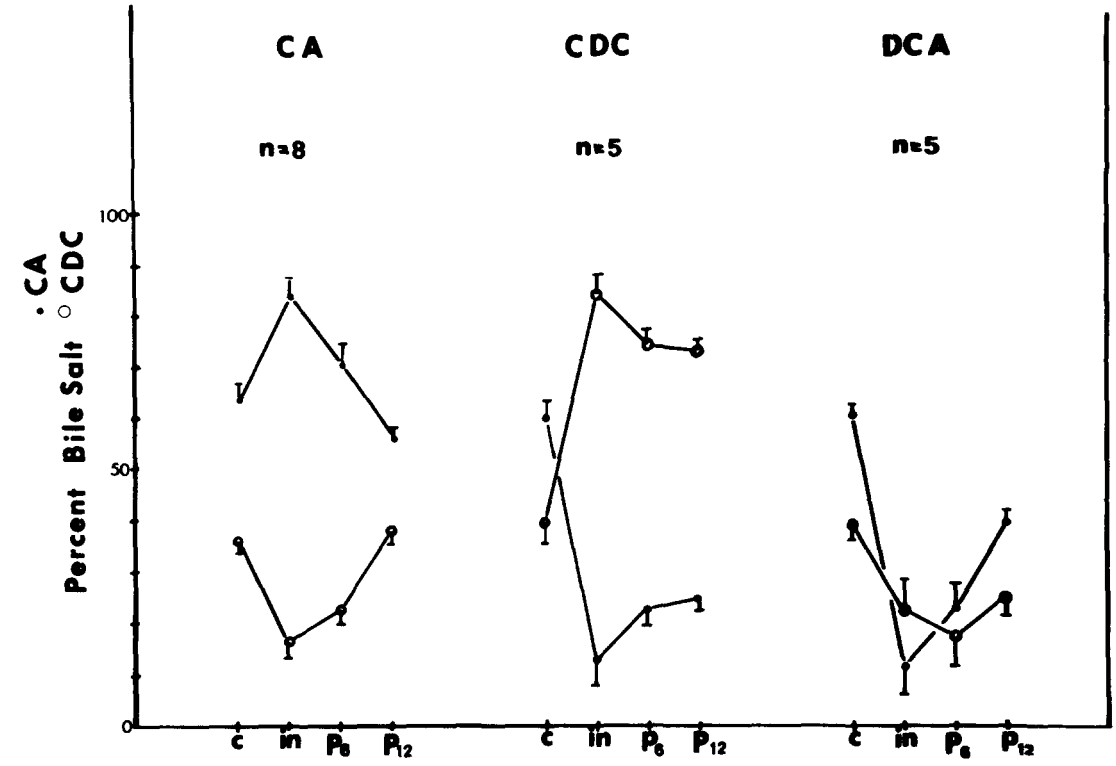

Fig. 4. Percent CA (●) and CDC (○) bile salts in bile are plotted for control (C); end of infusion (in); post 6 hr (P_6); and post 12 hr (P_{12}) infusion for three different bile acid pool infusions. n, Number of experiments; vertical extensions indicate one SEM.

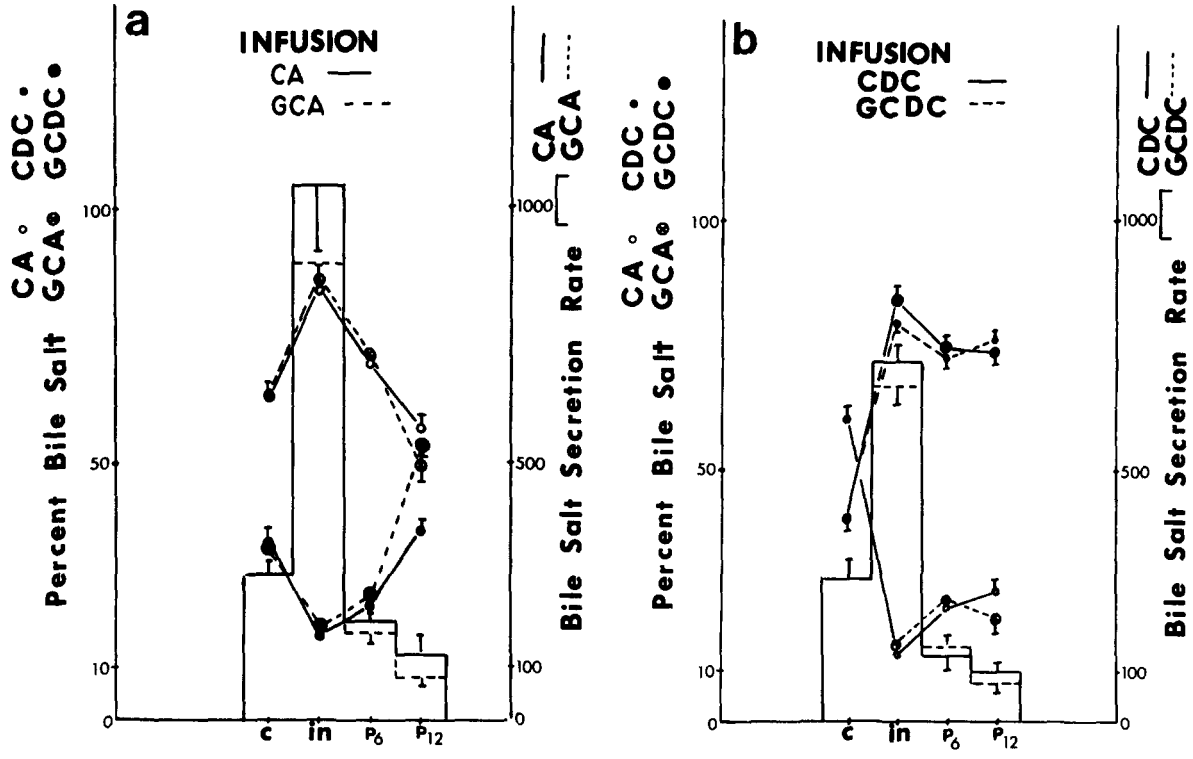

Fig. 5. Bile composition during glycine-conjugated bile acid infusions (Fig. 5a, GCA; Fig. 5b, GCDC) is joined by dashed lines, while that for unconjugated bile acids (CA or CDC) is joined by the solid lines. Bile salt secretion rate for CA is shown by a solid bar and GCA by dashed bars. Bile acid composition symbols are: free CA (○); conjugated CA (⊗); free CDC (●); and conjugated CDC (◉).

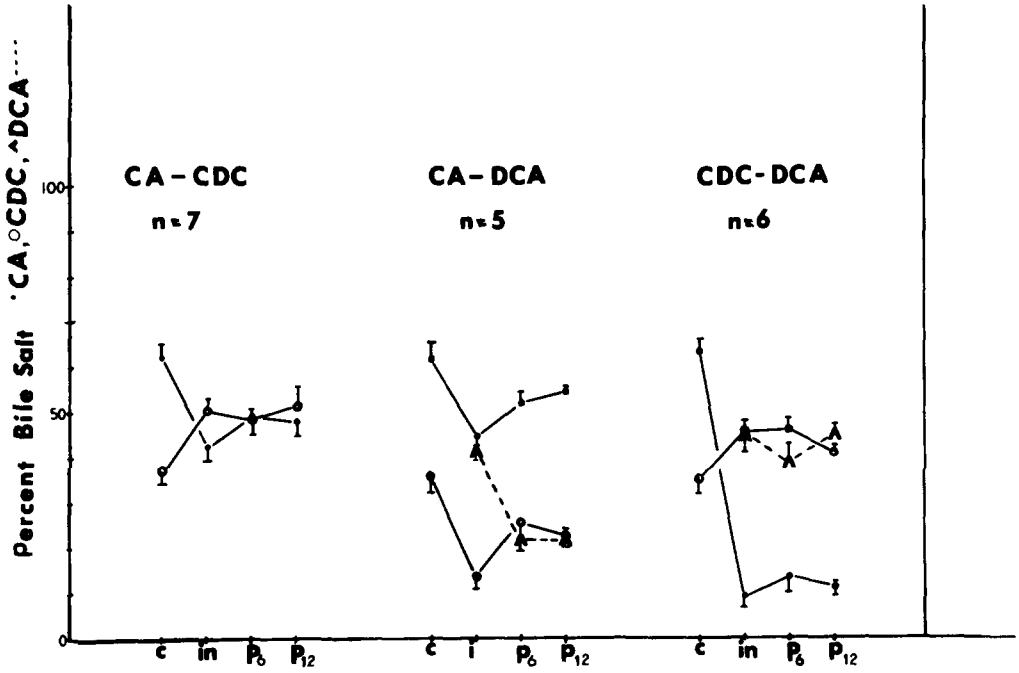

Fig. 6. Bile acid composition (i.e., relative percent) is shown for combined pool experiments using the same protocol outline as in Fig. 4. CA content is illustrated by closed circles and CDC by open circles. Vertical extensions represent one SEM. DCA content (▲) is shown joined by dashed lines during and following DCA infusions.

(see **Table 4**). While 500 mg of CA infusion had no inhibitory effect on bile acid synthesis, 1 g of CA infusion resulted in 21% inhibition which was enhanced 33% when the CA pool was increased to 2 g.

Moreover, there was potentiation of inhibition of cholesterol oxidation to bile acids when CDC was combined with CA, i.e., 250 mg CA + 250 mg CDC > 500 mg CA, $P < 0.05$.

Exogenous pool infusion studies with intact EHC (Table 5)

The infused pools in these experiments were made up of equal amounts (i.e., 1 g) of CA + CDC. CA and CDC accounted for 54.7% and 39.8% of bile acids in bile secreted at the beginning of pool infusion when synthesis rates were 80.4 and 58.5 μmol/hr for CA and CDC, respectively. Over the 18 hr of experimental observation, synthesis of 1447 μmol of CA and 1053 μmol of CDC would have been anticipated if no inhibition had occurred in these experiments. Pool expansion as determined by the isotope dilution technique showed that the CA pool increased by 870 μmol while that of CDC increased by 780 μmol. The higher specific activity of CDC that followed pool infusion also suggested that CDC synthesis had been inhibited more than that of CA and this effect persisted for 12 hr beyond the end of pool infusion.

TABLE 4. Exogenous pool studies—effect of dose on bile acid synthesis

Infusions[a]	Control[b]	Post 6 hr[b]	Difference[c]
	μmol/hr		
Single pool infusions			
CA 500 mg	245 ± 25	335 ± 36	+36%, $P < 0.01$
CA 1 g	267 ± 65	212 ± 58	−21%
CA 2 g	278 ± 28	127 ± 13	−54%, $P < 0.01$
Combined pool infusions			
CA + CDC 250 + 250 mg	264 ± 32	160 ± 15	−39%, $P < 0.01$
CA + CDC 500 + 500 mg	207 ± 29	122 ± 40	−41%, $P < 0.01$

[a] Infusions were for 2 hr at 600 μmol/hr.
[b] Value ± 1 SEM.
[c] Percent difference, and statistical significance compared to control (C).

TABLE 5. Bile acid specific activity during exogenous pool return in animals with intact EHC[a]

Bile Acid	Time (hr)						
	2	4	6	8	10	18	24
CA	1037 ± 450[b]	6629 ± 2133	8480 ± 2281	14016 ± 7265*	10040 ± 4436*	13731 ± 3775*	12453 ± 666*
CDC	1702 ± 744	13052 ± 3544	15915 ± 3718	21445 ± 10*	22806 ± 8323*	26852 ± 8059*	18504 ± 1801*

Values with an asterisk indicate attainment of constant bile acid specific activities.
[a] Both CA and CDC specific activities are shown for each rate of pool return.
[b] Value ± 1 SEM (mean of three experiments).

DISCUSSION

The inhibitory potential of individual bile salts for regulation of feedback control of bile acid synthesis is presently incompletely understood. Feedback inhibition of bile acid synthesis has been assessed by a number of methods including measurement of the activity of cholesterol 7α-hydroxylase, the rate-limiting enzyme involved in bile acid synthesis (2); bile salt secretion in bile fistula animals infused with bile salts by intravenous (20) or intestinal routes (21); acidic sterol balance (3) and radiochemical assessment of either bile salt turnover (22) or cholesterol oxidation to bile salts (9). The number of species studied have included rats (2), hamsters (23), rhesus monkeys (3), baboons (9), and man (24, 25). Bergstrom and Danielsson (21) first showed a progressive suppression of cholic acid synthesis by measuring cholic acid secretion in bile of bile fistula rats after intraduodenal infusion of taurochenodeoxycholic acid. Shefer et al. (2) later found that 7α-hydroxylase activity was inhibited when this same species was fed taurine-conjugated cholic acid but not taurine-conjugated dihydroxy bile acids. Schoenfield, Bonorris, and Ganz (23), on the other hand, found that both unconjugated primary bile acids inhibited 7α-hydroxylase activity when fed to hamsters. Redinger and Small (3) showed in the rhesus monkey model with exteriorized EHC that feedback inhibition of bile acid synthesis began when 6–7 mmol of bile salts per 24 hr was returned to the liver and became complete when 11–12 mmol/24 hr had been returned. Feedback inhibition of synthesis by intraduodenal cholic acid infusion was also demonstrated by $^{14}CO_2$ breath analysis by us (9) after labeling baboons with [26-^{14}C]cholesterol. Carulli et al. (26), found that short-term (7 day) feeding of CDC had no effect on 7α-hydroxylase, but Coyne et al. (27) showed a 50% decrease in enzyme activity after long-term feeding in man. Recently, Pries et al. (20) showed that taurine-conjugated cholic acid was more effective than unconjugated cholic acid in inhibiting bile acid synthesis as measured by bile salt output in the rat bile fistula preparation. Species and methodologic differences in bile acid specificity have obviously contributed to some of these differences reported for inhibition of bile acid synthesis.

We therefore sought to quantitate the differences in inhibitory potential of various bile salts in the baboon since this primate has a bile acid profile which is similar to that of man, and forms cholesterol gallstones (14) after chronic bile salt pool depletion as do patients with ileal disease (28). All methods we employed indicated that there was feedback inhibition of BA synthesis and those that produced quantitative data (i.e., bile salt mass output and cholesterol oxidation measurements) gave comparable results. During derepression, CA synthesis remained higher than did that of CDC suggesting independent feedback regulation for these two bile acids. This speculation was further supported when we established that repression of bile acid synthesis was greater for CDC than CA. We also found that feedback inhibition took 4–6 hr, which was less than half the time taken for derepression of bile acid synthesis (8). Changes in bile acid profile were not noted for up to 18 hr after interruption, but were clearly noted as early as 6 hr with feedback inhibition of BA synthesis. Furthermore, we found that the degree of inhibition was related to the rate of bile acid mass infused into our animals.

As in the Coyne et al. studies in man (27) and Bergstrom and Danielsson's studies in rats (21), we found that CDC had a greater inhibitory effect than CA on total bile acid synthesis. While both primary bile acids appeared to optimally inhibit their own synthesis, we also found that CDC was both capable of inhibiting CA synthesis and of potentiating total inhibition of bile acid synthesis when combined with CA. Pries and co-workers (20) found that, after intravenous bile acid administration, bile acid inhibition showed a progressively linear response to increases in bile acid infusions. In our studies an infusion rate as low as 3.3 to 5 μmol of CA/hr per 100 g body weight resulted in inhibition of total bile acid synthesis. However, when CDC was combined with CA, a rate as low as 1.7 μmol/hr per 100 g body weight was effective in inhibiting synthesis. The graded response of feedback inhibition of BA synthesis to increasing mass of bile acid

infusions in baboons was similar to that found by Pries et al. in rats (20), even though the route of bile acid administration was different (i.e., intravenous vs intraduodenal). We also showed in baboons, as Pries et al. showed in rats (20), that conjugated bile acids were more effective in inhibiting BA synthesis than their unconjugated counterparts. We studied glycine-conjugated bile salts because they were the predominant conjugated forms in baboons, while Pries et al. (20) infused the predominant conjugate (taurine) found in rats. We do not have data relating to taurine-conjugated bile acid infusions in the baboon, but were convinced that our animals did not become taurine-depleted during 100% bile diversion as judged by TLC of conjugated bile acids in bile. This was so, no doubt, because animals were kept at 100% bile collections for no more than 3 to 4 days before their previously collected endogenous bile acid pools were returned.

The reason that both dihydroxy bile acid infusions were more effective than CA in inhibiting bile acid synthesis may have been related to: *1*) the site and rate at which these bile acids were absorbed from the intestine; *2*) their rate of hepatic uptake; *3*) intrahepatic transport; and/or *4*) stereospecific effect on cholesterol 7α-hydroxylase activity. Both glycine-conjugated and unconjugated dihydroxy bile acids are absorbed by passive non-ionic diffusion along the entire length of the whole small intestine as opposed to primarily active ileal transport for trihydroxy cholic acid (29). Therefore, since more short-circuiting of EHC should occur (30) after CDC or DCA infusion compared to that of CA, a faster return to liver of CDC and DCA could result in more rapid feedback inhibition of synthesis by these dihydroxy bile acids. However, we also showed that glycine-conjugated CA was also more effective in inhibiting bile acid synthesis than unconjugated CA which is absorbed at a similar site. Trihydroxy bile acids are also reported to be cleared more efficiently by the rat liver than either dihydroxy bile acids (31) and have a maximal transport capacity two times that of dihydroxy bile acids which is independent of conjugation (32). It therefore appears likely that a great deal of the differences in inhibitory action by individual bile acids on bile synthesis was related to molecular specificity of these BA on cholesterol 7α-hydroxylase activity. However, the exact nature of this intracellular regulatory action needs to be further investigated by differently designed experiments.

In view of our findings, it is likely that both intestinal and hepatic mechanism are involved in the inhibitory specificity of bile acid feedback control (33). These actions are coordinated to allow greater feedback regulation of CDC synthesis and explain the higher content of CA in baboon bile. Such mechanisms may also be operating in man and a greater appreciation of their effects could be beneficial in offering optional strategies for bile acid therapy of gallstone dissolution.

This study was supported by Grant AM21986 from the National Institutes of Health and the Medical School Research Grant, University of Louisville.

Manuscript received 29 September 1981.

REFERENCES

1. Redinger, R. N. 1983. Effects of bile acid structure on bile acid synthesis in the baboon. *Clin. Invest. Med.* **6:** 51 (Abstract).
2. Shefer, S., S. Hanser, I. Berkersky, and E. H. Mosbach. 1970. Biochemical site of regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **11:** 404–411.
3. Redinger, R. N., and D. M. Small. 1973. Primate biliary physiology. VIII. The effect of phenobarbital upon bile salt synthesis and pool size, biliary lipid secretion, and bile composition. *J. Clin. Invest.* **52:** 161–172.
4. Small, D. M., R. H. Dowling, and R. N. Redinger. 1972. The enterohepatic circulation of bile salts. *Arch. Intern. Med.* **30:** 552–573.
5. Redinger, R. N., and D. M. Small. 1972. Bile composition, bile salt metabolism, and gallstones. *Arch. Intern. Med.* **30:** 618–630.
6. Hofmann, A. F. 1976. The enterohepatic circulation of bile acids in man. *Adv. Intern. Med.* **21:** 501–534.
7. Dowling, R. H. 1972. The enterohepatic circulation. *Prog. Gastroenterol.* **62:** 122–140.
8. Redinger, R. N., J. Hawkins, and D. M. Grace. 1984. The economy of the enterohepatic circulation of bile acids in the baboons. 1. Studies of controlled enterohepatic circulation of bile acids. *J. Lipid Res.* **25:** 428–436.
9. Redinger, R. N., L. Chow, and D. M. Grace. 1978. Cholesterol oxidation in primates by simultaneous sterol balance and breath analysis. *Am. J. Physiol.* **235:** R55–R63.
10. Redinger, R. N., A. H. Hermann, and D. M. Small. 1973. Primate biliary physiology. X. Effects of diet and fasting on biliary lipid secretion and relative composition and bile salt metabolism in the rhesus monkey. *Gastroenterology.* **64:** 610–621.
11. Wolfe, B. M., R. N. Redinger, E. B. Marliss, and D. M. Grace. 1983. Effects of dietary substitution of mixed amino acids for glucose on the splanchnic metabolism of plasma triglycerides, cholesterol, carbohydrates, and amino acids in conscious fed baboons. *Metabolism.* **32:** 403–412.
12. Guide for the Care and Use of Laboratory Animals. DHEW Publication No. 73-23, NIH.
13. Dowling, R. H., E. Mack, J. Picott, et al. 1968. Experimental model for the study of the enterohepatic circulation of bile in rhesus monkeys. *J. Lab. Clin. Med.* **72:** 169–176.
14. Redinger, R. N., and D. M. Grace. 1978. Cholesterol gallstones and biliary lipid metabolism in the primate. *Gastroenterology.* **74:** 201–204.
15. Dowling, R. H., E. Mack, and D. M. Small. 1970. Effects of controlled interruption of the enterohepatic circulation of bile salts by biliary diversion and by ileal resection on bile salt secretion, synthesis, and pool size in the rhesus monkey. *J. Clin. Invest.* **49:** 232–242.
16. Sperry, W. M., and M. Webb. 1950. A revision of the

Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187:** 97–106.
17. Talalay, P. 1960. Enzymatic analysis of steroid hormones. *Methods Biochem. Anal.* **8:** 119–143.
18. Vlahcevic, Z. R., C. C. Bell, Jr., and I. Buhac, et al. 1970. Diminished bile acid pool size in patients with gallstones. *Gastroenterology.* **59:** 165–173.
19. Davis, R. A., J. P. Showalter, and F. Kern, Jr. 1975. Measurement of bile acid synthesis by $^{14}CO_2$; the metabolism of propionyl CoA. *Steroids.* **26:** 408–421.
20. Pries, J. M., A. Gustafson, D. Wiegand, and W. C. Duane. 1983. Taurocholate is more potent than cholate in supression of bile salt synthesis in the rat. *J. Lipid Res.* **24:** 141–146.
21. Bergstrom, S., and H. Danielsson. 1958. On the regulation of bile acid formation in the rat liver. *Acta Physiol. Scand.* **43:** 1–7.
22. Einarsson, K., K. Hellstrom, and M. Kallner. 1973. Feedback regulation of bile acid formation in man. *Metabolism.* **22:** 1477–1483.
23. Schoenfield, L. J., G. G. Bonorris, and P. Ganz. 1973. Induced alterations in the rate-limiting enzymes of hepatic cholesterol and bile acid synthesis in the hamster. *J. Lab. Clin. Med.* **82:** 858–868.
24. Danzinger, R. G., A. F. Hofmann, J. L. Thistle, and L. J. Schoenfield. 1973. Effect of oral chenodeoxycholic acid on bile acid kinetics and biliary lipid composition in women with cholelithiasis. *J. Clin. Invest.* **52:** 2809–2821.
25. La Russo, N. F., N. E. Hoffman, A. F. Hofmann, T. C. Northfield, and J. L. Thistle. 1975. Effect of primary bile acid ingestion on bile acid metabolism and biliary lipid secretion in gallstone patients. *Gastroenterology.* **69:** 1301–1314.
26. Carulli, N., M. Ponz De Leon, F. Zirconi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria. 1980. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short-term feeding of chenodeoxycholic and ursodeoxycholic acid. *J. Lipid Res.* **21:** 35–43.
27. Coyne, M. J., G. G. Bonorris, L. I. Goldstein, and L. J. Schoenfield. 1976. Effect of chenodeoxycholic acid and phenobarbital on the rate-limiting enzymes of hepatic cholesterol and bile acid synthesis in patients with gallstones. *J. Lab. Clin. Med.* **87:** 281–291.
28. Heaton, K. W., and A. E. Read. 1969. Gallstones in patients with disorders of terminal ileum and disturbed bile salt metabolism. *Br. Med. J.* **3:** 494–496.
29. Dietschy, J. M. 1968. Mechanisms for the intestinal absorption of bile acids. *J. Lipid Res.* **9:** 297–309.
30. Angelin, B., K. Einarsson, and K. Hellstrom. 1976. Evidence for the absorption of bile acids in the proximal small intestine for normo- and hyperlipidemic subjects. *Gut.* **17:** 420–425.
31. Aldini, R., A. Roda, A. M. M. Labate, G. Cappelleri, E. Roda, and L. Barbara. 1982. Hepatic bile acid uptake: effect of conjugation, hydroxyl and keto groups, and albumin binding. *J. Lipid Res.* **23:** 1167–1173.
32. Reichen, J., and G. Paumgartner. 1975. Kinetics of taurocholate uptake by the perfused rat liver. *Gastroenterology.* **68:** 132–136.
33. Mok, H. Y. I., K. Von Bergmann, and S. M. Grundy. 1977. Regulation of pool size of bile acids in man. *Gastroenterology.* **73:** 684–690.