Conversion of Cholesterol to Bile Acids in Man.** (26916)

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Previous reports have shown that bile acids are final products of cholesterol degradation (for recent review see(1)), that rate of formation of bile acids is governed in part by reabsorption of bile acids from small intestine (2) and that half-lives of bile acids can be determined under physiological conditions in animals(3) as well as in man(4). Recently the rate of equilibration between cholesterol and bile acid pools has been studied(5,6). The present study was designed to determine rate of conversion of cholesterol to bile acids as well as sizes of cholesterol and bile acid pools in 2 hypercholesterolemic patients maintained solely on liquid formulas containing sterol-free triglycerides of known composition. By use of cholesterol-4-C^{14} and cholic acid-U-H^3 it was possible to determine rate of equilibrium between cholesterol and cholic acid and at the same time to measure cholic acid production.

Methods. 1. *Clinical.* Patient 1: 26 years, non-obese white male; 164 cm, 62 kg; hypercholesteremia; history of coronary insufficiency, but not of coronary occlusion or cardiac failure; arcus senilis, no xanthomatosis; no evidence of diabetes or other metabolic disorders; normal cholecystograms and EKG. Uneventful course on metabolic ward for 23 weeks.

Patient 2: 22 years, non-obese white female; 172 cm, 52 kg; hypercholesteremia and tendon xanthomatosis; no cardiovascular abnormalities, diabetes or other metabolic disorders; familial history of hypercholesteremia and early coronary disease. Uneventful course on metabolic ward for 26 weeks.

Both patients were fed solely on orally administered liquid formulas and maintained constant body weights (for complete regimen see ref. 7). Milk protein, fat and simple carbohydrates constituted 15, 40 and 45% of total calories. Entire dietary fat was synthetic triglycerides prepared from highly purified fatty acids, free of sterols (Table I). Daily intakes:
Patient 1–37.3 cal./kg, 103 g triglyceride A;
Patient 2–36.2 cal./kg, 87.6 g triglyceride B.

TABLE I. Composition of Synthetic Dietary Triglycerides.*

Fatty acids	Triglyceride A	Triglyceride B
Palmitic	57.4%	2.7%
Palmitoleic	.9	.3
Stearic	—	1.2
Oleic	3.6	60.3
Linoleic	35.5	35.5
Others	2.6	—
Iodine value	69	115
Free fatty acids	.2%	.3%
Hydroxyl value	1.6	0
Non-saponifiable	.4%	.4%
Trans acids	.7%	0
Conjugated dienes	.6%	.6%

* Prepared by Merck and Co., Rahway, N. J. (Dr. Erwin Schoenewalt). Fatty acid analyses by gas-liquid chromatography(8); other analyses performed by Procter and Gamble Co., Cincinnati, Ohio (Dr. Fred Mattson).

On day "zero" isotopic materials were administered by mouth: cholesterol-4-C^{14} (100 μC) and cholic acid-U-H^3 (5 μC) prepared by Wilzbach technic(9). Thereafter, bloods were drawn over a period of 3 weeks. Bile was aspirated through a duodenal tube after injection of 3 mg of cholecystokinin.* 2. *Chemical.* Serum lipids were separated and cholesterol isolated according to(10). Bile acids were isolated and separated(11,12,13) and analyzed by paper chromatography† (14). Isotope determinations were performed by gas-phase counting(15).

Results. The patients did well throughout the experimental period. Duodenal aspirations were most satisfactorily accomplished by introducing the tube the night before the aspiration. Total body weights remained unchanged. Values for serum lipid

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TABLE II. Serum Lipid Levels Before and During Isotope Study.*

		Days before and after administration of labeled cholesterol and cholic acid										
		-27	-20	-13	-6	-3	+1	+8	+11	+15	+18	+22
Patient 1	Total cholesterol	350	341	346	348		350	305	312	295	287	280
"	Phospholipids	311	291	288	298		285	292	240	235	239	222
"	Triglycerides	236	311	345	344		334	146	255	165	261	236
Patient 2	Total cholesterol		270	285	255	238	267	279		271		273
"	Phospholipids		220	238	204	215	221	251		265		224
"	Triglycerides		163	195	138	151	196	179		159		162

* All values expressed as mg/100 ml serum.

levels(7) prior to and during the test period are given in Table II. In Patient 1 all levels decreased during the experimental period, while in Patient 2 there was no significant change.

Fig. 1 and 2 show the specific activities of serum cholesterol (free and esterified), bile cholesterol and cholic acid in the 2 patients. The specific activity curves for cholesterol fractions are not significantly different from those observed in normal cases(5,6). Cross-over of cholesterol and cholic acid specific activity curves occurred at 12 and 16 days, respectively, which is considerably later than the 4-5 days observed in healthy students of the same age(6). Fig. 3 and 4 are semi-logarithmic plots of specific activities of H^3-cholic acid from which pool size, half-lives and daily production have been calculated (Table III).

In 8 normal students previously studied the half-life of cholic acid was 2.8 days (1.2-4.2) and daily production of cholic acid 0.36 g (0.26-0.69 g). In the present cases the half-lives were 6.5 and 10.2 days, corresponding to daily productions of 0.087 g and 0.093 g, respectively. The total bile acid pool calculated from cholic acid pool and relative composition of bile was 1.70 and 2.30 g, essen-

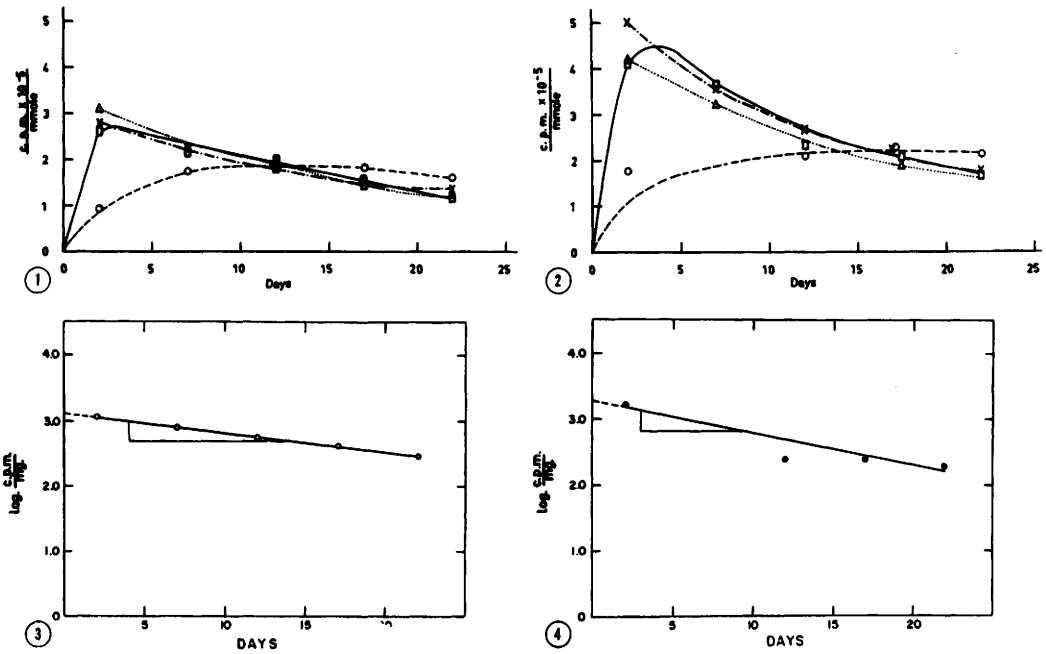

FIG. 1. Specific activities of cholesterol and bile acids after administration of cholesterol-4-C^{14} (case 1). ×–•–•–×, serum free cholesterol; □——□, serum esterified cholesterol; △•••△, biliary cholesterol; ○– – –○, biliary cholic acid.

FIG. 2. Specific activities of cholesterol and bile acids after administration of cholesterol-4-C^{14} (case 2). For symbols, see Fig. 1.

FIG. 3. Semi-log plot of specific activities of biliary H^3-cholic acid (case 1).

FIG. 4. Semi-log plot of specific activities of biliary H^3-cholic acid (case 2).

TABLE III. Bile Acid Turnover Data(4).

	Half-life (days)	Pool size (g)			Daily production of cholic acid (g)
		Cholic acid	Chenodeoxycholic acid	Deoxycholic acid	
Case 1	10.2	1.28	1.32	—	.087
" 2	6.5	.87	.50	.93	.093

tially within normal range (3.38 g, (1.88–4.97)(4)). Thus, decreased production of bile acids in these cases is caused mainly by prolongation of half-lives and not by decrease in pool size. It is interesting to observe that no detectable amount of deoxycholic acid was seen in Case 1, although this bile acid has been found in all other duodenal samples studied(16). Since this bile acid is formed from cholic acid by intestinal bacteria(17), it is possible that changes in intestinal flora caused by the diet are responsible for its absence in this case. However, Blomstrand (18) has noted the absence of deoxycholic acid in bile of a hypercholesteremic patient on *ad libitum* diet.

Previous experience has shown that complete analysis of fecal bile acids and sterols on regular diets presents great difficulties. The use of a synthetic sterol-free diet in these cases has been essential to analysis of fecal bile acids and sterols. These results will be published separately.

Discussion. Although it is interesting to speculate on the possible relation between decelerated cholesterol degradation and hypercholesterolemia in these patients, one should bear in mind that the formula diet *per se* may cause changes of bacterial flora that result in prolonged half-lives of bile acids. For example, it is known that germ-free animals show changes similar to those observed here, *i.e.*, normal pool size and prolonged half-lives of bile acids(19). More definitive answers to the basic question (rates of production *vs.* degradation and excretion (20)) await extension of the present experimental approach in further patients fed diets causing alternately high and low serum cholesterol levels.

Summary. A technic is described for simultaneous determination in man of equilibration of cholesterol with bile acids and of bile acid turnover. Two hypercholesterolemic patients on formula diets containing synthetic fats showed prolonged bile acid half-lives with decelerated cholesterol degradation; cholic acid production was only 0.09 g per day (4-fold reduction from normal levels). Deoxycholic acid was totally absent from the bile in one case.

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1. Bergström, S., Danielsson, H., Samuelsson, B., in *Metabolism of Lipids,* ed. by Bloch, K., John Wiley and Sons, New York, 1960.

2. Bergström, S., Danielsson, H., *Acta Physiol. Scand.,* 1958, v43, 1.

3. Lindstedt, S., Norman, A., *ibid.,* 1956, v38, 121.

4. Lindstedt, S., *ibid.,* 1957, v40, 1.

5. Rosenfeld, R. S., Hellman, L., *J. Clin. Invest.,* 1959, v38, 1334.

6. Lindstedt, S., *Fed. Proc.,* 1959, v18, 274.

7. Ahrens, E. H., Jr., Hirsch, J., Insull, W., Jr., Tsaltas, T. T., Blomstrand, R., Peterson, M. L., *Lancet,* 1957, v1, 943.

8. Farquhar, J. W., Insull, W., Jr., Rosen, P., Stoffel, W., Ahrens, E. H., Jr., *Nutr. Rev.,* 1959, v17, no. 8, part II.

9. Wilzbach, K., *J. Am. Chem. Soc.,* 1957, v79, 1013.

10. Garton, G. A., Duncan, W. R. H., *Biochem. J.,* 1957, v67, 340.

11. Bergström, S., Sjövall, J., *Acta Chem. Scand.,* 1951, v5, 1267.

12. Sjövall, J., *Acta Physiol. Scand.,* 1953, v29, 232.

13. Norman, A., *Acta Chem. Scand.,* 1953, v7, 1413.

14. Sjövall, J., *Clin. Chim. Acta,* 1959, v4, 252.

15. Glascock, R., *Isotopic Gas Analysis for Biochemists,* Academic Press, Inc., New York 1954.

16. Sjövall, J., *Clin. Chim. Acta,* 1960, v5, 33.

17. Lindstedt, S., Sjövall, J., *Acta Chem. Scand.,* 1957, v11, 421.

18. Blomstrand, R., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 126.

19. Gustafsson, B. E., Bergström, S., Lindstedt, S., Norman, A., *ibid.,* 1957, v94, 578.

20. Hellman, L., Rosenfeld, R. S., Insull, W., Jr., Ahrens, E. H., Jr., *J. Clin. Invest.,* 1957, v36, 898.

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