3. Toussaint, A. J., Muschel, L. H., *Nature*, 1959, v183, 1825.

4. D'Herelle, F., *Compt. Rend. Soc. Biol. Paris*, 1923, v88, 723.

5. Keller, R., Engley, F. B., Jr., *J. Immunol.*, 1958, v98, 577.

6. Keller, R., Zatzman, M. L., *ibid.*, 1959, v83, 167.

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Cholesterol-4-C^{14} and Bile Acids in the Guinea Pig.* (26277)

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Cholic acid (3*a*, 7*a*, 12*a*-trihydroxycholanic acid) has been isolated from the bile of many animal species. Because of the reported absence of 3*a*, 7*a*, 12*a*-trihydroxycholanic acid in the bile, the guinea pig has been termed "unique"(1). Recently we have found that 3*a*, 7*a*, 12*a*-trihydroxycholanic acid is the most abundant bile acid in the adult guinea pig, although absent in the immature animal(2). In determining the bile acid pattern of a particular species, several aspects have emerged: a) conversion of sterols into several bile acids having species-variable interconversion(1), b) oxidation of side chain in relation to 12-hydroxylation, and c) modification of hydroxyl groups during enterohepatic cycling(3). In studies on formation of bile acids the bile has been obtained from gall bladders and from biliary tract fistulae. In preliminary studies we found that the voluminous flow of bile from biliary fistulae resulted in early death of the guinea pigs. Recycling of bile or replacement of bile removed from the catheterized biliary tract permitted long term survival of the guinea pigs and the present study of conversion of cholesterol-4-C^{14} into labeled bile acids under favorable physiological conditions and at different phases of the enterohepatic cycling.

Methods. Young adult guinea pigs of either sex, approximately 5 months of age and weighing 500-700 g, were used in this study. A bile fistula was performed as described previously(2) in all animals. Forty-eight hours after operation, cholesterol-4-C^{14} was injected into the ear vein. For study of continuous enterohepatic cycling the catheters remained connected and small samples of bile were removed periodically. For study of freely flowing bile the 2 catheters were not connected until the ninth hour after administration of cholesterol-4-C^{14}. Aliquots of the samples of the free-flowing bile collected from 0-1, 1-3, and 3-6 hours after injection were given to different guinea pigs intraduodenally *via* common bile duct catheter (Fig. 1). The free-flowing bile from these animals was then collected for 9 hours. In all studies of free biliary flow a volume of normal unlabeled bile equal to that diverted was supplied through the duodenal fistulae. After 9 hours of free flow the catheters were connected and thereafter small daily samples of bile were taken in all animals.

For the continuous enterohepatic cycling 0.450 μC (29.5 μg), and for 9 hours free flow 0.900 μC (59 μg) were used. Radioactivity in the bile samples infused intraduodenally into other guinea pigs was: 0-1 hr, 0.0651 μC; 1-3 hr, 0.0671 μC; 3-6 hr, 0.0334 μC.

Bile acids were separated and identified by procedures used previously(2). After removal of the conjugated bile acids from the columns, residual radioactive materials ("neutral sterols") were eluted with chloroform. Aliquots of the initial bile samples, extracts and fractions of column effluents were plated on aluminum planchets and C^{14} determined with a windowless flow counter with sufficient accumulated counts per sample for 1% standard error. The paper chromatograms were scanned for radioactivity

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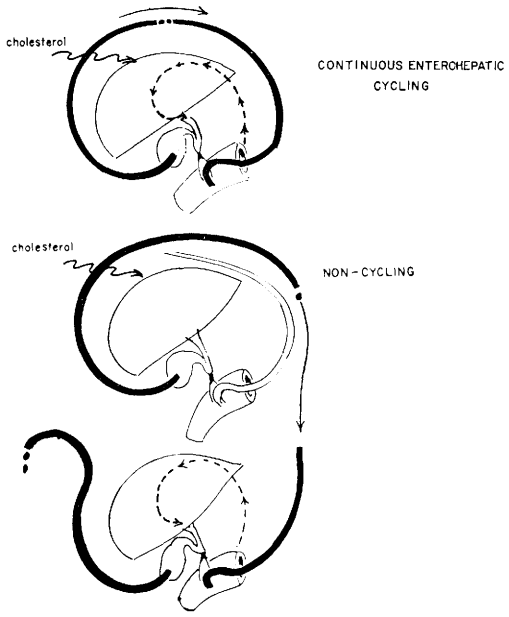

FIG. 1. Gall bladder and common duct catheterization with ligated common bile duct for determination of effects of continuous recycling, ½ cycle or tissue to bile outflow, and single enterohepatic cycle.

with a gas flow window counter. Those samples containing insufficient radioactivity for individual processing were pooled.

Results. After intravenous injection of cholesterol-4-C^{14} in the guinea pig, 3 bile acids were isolated from all bile samples: 3*a*, 7*a* - dihydroxycholanic (chenodeoxycholic), 3*a* - hydroxy, 7 - ketocholanic (7 - ketolithocholic) and 3*a*, 7*a*, 12*a*-trihydroxycholanic (cholic) acid. Of these bile acids, 3*a*, 7*a*-dihydroxycholanic and 3*a*-hydroxy, 7-ketocholanic acid appeared more rapidly and in greater concentration (Fig. 2, 3, 4). Although 3*a*, 7*a*, 12*a*-trihydroxycholanic acid appeared more slowly in the bile, it gradually increased without attaining the relative quantity of the other 2 bile acids during the first 9 hours of either enterohepatic cycling or free flow from the fistulae. After one or more days of enterohepatic cycling 3*a*, 7*a*, 12*a*-trihydroxycholanic acid became the predominant labeled bile acid and maintained a high level for 2 weeks or more after the original injection of labeled cholesterol (Table I). Thus, formation of all 3 bile acids was independent of enteric passage or microorganisms, but the high level of 3*a*, 7*a*, 12*a*-trihydroxycholanic acid observed in the adult guinea pigs was found, under the conditions of the present experiment, only after enterohepatic cycling.

Cholesterol-4-C^{14} was converted rapidly into bile acids with 26.7% of the label being excreted as bile acids or "neutral sterols" during the first 9 hours of free biliary flow (Fig.

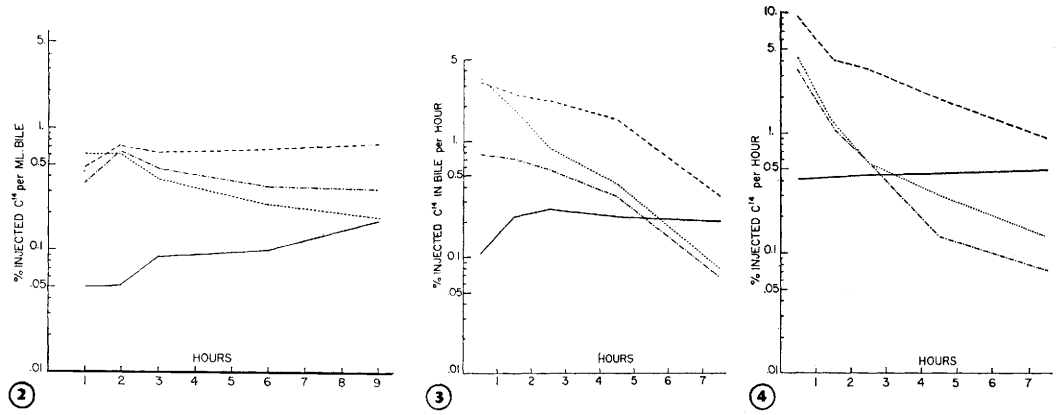

FIG. 2. Appearance of labeled bile acids and "neutral sterols" as % of inj. cholesterol-4-C^{14}/ml in bile during first 9 hr of continuous enterohepatic cycling. ———— 3*a*, 7*a*, 12*a*-trihydroxycholanic acid. ———— 3*a*, 7*a*-dihydroxycholanic acid. ·-·-·- 3*a*-hydroxy, 7-ketocholanic acid. ———— "neutral sterols."

FIG. 3. Labeled bile acids and "neutral sterols" in non-cycling (free-flow) bile for 9 hr after intrav. administration of cholesterol-4-C^{14}. Explanation of graph as in Fig. 2.

FIG. 4. Biliary excretion of bile acids and "neutral sterols" for 9 hr after intraduodenal administration of labeled bile collected from another animal (0-1 hr sample of Fig. 3). Explanation of graph as in Fig. 2.

TABLE I. Labeled Compounds in Bile during Enterohepatic Cycling as % of Total Radioactivity in Sample and as % of Injected or Infused C^{14}, Adjusted by Radioactivity Removed in Bile Samples: A, after intravenous injection of cholesterol-4-C^{14} and continuous cycling; B, after intravenous injection of cholesterol-4-C^{14} and free bile flow for 9 hr; C, D, and E, after intraduodenal infusion of labeled bile collected 0-1, 1-3 and 3-6 hr respectively.

Exp. condition	Time of enterohepatic cycling, hr	3α, 7α, 12α-trihydroxycholanic acid		3α, 7α-dihydroxycholanic acid		3α-hydroxy, 7-ketocholanic acid		"Neutral sterols"	
		% C^{14} in sample	% inj. dose/1.0 ml	% C^{14} in sample	% inj. dose/1.0 ml	% C^{14} in sample	% inj. dose/1.0 ml	% C^{14} in sample	% inj. dose/1.0 ml
A	9- 24	19.0	.019	40.0	.041	24.0	.024	17.0	.017
	24-240	81.0	.037	12.5	.019	3.5	.005	3.0	.004
	240-432	92.8	.090	5.1	.004	.3	.0002	1.8	.0016
B	9- 24	33.1	.030	40.4	.037	10.0	.009	16.5	.013
	24- 96	51.0	.056	36.0	.038	5.6	.005	7.4	.008
C	9- 24	41.4	.199	40.0	.192	10.6	.050	8.0	.038
	24- 96	48.0	.135	39.0	.109	9.3	.026	3.7	.010
D	9- 24	45.6	.215	42.0	.198	7.3	.033	5.1	.023
	24-192	62.1	.214	27.0	.093	6.2	.020	4.7	.016
E	9- 48	50.0	.350	41.0	.288	7.7	.053	1.3	.008
	48-192	62.7	.392	30.3	.189	6.1	.037	.9	.005

3). The conjugated bile acids and "neutral sterols" were rapidly absorbed from the bowel, with 42.25%, 31.32% and 40.39% of the labeled substances being excreted in the bile during the first 9 hours after introduction of 0-1, 1-3 and 3-6 hour samples, respectively. The relative proportion of labeled bile acids infused intraduodenally was similar to that excreted in the bile during the first 9 hours, although a relatively greater amount of 3α-hydroxy, 7-ketocholanic acid than of the other 2 bile acids appeared initially. The proportion of labeled "neutral sterols" excreted to that infused was far less than that of the bile acids.

The major part of the labeled bile acids separated by column and paper chromatography was conjugated with taurine. However, during the first few hours after injection of cholesterol-4-C^{14}, slight radioactivity was found in chromatographic regions of glycine conjugates. After separation of these fractions and after alkaline hydrolysis, the minute absolute amount of radioactivity prevented processing of different samples but chromatographic separation of pooled samples on column and paper revealed 2 labeled materials having the approximate mobility of 3α, 7α-dihydroxycholanic and 3α-hydroxy, 7-ketocholanic acid. Although color tests with phosphomolybdic and phosphotungstic acid on paper chromatogram were negative and no titratable material was present in the effluent from the column, the 2 labeled compounds cocrystallized with the respective unlabeled 3α, 7α-dihydroxycholanic or 3α-hydroxy, 7-ketocholanic acid isolated from guinea pig bile and identified as previously reported(2). Furthermore, the C^{14}/mg remained constant after several recrystallizations, as also observed with the free bile acids from taurine conjugates. The acetylated(4) bile acids also retained constant C^{14}/mg. No C^{14} was found in the bile samples other than that present in the taurine or glycine conjugated bile acids and the "neutral sterols."

Discussion. This study on conversion of cholesterol-4-C^{14} into labeled bile acids confirms the previous report(2) on the predominance of 3α, 7α, 12α-trihydroxycholanic acid in the bile of the adult guinea pig. Although labeled 3α, 7α, 12α-trihydroxycholanic acid occurred independently of enteric passage, it appeared in low but gradually increasing concentration in free flowing bile, becoming the predominant bile acid only after one or more days of enterohepatic cycling. However, the actual role of enteric passage or microorganisms remains uncertain, since relative biliary levels of labeled 3α, 7α, 12α-trihydroxycholanic acid were similar in both free flow and continuous enterohepatic cycling experiments during the first 9 hours (Fig. 2, 3). Such data suggest that intra-enteric conversion of

"neutral sterols" and the other 2 bile acids to 3α, 7α, 12α-trihydroxycholanic acid, if any, was not marked.

The long persistence of a relatively high concentration of labeled 3α, 7α, 12α-trihydroxycholanic acid following injection of cholesterol-4-C^{14} indicated that it is the predominant end-product of cholesterol metabolism in the bile of adult guinea pigs. According to Bergström(3) 12-hydroxylation of bile acid precursors in the rat do not occur after the side chain has been oxidized. Comparable to the effects of hyperthyroidism in rat (5) the heightened metabolic processes in rapidly growing young guinea pigs may favor the rapid side chain oxidation resulting in elaboration and excretion of dihydroxycholanic rather than trihydroxycholanic acid.

The rapid enteric passage and biliary excretion of conjugated bile acids was not unexpected with large circulating enterohepatic pool of bile acids and rate of bile acid conversion from cholesterol in the guinea pig was similar to that reported in other animals(3). The augmented cholesterol to bile acid conversion observed in animals with non-replaced loss of bile acids from fistulae(3) was minimized in the present study by substitution of normal bile for that removed from the fistulae.

After the marked depletion of 3α, 7α, 12α-trihydroxycholanic and other bile acids from the enterohepatic pool by flow from a biliary fistula, bile acids would be replaced by 3α, 7α-dihydroxycholanic and 3α-hydroxy, 7-ketocholanic acid and only small amounts of 3α, 7α, 12α-trihydroxycholanic acid. If the failure of replacement or cycling led to continued loss of bile acids from fistula, values for 3α, 7α, 12α-trihydroxycholanic acid in the bile would be minimal.

Danielsson and Kazuno(6), have suggested that 3α-hydroxy, 7-ketocholanic acid was derived from 3α, 7α-dihydroxycholanic acid in the guinea pig intestine. The present study indicates that both of these bile acids as well as 3α, 7α, 12α-trihydroxycholanic acid can be derived from tissue conversion of cholesterol.

Summary. In adult guinea pigs with biliary tract fistulae and stable enterohepatic bile acid pool, cholesterol-4-C^{14} was converted into 3 labeled bile acids (3α, 7α, 12α-trihydroxycholanic, 3α, 7α-dihydroxycholanic and 3α-hydroxy, 7-ketocholanic acid). This conversion occurred independently of enteric passage, 3α, 7α, 12α-trihydroxycholanic (cholic) acid appearing in relatively low but gradually increasing concentration during the first 9 hours of free flow and of continuous cycling. After one or more days of continuous enterohepatic cycling, 3α, 7α, 12α-trihydroxycholanic acid was the predominant labeled bile acid, maintaining a relatively high level until termination of the experiment 18 days after injection of cholesterol-4-C^{14}.

1. Haslewood, G. A. D., *Physiol. Rev.*, 1955, v35, 178.

2. Perič-Golia, L., Jones, R. S., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 337.

3. Bergström, S., *Ciba Foundation Symposium, Biosynthesis of Terpenes and Sterols,* Little, Brown and Co., Boston, 1959, 185.

4. Harold, F. M., Jayko, M. E., Chaikoff, I. L., *J. Biol. Chem.*, 1955, v216, 439.

5. Thompson, J. C., Vars, H. M., *Am. J. Physiol.*, 1954, v179, 405.

6. Danielsson, H., Kazuno, T., *Acta Chem. Scand.*, 1959, v13, 1137.

Received October 3, 1960. P.S.E.B.M., 1961, v106.