Esterification of injected epicoprostanol in a human subject

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ABSTRACT That a necessary requirement for in vivo esterification of 3-hydroxy sterols is the equatorial conformation of the hydroxyl group has been substantiated by the administration of epicoprostanol-3β-^{3}H (3-OH equatorial) to a man with a complete biliary fistula. Radioactive epicoprostanol was identified in both free and esterified sterols of plasma. The specific activity of biliary sterols was much higher than that of plasma sterols; this indicated a preferential removal of epicoprostanol from circulating sterols. The appearance of tritium in body water demonstrated that a portion of the labeled epicoprostanol underwent oxidation at C-3.

SUPPLEMENTARY KEY WORDS 5β-cholestan-3α-ol · metabolism · equatorial configuration

In studies designed to define certain requirements for sterol esterification in man, investigations from this laboratory demonstrated that absorbed coprostanol (3-OH axial) circulated only in the free form, while cholestanol, which is similar in conformation at C-3 to cholesterol (3-OH equatorial), was efficiently esterified (1). Earlier, in animal studies, Hernandez, Chaikoff, Dauben, and Abraham (2) and Swell, Stutzman, Law, and Treadwell (3) had shown that epicholesterol (3-OH axial) was not esterified. Thus, it appeared that the equatorial conformation of the C-3 hydroxyl (and not its α- or β-orientation) was a necessary requirement for the process. If this, indeed, obtains, then epicoprostanol (3-OH equatorial), a sterol with structural characteristics at C-3 and C-5 "foreign" to circulating sterols, should nevertheless undergo esterification in vivo. Epicoprostanol-3β-^{3}H was administered by vein to a patient with a bile fistula and esterification of circulating labeled free sterol proceeded readily. A significant amount of radioactivity was excreted in the feces, which provided additional evidence for the intraluminal secretion of sterols by the intestine.

The following trivial names have been used: epicoprostanol, 5β-cholestan-3α-ol; coprostanol, 5β-cholestan-3β-ol; cholestanol, 5α-cholestan-3β-ol; epicholesterol, Δ^5-cholesten-3α-ol.

METHODS

The patient was a 70 yr old male with a surgically-established complete external bile fistula following laparotomy for carcinoma of the stomach metastatic to the gall bladder. He had been operated on 2 months prior to the study and was in fair clinical condition. The bile volume was about 350 ml per day; liver function tests were normal and serum cholesterol was 143 mg/100 ml (31% free). The external drainage of the bile was judged complete because the stools were acholic and contained no bile acids, as judged by gas–liquid chromatography. Epicoprostanol-3β-^{3}H, 14.37 mg, 5.04 × 10^8 cpm, was dissolved in 2.0 ml of ethanol; the solution was mixed with 30 ml of 5% dextrose, and the microsuspension was administered by vein over a 3 min period. Blood samples were obtained at specified times over 8 days. Bile was collected during three 4 hr intervals, then in one collection for the following 12 hr, and subsequently daily for 8 days; during this time, five stool collections and daily urine collections were obtained.

Radiochemical Purity of Epicoprostanol-3-^{3}H

The labeled material was prepared by reduction of coprostanone with lithium aluminum hydride-^{3}H. The preparation and the determination of the radiochemical homogeneity have been reported (4); epicoprostanol-3-^{3}H was over 90% pure, the only contaminant being coprostanol-3-^{3}H.

Plasma Sterols

Free sterol and sterol esters were obtained by deproteinization of the plasma, extraction of the lipids, and column chromatography. Sterols from the ester fraction were isolated after saponification and chromatography on alumina. These procedures, which have been verified in other studies (4–6), have been previously described (7).

Free sterols from the 1st, 4th, and 8th day, obtained as described above, were combined and a portion was diluted with 50 mg each of nonradioactive cholesterol and epicoprostanol; the mixture was chromatographed on alumina. The column was eluted in benzene–petroleum ether 3:7 and the epicoprostanol appeared first. The combined epicoprostanol fractions contained 11,600 cpm and the combined cholesterol fractions, 950 cpm. Each fraction was then separated into 3α-hydroxy- and 3β-hydroxysteroids by means of digitonin (8, 9). Over 98% of the radioactivity in epicoprostanol remained in the α-fraction while only 18% of the radioactivity in cholesterol was digitonin-precipitable. Thus, at least 92% of the radioactivity in free sterols from plasma was present as epicoprostanol. Sterol from the ester fractions was treated in an identical manner. Over 97% of the radioactivity remained with epicoprostanol.

Bile Steroids

Bile was mixed with concentrated KOH to afford a 15% alkali solution and refluxed for 4 hr. The nonsaponifiable material was chromatographed on alumina to yield a crystalline sterol fraction.

The aqueous alkaline layer was acidified and extracted with diethyl ether. The crude bile acids were separated from nonpolar material, converted to methyl esters, acetylated, and chromatographed on silica gel by established methods (10); a diacetoxycholanoic ester fraction and a triacetoxycholanoic ester fraction were obtained.

Feces

Stool was dried, extracted, and fractionated according to published procedures (11) to yield a nonpolar, neutral fraction and an acid fraction in which bile acids could not be detected by gas–liquid chromatography (12); 10 mg/day of bile acids could have been measured by these methods. The neutral fraction from the first day's collection was saponified and the crude sterols were chromatographed on aluminum oxide (13); over 80% of the radioactivity was found in the early coprostanol eluates. A small amount of this fraction (68,000 cpm, 2.56 mg) was diluted with 53 mg of coprostanol and 50 mg of epicoprostanol. The mixture was separated by digitonin as described above; over 98% of the radioactivity remained with epicoprostanol.

Plasma Water and Urine

Plasma was diluted 1:3 with water and distilled in vacuo. Urine was distilled without dilution. The radioactivity in the distillates was measured; the results are illustrated graphically in Fig. 1.

Radioactive Measurements

Samples were assayed in a liquid scintillation spectrometer. Polar substances or aqueous solutions were measured in the diotol scintillation mixture of Herberg (14); suitable interconversion factors were used (15) so that all radioactivity measurements, expressed as cpm, are comparable.

RESULTS AND DISCUSSION

After 9 days, the cumulative excretion of labeled bile sterols (Fig. 2), which totaled 3.85% of injected radioactivity, amounted to 81% of the total biliary radioactivity. Dihydroxy bile acids contained 0.77% and trihydroxy bile acids 0.10% of injected radioactivity. This is in contrast to the distribution of biliary radioactivity after the administration of cholesterol-4-^{14}C, when 50–90% is found in bile acids (10, 16). This difference is probably related to oxidation of epicoprostanol-3β-^{3}H at C-3 since any resulting ketonic intermediate transformed to bile acids would be devoid of radioactivity. It can be calculated from the data in Fig. 1, the volume of urine output, and body water content, that 9 days after the injection, the tritium in body water plus that excreted in the urine amounted to about 8% of the injected material. If the loss of approximately 1 liter of water per day through breath and skin is included, this figure would be raised to 10%.

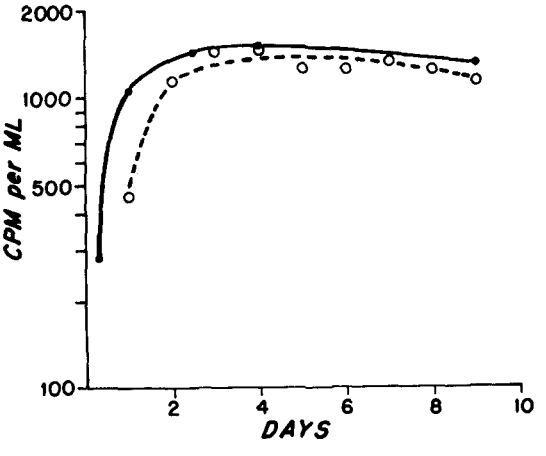

FIG. 1. ^{3}H radioactivity in water of urine (O--O) and plasma (●—●). Plasma was diluted 1:3 with water before distillation and data are related back to original volume. Urine was distilled without dilution.

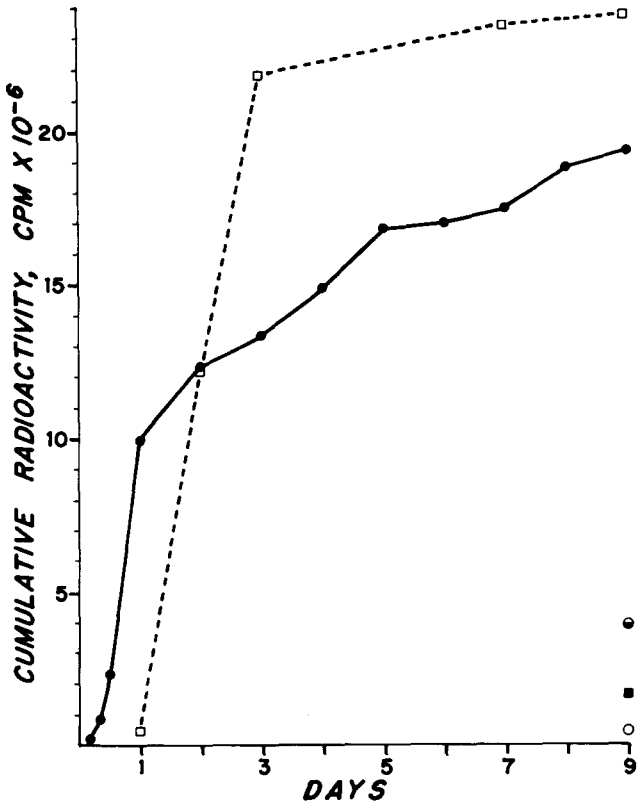

FIG. 2. Cumulative excretion of radioactivity in biliary and fecal sterols after injection of epicoprostanol-3β-^{3}H. □---□, fecal sterols (4.67% of injected radioactivity at 9 days); ●—●, bile sterols (3.85 %). Radioactivity was also measured in bile acids from bile and feces; the totals after 9 days were, for bile dihydroxycholanoic acids (◒) 0.77%, for bile trihydroxycholanoic acids (○) 0.10%, and for fecal acids (■), 0.32% (the feces were acholic and these acids may not have been bile acids).

Fig. 2 also shows the cumulative excretion of radioactivity in feces during the period of study. Over 90% of the tritium was found in the sterol fraction, and reverse isotope dilution techniques demonstrated that at least 95% of this was associated with epicoprostanol. As would be expected in a subject with acholic stools containing no detectable bile acids, the amount of radioactivity in the acid fraction (6% of the fecal radioactivity) was small. It is possible that most of the fecal radioactivity resulted from intestinal secretion of sterol.

8 days after the administration of the tritiated material, 17.8% of the radioactivity was accounted for in bile, feces, urine, plasma water, and circulating sterol. There is no ready explanation for the inability to recover more of the tritium except for the possibility of rapid removal and sequestration of this abnormal sterol from the circulation by the liver, lungs, and other tissues.

The specific activities of plasma and biliary sterols during the 8 days following the administration of the labeled sterol are recorded in Table 1. It should be emphasized that the source of radioactivity in these fractions

TABLE 1 SPECIFIC ACTIVITY OF STEROLS OF PLASMA AND BILE

Plasma Sterols				
Time	Free	Ester	Time	Bile Sterols
hr	*cpm/mg* × 10⁻²*		*hr*	*cpm/mg × 10⁻²*
0.048 (3 min)	258	2		
0.5	207	4		
3.5	315	15	0–4	109
8	92	10	4–8	1010
12	75	9	8–12	1380
24	90	44	12–24	1350
36	58	24	24–48	545
60	13	11		
72	7	38	48–72	150
96	11	10	72–96	98
			96–120	132
144	6	13	120–144	18
			144–168	27
192	5	8	168–192	55
			192–216	46

* cpm/mg represents radioactivity of epicoprostanol per mg of sterol.

is the small amount of epicoprostanol-3β-^{3}H that is mixed with plasma and biliary cholesterol, and since these substances do not necessarily behave identically during the chromatographic procedures involved in their isolation, no significance should be attached to the failure of the data to fit a smooth curve. A similar situation was observed in plasma after the administration of labeled coprostanol (1). Nevertheless, cholesterol and epicoprostanol are sufficiently related in their physical properties to allow for broad comparisons between the specific activities of the three sterol compartments examined.

After 4 hr (Table 1) the specific activity of bile sterol always exceeded that of plasma sterol sampled during the period of bile collection. As it is known that cholesterol of plasma, bile, and probably liver and intestine interchange rapidly (10), it is likely that circulating epicoprostanol was excreted at a greater rate than cholesterol. This may be a consequence of a weak binding between plasma lipoprotein and epicoprostanol, whose A/B *cis* conformation leads to a large departure from the planar configuration of the A/B rings in cholesterol.

A most significant finding, as shown by data in Table 1, was the extensive esterification of circulating epicoprostanol. After 2 days the specific activity of plasma ester approached that of free sterol; within the limitations of the experiment, this was grossly similar to the behavior of labeled cholesterol (17) and cholestanol (1). That the labeled material of the circulating free and ester sterols was epicoprostanol was demonstrated by the recovery of 92% and 97%, respectively, of the radioactivity in this substance (see Methods). The esterification was an in vivo process and not of microbiological origin since little, if any, absorption of sterol esters from

the intestine takes place and recent work has shown that suspensions of feces that efficiently esterified cholesterol and coprostanol did not esterify epicoprostanol (4).

Thus although epicoprostanol, with the 3α-OH, 5β-H configuration, is a sterol foreign to the circulation, the equatorial conformation of the hydroxyl group permitted ready in vivo esterification in this subject in a manner similar to that of the naturally occurring cholesterol and cholestanol. The generality of this observation and the type of esters formed remain questions for future investigations.

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