Biologic Stability of Tauro-23-[^{75}Se] Selena-25-Homocholic Acid

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The stability of tauro-23-[^{75}Se]selena-25-homocholic acid (SeHCAT) towards deconjugation by the enzyme cholylglycine hydrolase was compared with that of taurocholate: whereas taurocholate underwent 58% deconjugation within 2 hr, SeHCAT suffered only 8% deconjugation plus 5% conversion to an unknown product within 24 hr. Incubation of SeHCAT under anaerobic conditions for 48 hr at 37°C with human fecal organisms resulted in considerable deconjugation, 7α-dehydroxylation, and dehydrogenation. Twenty-four hours after the simultaneous administration of SeHCAT and tauro-[24-^{14}C]cholate to a rabbit the recovery of ^{75}Se in bile was 90% of that of ^{14}C. Forty-eight hours following administration of SeHCAT to a second rabbit residual bile radioactivity revealed 80% deconjugation and dehydroxylation and 60% reconjugation with glycine. Although SeHCAT is more resistant than taurocholate towards modification by fecal bacterial enzymes, within the rabbit it follows the principal metabolic pathways of the natural bile acids.

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The absorption and excretion of the synthetic bile acid analog, tauro-23-[^{75}Se]selena-25-homocholic acid (SeHCAT), has been investigated in both rat and man in order to assess its use as an agent for investigating the functional integrity of the ileum (*1,2*). It has been evaluated in tests of ileal function in patients suffering inflammatory bowel disease and in those who have undergone gastrointestinal surgery; it has also been applied to the diagnosis of bile acid malabsorption in patients with diarrhea of unknown etiology and diarrhea resulting from pelvic irradiation (*3–6*). Gamma camera imaging of the enterohepatic system with SeHCAT allows the dynamics of the bile acid circulation to be investigated (*7*).

Bile acids, both free and conjugated, are absorbed from the gut principally by an active transport process in the distal ileum. Free bile acids are passively absorbed to some extent throughout the small and large bowel. The mechanisms of absorption of both free and conjugated bile acids have been studied extensively by a number of investigators (*8–10*). Both conjugation and degree of hydroxylation affect these absorption processes (*11*). During their passage through the gut, bile acids are subjected to bacterial action whereby they undergo deconjugation, dehydroxylation and oxidation (Fig. 1). In the liver they are reconjugated with either glycine or taurine and in some species may be rehydroxylated. Such processes may modify the enterohepatic circulation of bile acids. In normal man the principal component of the enterohepatic circulation of bile acids may be considered to be ileohepatic (*12,13*). In certain pathologic conditions such as ileal dysfunction resulting from Crohn's disease, in which large quantities of bile acids pass into the colon, and in the stagnant loop syndrome of the proximal small bowel, bacterial modification of bile acids may give rise, through passive nonionic diffusion processes, to an elevated subsidiary colohepatic or jejunohepatic circulation of free bile acids (*14–17*). The sensitivity of an ileal function test based on a conjugated bile acid may thus be affected by the exposure to intestinal microorganisms.

The purpose of the work described here was to examine the biologic stability of SeHCAT towards fecal bacterial enzymes. The rabbit was chosen as an in vivo model for studies since its liver contains no 7α-hydroxylating enzyme and it reconjugates free bile acids with glycine (*18*). Bacterial deconjugation and dehydroxylation would therefore not be masked by subsequent metabolism in the liver.

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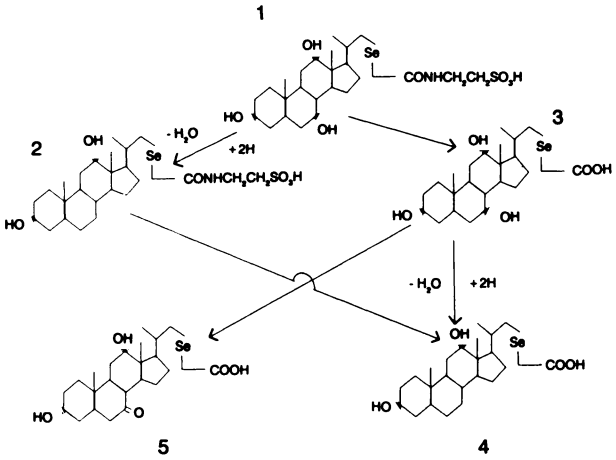

FIGURE 1
The modification of bile acids by gut bacteria. 1. Tauro-23-selena-25-homocholic acid, TSHC; 2. Tauro-23-selena-25-homodeoxycholic acid, TSHDC; 3. 23-selena-25-homocholic acid, SHC; 4. 23-selena-25-homodeoxycholic acid, SHDC; 5. 3α,12α-dihydroxy-7-oxo-23-selena-25-homocholanoic acid.

MATERIALS AND METHODS

Chromatography

Materials were purified either by preparative layer chromatography on 1-mm Merck silica gel thin layer chromatography (TLC) plates or by preparative high performance liquid chromatography (HPLC). Products were characterized on 0.25-mm Merck silica gel 60 F_{254} TLC plates. Plates were scanned for radioactivity by a 100-channel TLC analyzer; they were visualized by exposure to iodine vapor and by autoradiography on Kodak "Kodirex" film. The following solvent systems were used: chloroform-methanol (2:1) (SS.1); chloroform-methanol (3:1) (SS.2); n-butanol-water-acetic acid (60:25:15) (SS.3); ethyl acetate-acetic acid (2:1) (SS.4); chloroform-methanol-acetic acid-water (65:20:10:5) (SS.5); butanol-water-acetic acid (10:1:1) (SS.6); cyclohexane-ethyl acetate-acetic acid (7:23:3) (SS.7); and chloroform-ethyl acetate-acetic acid (35:115:15) (SS.8). Selenium-75- (^{75}Se) labeled and nonradioactive markers for tauro-23-selena-25-homocholic acid (TSHC), tauro-23-selena-25-homodeoxycholic acid (TSHDC), glyco-23-selena-25-homocholic acid (GSHC), 23-selena-25-homocholic acid (SHC) and 23-selena-25-homodeoxycholic acid (SHDC) were synthesized and supplied by Amersham International plc (*19*), as were carbon-14- (^{14}C) labeled taurocholate (TC), glycocholate (GC), cholate (C) and deoxycholate (DC). Nonradioactive samples of taurocholate, glycocholate, glycodeoxycholate (GDC), cholate and deoxycholate were obtained from Sigma Chemical Corporation, St. Louis, MO.

Preparative HPLC was carried out on equipment comprising two Altex 110A pumps controlled by an Altex Model 420 microprocessor, a Pye Unicam LC-uv spectrophotometer set at 210-220 nm, a gamma scintillation detector with an ESI Nuclear ratemeter, and a Linseis recorder. Gradient elution information was fed directly from the microprocessor to the recorder. Metabolites of SeHCAT were separated on a column of Ultrasphere ODS (Altex), 0.9 cm × 50 cm which was eluted at 1.5 ml/min with a linear gradient of methanol-water (20:80) running to methanol-water (95:5).

Enzymatic Hydrolysis of SeHCAT with Cholylglycine Hydrolase

The enzymatic hydrolysis of SeHCAT was compared with that of tauro-[24-^{14}C]cholic acid under strictly controlled conditions. Stock solutions were prepared as follows: A: 0.025*M* sodium acetate buffer, pH 5.6; B: 0.78% aqueous 2-mercaptoethanol solution; C: 1.86% aqueous EDTA solution; D: cholylglycine hydrolase (ex Clostridium perfringens, Sigma Chemical Corp.), 30 units/ml buffer. SeHCAT was purified by preparative TLC on 1-mm Merck silica gel using chloroform-methanol (2:1). Lyophilized SeHCAT, 1.4 μmol, 134 μCi (4.96 MBq) plus 268 μl of solution A, 134 μl of solution B, 134 μl of solution C, and 67 μl of water were equilibrated at 37°C for 15 min. Solution D, 67 μl, was then added and incubation of the mixture was continued at 37°C. Samples, 50 μl, of the incubation mixture were withdrawn after 0, 2, 4, 6, 23, 26, 31, and 47 hr. Each sample was added to 50 μl of 20% aqueous trichloroacetic acid and the solution was then lyophilized. The products of the reaction, after dissolving in 50 μl of methanol, were characterized by TLC on Merck silica gel using solvent systems SS.3 and SS.4. The plates were autoradiographed and scanned. The same hydrolytic procedure was applied to 0.85 μmol, 50 μCi (1.85 MBq) of lyophilized tauro-[24-^{14}C]cholic acid and reagent solutions in similar proportions. The incubation mixture was sampled at 0, 5, 10, 15, 30, 45, 60, 90, and 120 min and analyzed as for SeHCAT.

Metabolism of SeHCAT by Anaerobic Bacteria

The following microorganisms were used: (i) *Bacteroides fragilis,* (ii) *Bacteroides vulgatus,* (iii) *Clostridium perfringens,* and (iv) a suspension of mixed human fecal bacteria. These microorganisms were grown under strictly anaerobic conditions in PPY broth (*20*) which contained 2% w/v Proteose peptone (Difco), 1% w/v yeast extract (Oxoid), and 0.5 w/v sodium chloride in distilled water at pH 7.4. The broth was supplemented, after sterilization, and prior to inoculation, with sodium carbonate (0.04% w/v), cysteine hydrochloride (0.075% w/v), hemin (5 μg/ml), and menadione (1 μg/ml).

To 1 ml of sterile PPY broth in each of five tubes was added 0.5 ml of one of the culture concentrates of each microorganism; to the fifth control tube was added a further 0.5 ml of the sterile medium. SeHCAT, 1.05 μmol, 0.95 μCi (0.035 MBq) in 0.1 ml of a sterile aqueous solution was added to each tube and the tubes then incubated under strictly anaerobic conditions at 37°C for 48 hr. After the addition of 500 μl of 20% trichloroacetic acid to each tube the contents were filtered through sintered glass and the filtrates lyophilized. The products were dissolved in 1 ml of methanol and a 50-μl aliquot analyzed by TLC on Merck silica gel using solvent system SS.8. Standard markers of SeHCAT, SHC, SHDC and 3α,12α-dihydroxy-7-oxo-cholanoic acid were used for comparison. The plates were scanned and visualized.

Metabolism of SeHCAT in the Rabbit

A solution was prepared containing tauro-[24-^{14}C]cholic acid, 50 μCi, 59 μCi/μmol (1.85 MBq, 2.18 MBq/μmol) and SeHCAT, 57.5 μCi, 98.7 μCi/μmol (2.13 MBq, 3.65 MBq/μmol) in 2.05 ml of isotonic saline. A portion of this solution, 1.5 ml, was administered intravenously to a rabbit. The rabbit

was maintained on a normal diet and after 24 hr was killed. The gall bladder was removed and bile from it was collected and stored at −20°C.

Samples of bile were decolorized with hydrogen peroxide and diluted aliquots were counted for ^{14}C and ^{75}Se in the beta and gamma counting channels of a Nuclear Enterprises 8312 counter. Triton X was used as the liquid beta scintillant. The original injection solution administered to the rabbit was suitably diluted and counted similarly. A standard ^{75}Se solution was counted in both the beta and gamma channels and a correction factor was determined for the ^{75}Se counts appearing in the beta channel. The ratio

$$\frac{^{14}C/^{75}Se \text{ collected in bile}}{^{14}C/^{75}Se \text{ administered}}$$

was calculated from counts corrected for background, decay and spillover between channels.

Samples of rabbit bile were extracted with 10 volumes of boiling ethanol. The filtered ethanolic solution was evaporated and the residue, after extraction with light petroleum (60–80°C), was dissolved in methanol. The methanolic solution of bile salts was examined by thin layer chromatography against known radioactive markers using solvent systems SS.2, SS.3, SS.4 and SS.5. Plates were autoradiographed for 4–5 days and again for 7 days with the interposition of a sheet of paper of sufficient thickness to absorb ^{14}C beta radiation.

A second rabbit was injected intravenously with SeHCAT, 496 μCi, 159 μCi/μmol (18.4 MBq, 5.88 MBq/μmol) in 1 ml of isotonic saline. The SeHCAT had been previously purified by preparative HPLC. The rabbit was maintained on a normal diet and, in order to increase SeHCAT metabolism, was killed at the increased time interval of 48 hr after injection. The gall bladder was removed and the bile recovered; it contained 91 μCi (3.37 MBq) of seleno bile acids labeled with ^{75}Se. The bile was treated with 10 volumes of boiling ethanol. The ethanolic extract, after filtration, was reduced in volume and stored at −20°C. A portion of this partially purified bile was further purified by extraction into n-butanol from aqueous solution at pH <1.0. The butanol extract was evaporated under reduced pressure and the residue dissolved in methanol to yield a solution containing 76 μCi (2.81 MBq) of ^{75}Se-labeled seleno bile acids. A portion of this methanolic solution was subjected to preparative HPLC. The five main fractions obtained were measured for radioactivity in an ion-chamber. Both the methanolic solution of purified bile acids and the separate fractions obtained by HPLC were examined by thin layer chromatography using solvent systems SS.3, SS.4, SS.5, SS.6, and SS.7. Nonradioactive markers of glycodeoxycholate, tauro-23-selena-25-homocholate, tauro-23-selena-25-homodeoxycholate, 23-selena-25-homocholate and 23-selena-25-homodeoxycholate were used for comparison. Plates were visualized by autoradiography over varying periods from 4–11 days, and also by exposure to iodine vapor. Hydrolysis of bile acid conjugates to enable characterization of components was carried out by heating them in 2*M* aqueous sodium hydroxide at 125°C for 16 hr.

RESULTS

Table 1 shows the Rfs for the enzymatic hydrolysis products of both tauro-[24-^{14}C]cholate and SeHCAT.

TABLE 1
Rfs of Enzymatic Hydrolysis Products of Tauro-[24-^{14}C]-cholic acid and Tauro-23-[^{75}Se]Selena-25-Homocholic Acid

Compound	Hydrolysis product Rfs: Eluent SS.3	Hydrolysis product Rfs: Eluent SS.4	Characterization of product
Tauro-[24-^{14}C]-cholic acid	0.44	0.02	TC
	(0.60)	(0.25)	Minor product; ?
	0.82	0.73	Major product; C
	(0.89)	(0.94)	Minor product; DC
SeHCAT	0.55	0.03	TSHC
	0.76	0.34	Major product; TSHDC?
	0.89	0.73	Major product; SHC
	(0.96)		Minor product; SHDC?

There was one major product of the hydrolysis of tauro-[24-^{14}C]cholate, i.e., [24-^{14}C]cholic acid, together with some very minor components. SeHCAT on the other hand yielded predominantly two products, one of which was 23-[^{75}Se]selena-25-homocholic acid and the other a product of lower Rf which was present to the extent of ~5%. The product of lower mobility was not characterized but its Rf (0.34 in SS.4) indicated it to be a taurine conjugate. It must have resulted from the presence of a contaminating enzyme, e.g., either a hydroxycholanoyl dehydrogenase or a 7α-dehydroxylase. Since the hydroxycholanoyl dehydrogenases require the presence of $NAD(P)^+$ in cell-free systems and have pH optima above pH 8.5 (*21*) it is more likely that this product was tauro-23-selena-25-homodeoxycholate although the possibility of its being a keto product cannot be discounted.

Figures 2 and 3 show the deconjugation by cholylglycine hydrolyase of taurocholic acid and SeHCAT respectively. Whereas taurocholic acid underwent 58% deconjugation within 2 hr, under the same experimental conditions SeHCAT underwent only 2.5% deconjugation at 2 hr rising to 8% at 24 hr; beyond this time the enzyme appears to have been largely inactivated.

Table 2 gives the Rfs and approximate percentages of the major metabolic products resulting from the incubation of SeHCAT with certain strains of anaerobic bacteria found in human feces. The chromatographs showed no change in the control sample of SeHCAT but in all other cases SeHCAT was metabolised to an extent of at least 75%. Although the pattern of metabolites in each case was similar their proportions varied: the principal metabolite, selenahomocholate (Rfs 0.34–0.41), was present to the extent of 25–52% while selenahomodeoxycholate (Rfs 0.91–0.95) was present to the extent of 2–19%. The metabolite appearing at Rfs 0.45–0.56 agreed closely in mobility with 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid and it is presumed that this metabolite was indeed a keto derivative of selenahom-

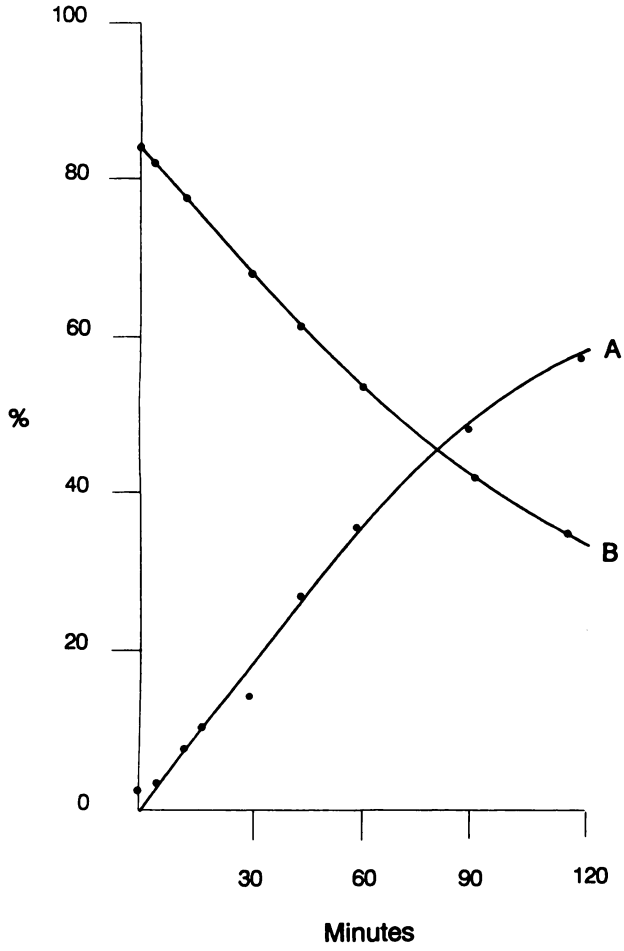

FIGURE 2
Hydrolysis of tauro-[24-^{14}C]cholic acid by *Cholylglycine hydrolase* derived from *Clostridium perfringens*. A: % cholic acid; B: % taurocholic acid.

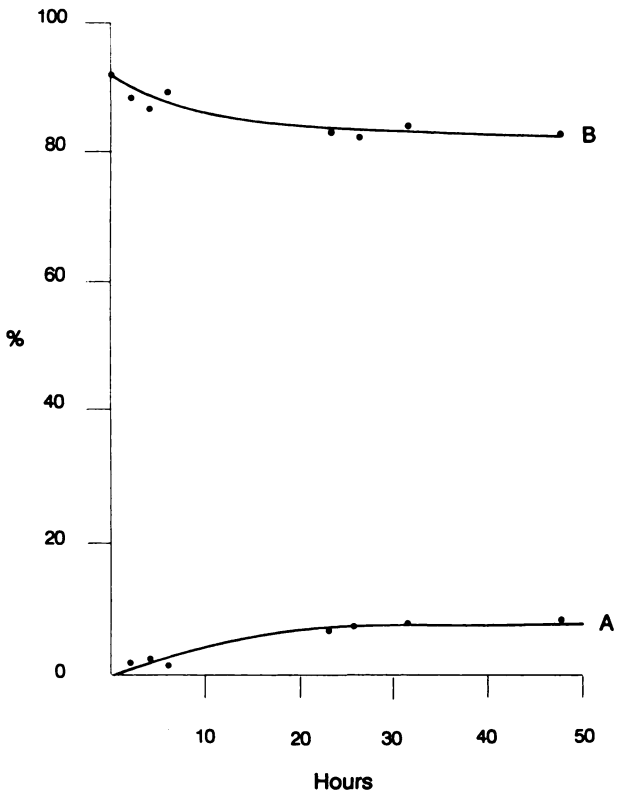

FIGURE 3
Hydrolysis of SeHCAT by *Cholylglycine hydrolase* derived from *Clostridium perfringens*. A: % selenahomocholate; B: % SeHCAT.

ocholate. Further minor metabolites occurred at Rfs both lower and higher than that of selenahomocholate.

In the experiment on the first rabbit the corrected ratio

$$\frac{^{14}C/^{75}Se \text{ collected in bile}}{^{14}C/^{75}Se \text{ administered}}$$

was calculated to be 1.11, corresponding to a ^{75}Se recovery which was 90% of the ^{14}C recovery. A qualitative assessment of the chromatographic data was made by comparing the autoradiographs from the unscreened with those from the screened plates and those visualized by iodine vapor. Solvent system SS.5 revealed the presence of minor amounts of unchanged [24-^{14}C] taurocholic acid and also minor amounts of [24-^{14}C] cholic, [24-^{14}C]deoxycholic and [24-^{14}C]glycocholic acids; the major ^{14}C-labeled metabolite had the same mobility as the major endogenous bile acid as revealed by exposure to iodine vapor, i.e., glycodeoxycholic acid (GDC). Tauro-23-[^{75}Se]selena-25-homocholic acid remained largely unchanged. There was some evidence for the formation of free acids but glycine conjugates appeared to be absent. The major metabolite had a Rf of 0.24 in SS.4, which corresponded to the Rf of tauro-23-[^{75}Se]selena-25-homodeoxycholic acid.

TABLE 2
Anaerobic Bacterial Metabolites of SeHCAT

Organism	Rfs of metabolites	Characterization and approx. % of metabolites	
(i) *Bacteroides fragilis*	0.00	SeHCAT;	8%
	0.39	SHC;	25%
	0.55	keto component?	24%
	0.92	SHDC	19%
(ii) *Bacteroides vulgatus*	0.00	SeHCAT;	14%
	0.34	SHC;	49%
	0.45	keto component?	33%
	0.95	SHDC;	4%
(iii) *Clostridium perfringens*	0.00	SeHCAT;	25%
	0.39	SHC;	52%
	0.56	keto component?	12%
	0.93	SHDC:	3%
(iv) Fecal suspension	0.00	SeHCAT;	14%
	0.41	SHC;	25%
	0.52	keto component?	34%
	0.91	SHDC;	2%
(v) Control	0.00	SeHCAT;	100%

In the examination of bile from the second rabbit the ^{75}Se-labeled metabolites were quantified. Figure 4 shows the radioactive scan obtained from preparative HPLC of the bile. Table 3 shows the amount of ^{75}Se in each fraction as a percentage of the total ^{75}Se collected in the five fractions, and also the composition of each fraction as determined by analytical TLC. The separation of the ^{75}Se-labeled seleno bile acid constituents was affected by the relatively large amounts of endogenous nonradioactive bile acids contained in all the fractions to a varying degree. Whereas fractions F1 and F5 each contained a single [^{75}Se]seleno bile acid, fractions F2, F3, and F4 contained mixtures of the [^{75}Se]seleno bile acids. The influence of the endogenous bile acids upon the separation of the [^{75}Se]seleno bile acids was reflected in the mobilities obtained with certain fractions in some TLC solvent systems. Streaking sometimes became a problem if the chromatograms were overloaded. Table 4 gives the Rf values in various solvent systems for each of the [^{75}Se]seleno bile acid constituents. Whereas constituents in F1, F2 (in part), F4 (in part) and F5 could be characterized by comparison with available markers, the mobility of the major constituent in F3 did not coincide with any available marker. In all solvent systems its mobility indicated it to be a glycine conjugate; it ran very close to glycodeoxycholate. Alkaline hydrolysis of a portion of F3 yielded a single component which was shown by TLC in SS.7 to be 23-[^{75}Se]selena-25-homodeoxycholic acid (Rf F3 (major product), 0.22; Rf selenahomodeoxycholate, 0.68). The available evidence therefore indicated that the major constituent of F3 was glyco-23-[^{75}Se]selena-25-homodeoxycholic acid (GSHDC).

The methanolic solution of purified bile acids was also subjected to TLC analysis against known markers in solvent system SS.5 (Fig. 5). In addition to the natural bile acid glycodeoxycholate (GDC), visualized with iodine vapour, four major ^{75}Se-labeled components appeared in the bile corresponding to tauroselenahomocholate (TSHC), tauroselenahomodeoxycholate (TSHDC), glycoselenahomodeoxycholate (GSHDC), and selenahomodeoxycholate (SHDC). Quantitative radioactive scanning of this plate gave composition values agreeing closely with those obtained by preparative HPLC and TLC (Table 5). The 50% GSHDC and 32% SHDC figures imply that more than 80% of the administered SeHCAT was both deconjugated and dehydroxylated and that 60% of the selenahomodeoxycholate was reconjugated with glycine in the liver. Apart from the four major radioactive components found in the bile some very minor components, probably <1%, appeared on the radioautograph in the region of the free bile acids. These may have been keto components. On the radioautographs of some TLC plates there was also an indication of the presence of both glycoselenahomocholate and selenahomocholate to the extent of ~2–3% (Fig. 1).

TABLE 3
Analysis of HPLC Fractions

Fraction F	% ^{75}Se in F	Characterization and % composition of ^{75}Se	Solvent systems; Rfs of components
F1	7.5	>98 TSHC	SS.3 (0.50) SS.5 (0.31)
F2	15.0	7 TSHDC 75 GSHDC 7 SHDC (~3 SHC) (~3 GSHC)	SS.3 (0.71, GSHDC) SS.5 (0.39, 0.88, 0.98)
F3	55.0	80 GSHDC 6 SHC 9 SHDC	SS.5 (0.87, 0.91, 0.98) SS.6 (0.62, 0.71, 0.78) SS.7 (0.22, 0.29, 0.69)
F4	2.5	>90 SHDC	SS.5 (0.97) SS.7 (0.66)
F5	20.0	>98 SHDC	SS.3 (0.90) SS.5 (0.96) SS.7 (0.69)

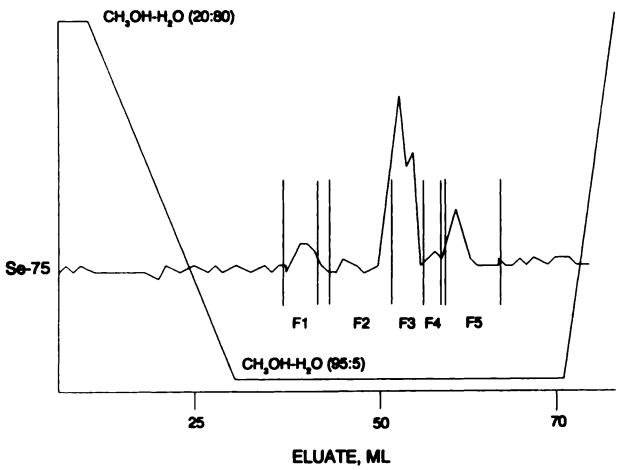

FIGURE 4
Radioactive scan obtained from preparative HPLC of bile from the second rabbit, killed 48 hr after i.v. injection with SeHCAT.

DISCUSSION

The carbon-nitrogen bond in the amide linkage of bile salts is extraordinarily stable to hydrolysis: it requires drastic alkaline conditions for its chemical hydrolysis and it is unusual in its ability to withstand cleavage by proteolytic enzymes. The carbon-selenium bond of the selenide linkage in SeHCAT would be expected to be chemically stable in vivo: it has been shown that SeHCAT is stable to heating in aqueous solution at 120°C for 30 min, and that heating in 2*M* aqueous-ethanolic potassium hydroxide at 120°C for 16 hr effects quantitative hydrolysis to the corresponding

TABLE 4
Rf Values of SeHCAT Metabolites

	Rf values and solvent systems				
	SS.3	SS.4	SS.5	SS.6	SS.7
TSHC	0.50	0.14	0.31 (0.29–0.33)		
TSHDC	0.56	0.27 (0.23–0.32)	0.39 (0.38–0.40)		
GSHC		0.60			
GSHDC	0.71	0.85	0.85 (0.76–0.89)	0.71 (0.62–0.79)	0.20 (0.18–0.22)
SHC	0.81 (0.79–0.83)	0.87 (0.81–0.93)	0.94 (0.91–0.96)		0.23 (0.22–0.24)
SHDC	0.90	0.95 (0.92–0.98)	0.98 (0.97–0.98)		0.68
GDC*	0.71		0.78 (0.72–0.85)		0.11 (0.09–0.12)

* Principal natural bile acid of rabbit

free acid. However, splitting of the amide bond of bile salts is effected by certain strains of intestinal microorganisms. P.P. Nair isolated an enzyme from *Clostridium perfringens* (ATCC-19574, Sinai-43-F-4) which is capable of deconjugating bile salts. He studied the relationship of side chain structure to enzyme activity, and came to the conclusion that a side chain longer than two carbon atoms beyond the C-N bond renders the substrate inactive towards the enzyme (*22,23*). Aries and Hill have examined enzymes from various strains of bacteria and, apart from the deconjugating enzymes, have been able to isolate extracts possessing enzyme activity catalyzing the oxido-reduction of the 7α- and 12α-hydroxy groups of bile acids, as well as the 7-dehydroxylase (*21*).

Our work described here shows that SeHCAT undergoes considerable metabolic transformation when incubated with anaerobic microorganisms and therefore provides a suitable substrate for the enzymes described by Aries and Hill. The range of products found are probably more representative of those to be found in the feces than in the bile. Incubation with the enzyme cholylglycine hydrolase showed that SeHCAT was very much more resistant towards deconjugation than was taurocholate, only 8% deconjugation being suffered by SeHCAT within 24 hr while 58% deconjugation occurred with taurocholate within 2 hr. The occurrence of a taurine-conjugated major secondary product in the case of SeHCAT, whether it was derived from a hydroxycholanoyl dehydrogenase or a 7α-dehydroxylase, would also appear to indicate that, as compared to taurocholate, the activity of the enzyme acting on the side chain was reduced relatively to that of the enzyme acting on the nucleus.

The principal bile acid synthesized by rabbit liver is cholic acid, which is conjugated almost entirely with glycine. Intestinal microorganisms 7α-dehydroxylate the cholate to deoxycholate, and since rabbit liver contains no bile acid 7α-hydroxylating enzyme, unlike the rat liver, bile acids from the gall bladder of a rabbit consist almost entirely of glycodeoxycholic acid. It was considered that the rabbit would provide a suitable model for studying the in vivo stability of SeHCAT to intestinal microorganisms; a comparison could be made with taurocholate, which over a number of enterohepatic circulations would be converted to glycodeoxycholate.

The analysis of bile from the first rabbit, showing the recovery of ^{75}Se to be 90% of that of ^{14}C, indicated that the overall efficiencies of intestinal absorption for each of these compounds and its metabolites are not markedly different. However, the qualitative differences seen in the metabolic products derived from them, as revealed by inspection of chromatographs and autoradiographs, tended to confirm the greater in vivo stability of SeHCAT as compared with taurocholate. The presence of a major metabolite of SeHCAT, corresponding to a 7α-dehydroxylated taurine conjugate, confirmed the deduction made previously that the different bile acid structure of SeHCAT resulted in a relatively greater resistance to side chain deconjugation than to nuclear modification. In the second rabbit, where the SeHCAT was allowed to circulate in the enterohepatic system for 48 hr before a quantitative estimate of biliary metabolites was made, there was a much greater degree of bacterial modification. In this case more than 80% of the administered SeHCAT was both deconjugated and dehydroxylated, while 60% of the selenahomodeoxycholate was reconjugated in the liver to appear in the bile as the glycine conjugate. The presence of more than 30% selenahomodeoxycholate in the bile suggests that the reconjugating enzyme in the rabbit liver is less active towards the seleno bile acids than it is towards the natural bile acids.

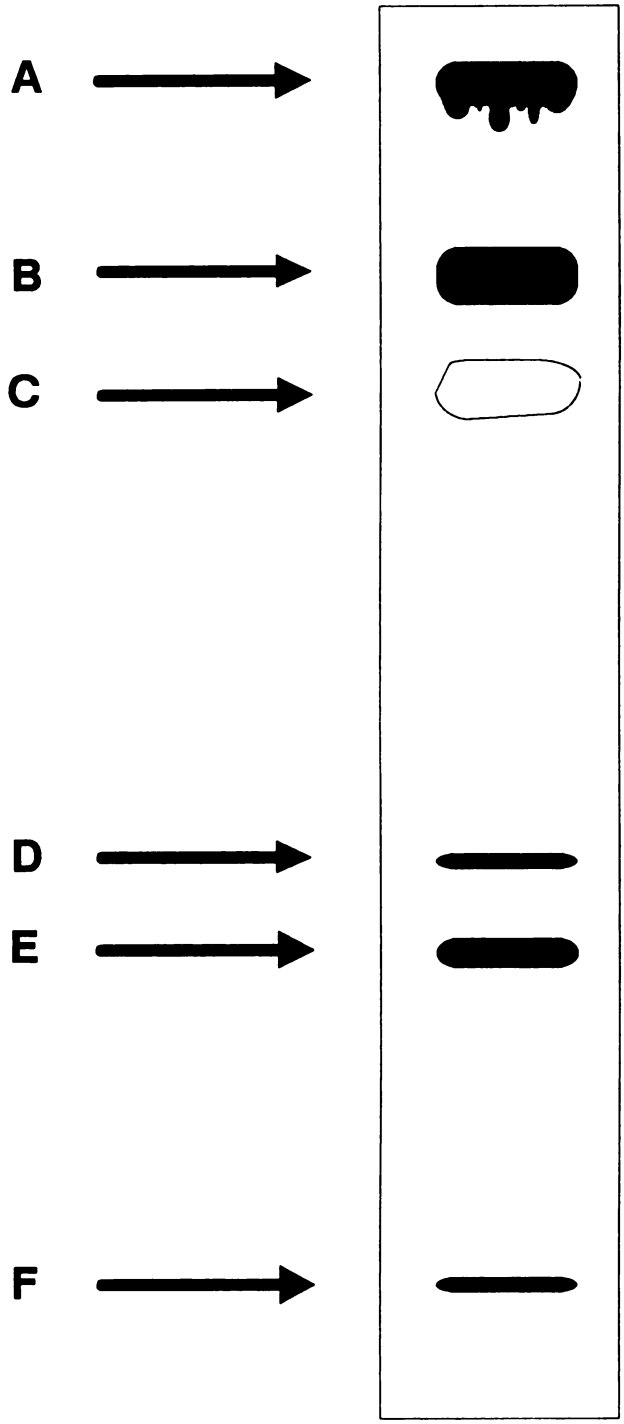

FIGURE 5
Radioautograph of TLC plate used to analyze the purified bile acids from the second rabbit, killed 48 hr after i.v. injection with SeHCAT. A: SHDC; B: GSHDC; C: GDC; D: TSHDC; E: TSHC; F: Origin.

In general, within the rabbit SeHCAT appears to follow the principal metabolic pathways of the natural bile acids. It is both deconjugated and dehydroxylated by microorganisms and the deconjugated product is also reconjugated in the liver. SeHCAT and its metabolites, however, are more resistant than the natural bile acids towards enzymatic modification. In the rabbit the exposure of SeHCAT to intestinal microorganisms will be assisted by coprophagy. In man the extent to which free seleno bile acids appear in the bile will depend upon both the degree of exposure of SeHCAT to the bacterial flora and the relative activities of the bacterial deconjugating and the liver reconjugating enzymes towards the seleno bile acid analogues. How these various metabolic transformations modify the enterohepatic circulation of SeHCAT and its metabolites, and influence its specificity for the ileum, is a complex question to answer and merits further investigation. However, as also shown elsewhere (*1*), the overall efficiency of intestinal absorption of SeHCAT and its metabolites would not appear to differ markedly from that of the natural bile acids.

TABLE 5
Distribution of ^{75}Se Among SeHCAT Metabolites in Rabbit Bile

^{75}Se metabolite*	% ^{75}Se metabolite	
	HPLC + TLC	TLC (SS.5)
TSHC	7.5	7.6
TSHDC	1.1†	3.4
GSHDC	55.0	49.7
SHDC	28.6	31.8

* 2-3% each of SHC and GSHC appeared also to be present.
† TSHDC was measured in HPLC fraction F2 only.

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