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Evidence for a Common Hepatic Cholesterol Precursor Site for Cholic and Chenodeoxycholic Acid Synthesis in Man[1] (39918)

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Recent studies (1–4) in the rat and man have provided evidence for the existence of a specific hepatocellular cholesterol compartment associated with the synthesis of bile acids. It has also been suggested (5, 6) that both primary bile acids (cholic[3] and chenodeoxycholic acids) are derived from this hepatic cholesterol site. This conclusion is based on the observation that, following the administration of labeled mevalonic acid and cholesterol to bile fistula rats, the specific activities of cholic and chenodeoxycholic acids were virtually identical. However, contrary to these findings, another report (7) in the rat with more frequent bile collections has shown that cholic acid and chenodeoxycholic acid specific activities were different after the administration of labeled mevalonic acid and cholesterol, suggesting that these bile acids may not be synthesized from the same hepatic cholesterol substrate pool.

The question of whether cholic and chenodeoxycholic acids arise from a specific cholesterol precursor site in man has not been resolved. This could be of significance in elucidating the regulation of bile acid and cholesterol metabolism in man and, in particular, whether both primary bile acids are derived via 7α-hydroxycholesterol or alternate pathways. In the present report, the homogeneity of the hepatic cholesterol substrate pool for bile acid synthesis has been investigated in bile fistula patients administered [^{14}C]cholesterol and labeled mevalonic acid.

Materials and Methods. Labeled compounds. The DL-[5-^{3}H]mevalonic acid and DL-[2-^{14}C]mevalonic acid (DBED salt) were obtained from New England Nuclear Corporation, Boston, Massachusetts. The mevalonic acid was liberated from the DBED salt by the addition of sodium bicarbonate. The DBED was extracted from the solution with diethylether. The aqueous solution of labeled sodium mevalonate was neutralized with an equimolar amount of HCl and diluted to a volume of 25 ml with sterile saline. The solution was passed through a Millipore filter (0.22 μm), assayed for ^{3}H and ^{14}C radioactivity, and then administered to the patient within 12 hr of preparation.

The [4-^{14}C]cholesterol was obtained from New England Nuclear Corporation, purified by silicic acid column chromatography, and stored at −15° in benzene. The radiopurity of the [4-^{14}C]cholesterol was checked by recovery of ^{14}C activity as the digitonide and by tlc on silica gel G, using a solvent system of petroleum ether/diethyl ether/acetic acid (89/11/3, v/v/v). On the day of the experiment, the benzene was removed and the [4-^{14}C]cholesterol was dissolved in 0.25 ml of absolute ethanol and slowly added to 20 ml of human serum albumin (Cutter Laboratories, Inc., Berkeley, Calif.). This solution was assayed for radioactivity and administered to the patient within 1 hr of preparation.

Patients. The patient with complete biliary diversion is an ideal model for critically

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[3] The systematic names of sterols referred to by trivial names: cholesterol, cholest-5-en-3β-ol; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; 7α-hydroxycholesterol, cholest-5-ene-3β,7α-diol; 26-hydroxycholesterol, cholest-5-ene-3β,26-diol.

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examining the precursor sites for cholic and chenodeoxycholic acids. In such a patient, there is no bile acid pool, and the secreted bile acids are derived directly from their hepatic cholesterol precursor sites. In addition, although bile acid synthesis is markedly stimulated, the proportion of cholic acid synthesis to chenodeoxycholic acid synthesis is normal.[4]

Each patient had a balloon-occludable T tube inserted in the common bile duct during cholecystectomy and choledocholithotomy for gallstone disease. The experiments were carried out 2–4 weeks after surgery, when liver function tests were normal. Approval from the Human Research Committee and informed consent from the patient were obtained prior to each study. Before the experiment was started, the T tube was allowed to drain externally for several days until the maximal output of bile was reached. At the time of administration of labeled compounds, the distal limb of the T tube was occluded by inflating the balloon with sterile saline and deflated 12 hr later. Inflation of the balloon was shown to completely divert all hepatic bile to the outside at the time of T-tube cholangiography in each patient. The inflated balloon ensured a complete bile fistula and complete collection of hepatic bile during this 12-hr period. In addition, virtually complete interruption of the enterohepatic circulation was confirmed prior to and throughout the entire study by the presence of acholic stools, the absence of secondary bile acids (3α-hydroxy-5β-cholanoic acid and 3α,12α-dihydroxy-5β-cholanoic acid) in the bile, and a constant flow of bile. Patients W.E. and T.T. received, in the morning, an intravenous infusion of DL-[5-^{3}H]mevalonic acid at a rate of 14 μCi/hr for 5 hr. Patient I.H. received, in the afternoon, a pulse injection of 70 μCi of DL-[2-^{14}C]mevalonic acid. Patients L.C. and T.T. each received, in the morning, simultaneous pulse injections of [^{3}H]mevalonic acid and [^{14}C]cholesterol. L.C. received 145 μCi of DL-[5-^{3}H]mevalonic acid and 30.5 μCi of [4-^{14}C]cholesterol. In patient T.T., bile was monitored for ^{3}H activity, following the constant infusion of [^{3}H]mevalonic acid, until the activity had fallen to twice background, at which time he was then administered 285 μCi of DL-[5-^{3}H]mevalonic acid and 50 μCi of [4-^{14}C]cholesterol. Bile was collected for 20 to 60-min intervals for 4 hr following administration of labeled compounds, and then for longer intervals until completion of the study.

Methods. Bile was extracted with 20 vol of 2:1 chloroform–methanol and the extract was washed with 0.2 vol of water (8). Bile acids (mass and radioactivity) were determined on the methanol–water phase by a combination of thin-layer and gas–liquid chromatography (9,10). Radioactivity was determined by liquid scintillation counting (Mark III, Searle Analytic). Cholic acid specific activities (dpm/μmole) were increased by 1/12 to account for ^{3}H loss when the 12α-hydroxyl group was inserted on the ring during its biosynthesis (11).

Results. Representative specific activity buildup curves for cholic acid and chenodeoxycholic acid during the constant infusion of [^{3}H]mevalonic acid are shown in Fig. 1. There was a rapid incorporation of the labeled precursor into the bile acids; both cholic and chenodeoxycholic acids had essentially the same specific activity curves. The ratios of cholic acid to chenodeoxycholic acid specific activities are shown in Table I; they were very close to unity for both patients during the 5-hr period. Representative specific activity time course curves for cholic acid and chenodeoxycholic

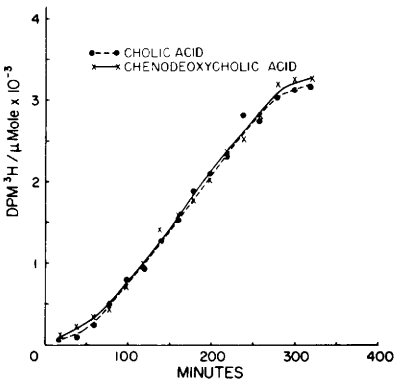

FIG. 1. Specific activity time course during the intravenous constant infusion of DL-[5-^{3}H]mevalonic acid (patient T.T.).

[4] Unpublished observations.

acid after the administration of a pulse of [^{14}C]mevalonic acid are shown in Fig. 2. There was a rapid buildup in the specific activity of both bile acids, followed by a rapid decay. The specific activity ratios of cholic acid:chenodeoxycholic acid following labeled mevalonic acid and [^{14}C]cholesterol administration are shown in Table II. The cholic acid and chenodeoxycholic acid specific activity ratios were nearly identical throughout the experiment in all patients studied.

Discussion. The findings of the present report clearly indicate that newly synthesized cholic and chenodeoxycholic acids secreted by the bile fistula patient have virtually identical specific activity time course curves following the administration of either [^{14}C]cholesterol or labeled mevalonic acid. These results provide direct evidence that cholic and chenodeoxycholic acids arise from the same hepatic cholesterol precursor site (compartment) in man, and are in contrast to the findings of Mitropoulos *et al.* (7), who suggested that, in the bile fistula rat, chenodeoxycholic acid may be formed from a compartment of cholesterol other than that from which cholic acid is derived. Isotopic equilibration of labeled, newly synthesized cholesterol throughout the total hepatic cholesterol pool has been shown to take several hours (2–4). If cholic and chenodeoxycholic acids were derived in part or in toto from separate cholesterol compartments, differences in their specific activities

TABLE 1. CHOLIC ACID-TO-CHENODEOXYCHOLIC ACID SPECIFIC ACTIVITY RATIOS DURING THE CONSTANT INFUSION OF [^{3}H]MEVALONIC ACID.

	Cholic/chenodeoxycholic specific activity ratio	
Time[a] (hr)	W.E.	T.T.
0–1	1.02	0.95
1–2	0.98	0.93
2–3	0.91	1.03
3–4	0.97	1.02
4–5	0.94	0.96

[a] Bile collection period after the constant infusion of [^{3}H]mevalonic acid was started.

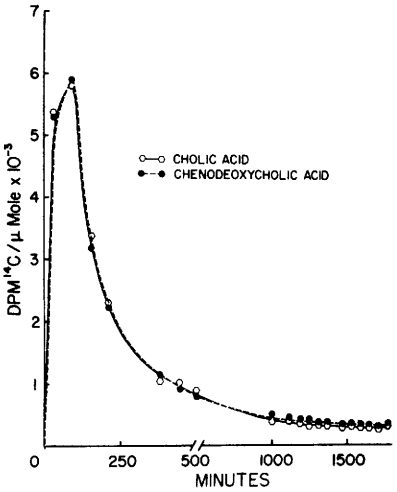

FIG. 2. Specific activity time course following the intravenous pulse administration of DL-[2-^{14}C]mevalonic acid (patient I.H.).

TABLE II. CHOLIC ACID-TO-CHENODEOXYCHOLIC ACID SPECIFIC ACTIVITY RATIOS FOLLOWING THE PULSE INJECTION OF LABELED BILE ACID PRECURSORS.

	Cholic/chenodeoxycholic specific activity ratio				
	[^{14}C]Mevalonic acid	[^{3}H]Mevalonic acid		[^{14}C]Cholesterol	
Time[a] (hr)	I.H.	L.C.	T.T.	L.C.	T.T.
0–1	1.02	0.99	1.13	0.99	1.08
1–2	0.98	0.93	0.98	0.90	1.02
2–3	1.06	0.98	1.00	0.99	1.05
3–4	1.04	0.96	0.92	0.90	0.89
4–6	0.97	0.83	1.06	0.88	0.90
6–8	1.11	0.94	1.16	0.96	0.91
8–10		0.92	1.10	1.00	1.01
10–12		0.93	0.94	0.94	1.07
12–14		0.90	0.99	0.93	1.12
14–16	0.92	0.96	1.00	1.01	1.04
16–20	0.99	0.94	0.96	0.94	1.09
20–24	0.96	0.98	0.95	1.01	1.04

[a] Bile collection period after the intravenous administration of labeled mevalonic acid and [^{14}C]cholesterol.

should have been most apparent in the first few hours following the administration of the labeled precursors. Such differences were not apparent in the early time period, even during a constant infusion of [^{3}H]mevalonic acid. However, the contribution of separate cholesterol precursor sites to cholic and chenodeoxycholic acids might be relatively more significant in subjects with an intact enterohepatic circulation and normal rate of bile acid synthesis.

Present concepts of the biosynthesis of cholic and chenodeoxycholic acids suggest the existence of a common pathway via 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one (12). However, an alternate pathway for the synthesis of chenodeoxycholic acid through 26-hydroxycholesterol has also been demonstrated in the bile fistula patient (13, 14). *In vitro* studies with human liver organelles have shown that the cholesterol-26-hydroxylase enzyme is predominantly in the mitochondria (15), whereas cholesterol-7α-hydroxylase is in the microsomes (16). Therefore, if chenodeoxycholic acid was synthesized via both pathways it would imply separate hepatic cholesterol precursor sites. In view of the identical specific activities for cholic and chenodeoxycholic acids found in the present study, it appears that the 26-hydroxycholesterol pathway makes very little contribution to chenodeoxycholic acid synthesis in man. Thus, our results are most consistent with the concept that 7α-hydroxycholesterol is the initial product in the biosynthesis of cholic and chenodeoxycholic acids.

Summary. Bile fistula patients were administered [^{14}C]cholesterol and labeled mevalonic acid by constant infusion (for 5 hr) or by intravenous pulse. Bile was collected at frequent time intervals. The specific activities of newly synthesized cholic and chenodeoxycholic acids were virtually identical at all time periods (up to 24 hr). These observations provide evidence for the presence of a common cholesterol substrate pool for primary bile acid synthesis in man. Both cholic and chenodeoxycholic acids appear to be synthesized via the 7α-hydroxycholesterol pathway.

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