Se-75-Labeled Bile Acid Analogs, New Radiopharmaceuticals for Investigating the Enterohepatic Circulation

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Four selenium-labeled free bile acids and four selenium-labeled conjugated bile acids, labeled with Se-75 at the C-19, C-22, C-23, or C-24 position, have been synthesized and their absorption and excretion compared with that of [24-^{14}C]cholic acid, following both oral and intravenous administration. All but one of the compounds is absorbed and excreted in bile to a significant extent. One compound, SeHCAT, has been selected for particular study. It is quantitatively absorbed from the gut at the same rate as cholic acid, and both are excreted into the bile at the same rate. It remains almost entirely confined to the enterohepatic circulation (the gut, liver, and biliary tree) and excretion is exclusively fecal. Whole-body retention, measured for 41 days, and tissue distributions suggest that the absorbed radiation dose would be small compared with that in many established tests. Such a compound offers the possibility of a simple, novel, and aesthetically acceptable method of investigating small-bowel disease. It therefore merits further investigation.

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Bile contains a mixture of mono-, di-, and tri-hydroxy acids, conjugated with taurine or glycine. The various free acids and their conjugates differ in a number of important respects, including the rates and sites of absorption and the extent to which they inhibit sodium reabsorption from the colon. No single tracer can be used as an index of all aspects of bile acid metabolism. It is therefore necessary first to define which natural process is to be traced. In the present study active ileal absorption is of particular interest.

Both free and conjugated bile acids are absorbed in the distal ileum by a specific, active, ionic transport process. The fraction of the bile salts that escapes reabsorption and passes into the large intestine is there subjected to two principal metabolic transformations by bacterial action: (a) deconjugation, resulting in the release of free bile acids; and (b) dehydroxylation with formation of one or more (secondary) bile acids. Free bile acids, both primary and secondary, are absorbed to some extent from both large and small bowel by passive nonionic diffusion. There is little nonionic diffusion of the glycine conjugates, and none of the taurine conjugates. Whether conjugated or unconjugated at the time of absorption, virtually all natural bile acids are conjugated in the liver before being excreted into the bile.

(24-^{14}C)cholic acid, (2,4-^{3}H)cholic acid, and their taurine conjugates have been used to study the intestinal absorption of bile salts under various pathological conditions (*1–6*). Fromm et al. have specified the need for a test substance that would be absorbed exclusively and actively in the ileum and whose physical and chemical states are not influenced by intraluminal factors (*3*). Such a substance could provide the basis of a test for assessing ileal absorptive function. However, the use of C-14- and H-3-labeled bile acids presents certain disadvantages, not the least being the need to collect and process feces. Hofmann (*7*) has emphasized the need for a labeled gamma-emitting compound that behaves like a bile acid and may be used to study enterohepatic circulation, especially ileal reabsorption.

The number of elements that could be used for this purpose, having both appropriate chemical properties

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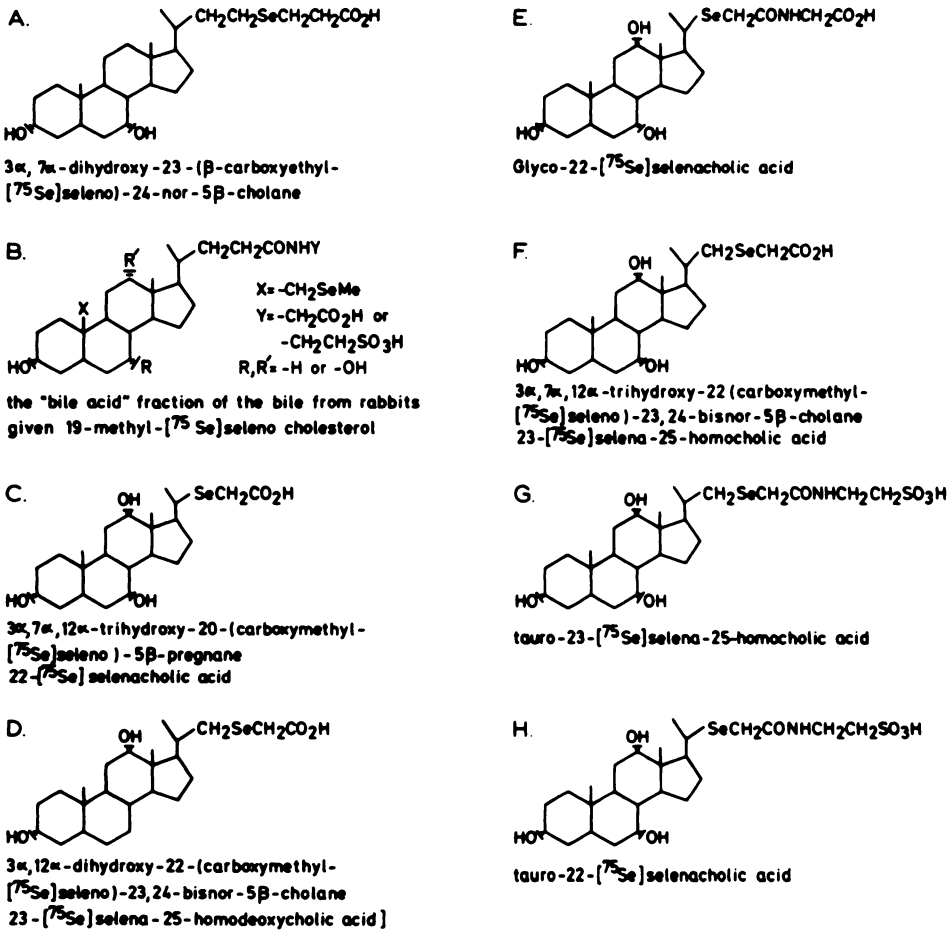

FIG. 1. Structural formulae of the compounds investigated.

and a convenient radionuclide, is limited. Potential emitters include selenium-75, tellurium-123m, iodine-131, and iodine-125. Bile acid analogs labeled with these nuclides, having biological half-times of 3 days and distributed in the body like natural bile acids, would result in absorbed doses to the small intestine of 11 mrads/μCi for Se-75, 16.1 mrads/μCi for Te-123m, 45.3 mrads/μCi for I-131, and 8.8 mrads/μCi for I-125 (Tuck, private communication).

However, the counting efficiency for selenium-75 is approximately twice those for tellurium-123m and iodine-131, and greater by an even larger factor in the case of iodine-125. A further disadvantage of iodine-131, in a study that may last 1–4 wk, is its shorter half-life. Thus to achieve similar counting statistics even at 1 wk, the absorbed dose from a compound containing I-131 would be substantially higher than that from one containing Se-75.

A further disadvantage of the iodine radioisotopes is the generally observed instability of aliphatic iodides in vivo. This instability, as evidenced by the I-131 cholesterols used for adrenal visualization, requires blocking of the thyroid gland to minimize uptake of liberated radioiodide. Thyroid-blocking agents inhibit bile acid synthesis, so their use would disturb the equilibrium under study. The superior imaging properties of tellurium-123m are not appropriate to the type of function study anticipated here, and the cost of the nuclide precludes its use.

Thus selenium-75 was considered to be the label of choice.

METHODS

Four bile acid analogs were synthesized, with selenium (as Se-75) replacing one of the methylene groups in the C17 side chain at positions C22, C23, and C24. A glycine conjugate of one of the acids, and taurine conjugates of two, were also synthesized. Our nomenclature for these compounds (Fig. 1 and Table 1) follows IUPAC guidelines. An eighth substance was prepared by administering 19-methyl-[^{75}Se]selenocholesterol intravenously to a rabbit with a cannulated bile duct. The acid component of the bile, which contained a mixture of bile acid analogs and conjugates, labeled with Se-75 in the C19 position, was separated. Compounds C, E, and H were very probably mixtures of the 20R and 20S isomers. All the compounds were synthesized and supplied by The

TABLE 1.

Designation	Full Chemical Name	"a" Nomenclature	Specific Activity
A	3α, 7α-dihydroxy-23-(β carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane	—	69.8 mCi/millimol
B	"Bile acid" fraction from a rabbit given 19-methyl-[^{75}Se]selenocholesterol	—	2500 mCi/millimol
C	3α, 7α, 12α-trihydroxy-20-(carboxymethyl-[^{75}Se]seleno)-5β-pregnane	22-[^{75}Se]selenacholic acid	95.3 mCi/millimol
D	3α, 12α-dihydroxy-22-(carboxymethyl-[^{75}Se]seleno)-23,24-bisnor-5β-cholane	23-[^{75}Se]selena-25-homodeoxycholic acid	83.2 mCi/millimol
E	—	glyco-22-[^{75}Se]selenacholic acid	86.9 mCi/millimol
F	3α, 7α, 12α-trihydroxy-22-(carboxymethyl-[^{75}Se]seleno)-23,24-bisnor-5β-cholane	23-[^{75}Se]selena-25-homocholic acid	73.0 mCi/millimol
G	—	Tauro-23-[^{75}Se]selena-25-homocholic acid (SeHCAT)	74.6 mCi/millimol
H	—	Tauro-22-[^{75}Se]selenacholic acid	87.8 mCi/millimol

TABLE 2. CHROMATOGRAPHIC SOLVENT SYSTEMS, ANALYTICAL R_f VALUES, AND RADIOCHEMICAL PURITIES

Solvent system	Compound	R_f	Radiochemical purity (%)
Chloroform, methanol (3:1)	F	0.42	93
	E	0.04	85
Chloroform, methanol (5:1)	A	0.65	97
	C	0.11	—
	D	0.36	95
	B	0.00	—
iso-octane, di-isopropyl ether, acetic acid (2:1:1)	A	0.41	—
	D	0.43	—
Dichloromethane, acetone, acetic acid (7:2:1)	C	0.22	95
Dichloromethane, acetone, acetic acid (7:2:1.5)	F	0.76	97
Dichloromethane, acetone, acetic acid (7:2:2)	G	0.05	—
n-butanol, water, acetic acid (60:25:15)	G	0.53	96.5
	H	0.51	92.5
n-butanol, water, acetic acid (10:1:1)	B	major 0.68	—
		minor 0.35	—

Radiochemical Centre (*8*). The radiochemical purities of the compounds were assessed by thin layer chromatography on 0.25-mm silica gel plates*; these were scanned by a 100-channel scanner and were visualized by both autoradiography and exposure to iodine vapor. Chromatographic solvent systems, analytical R_f values, and radiochemical purities are given in Table 2.

Animal studies. For each compound a mixture of C-14 cholic acid (sodium salt) and the test compound was dispensed in water with 2% ethanol added, at concentrations of 4 μCi/ml for the C-14 cholic acid and ~3 μCi/ml for each of the seleno compounds. The glycine conjugates were solubilized by the addition of 1.6 mg/nl sodium carbonate. The specific activities of the compounds are given in Table 1. All experiments were performed using young adult male rats. Those for the bile-duct cannulation experiments were an inbred Wistar strain reared in the Department of Biochemistry's animal facility. Those used for measurement of tissue distribution and whole-body counting were of a different, but inbred, Wistar strain or an outbred Sprague-Dawley derived strain.† The animals were fed standard rodent cake and water *ad libitum*.

Bile ducts were cannulated under ether anesthesia using a clean but not aseptic technique. After cannulation of the duct, the animals were kept in a restraining cage. These animals had their drinking water replaced by an electrolyte solution containing adequate quantities of sodium and potassium chlorides. One milliliter of the test mixture (~0.15 μmol of the test substance per kg body weight) was administered promptly at the end of the operation, either into the stomach by orogastric tube, or into a tail vein. The animals were kept at a temperature of 22°C; the lighting schedule was illumination from 8 a.m. to 8 p.m., otherwise darkness. Consecutive bile fractions were collected for 45- or 60-min intervals for 24 hr using an automatic sample collector connected to

TABLE 3. RECOVERY OF Se-75 RADIOACTIVITY IN BILE FOLLOWING ORAL ADMINISTRATION TO RATS WITH BILIARY CANNULAE*

	Compound	Recovery†
A	3α, 7α-dihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane	2.4 (0.88–3.8)
B	19-methylseleno bile salt mixture	23.5 (22.7–25.9)
C	22-[^{75}Se]selenacholic acid	28.0 (26.5–29.1)
D	23-[^{75}Se]selena-25-homodeoxycholic acid	31.2 (27.9–36.8)
E	glyco-22-[^{75}Se]selenacholic acid	49.3 (39.3–57.2)
F	23-[^{75}Se]selena-25-homocholic acid	103.0 (101.8–106.9)
G	Tauro-23-[^{75}Se]selena-25-homocholic acid (SeHCAT)	82.8 (79.1–91.1)
H	Tauro-22-[^{75}Se]selenacholic acid	80.7 (79.0–83.9)

* Expressed as % of the recovery of C-14 from simultaneously administered C-14 cholic acid.
† Each value is the mean and range of three animals, except six animals for Compound G.

the cannula. Samples were assayed for gamma radioactivity in an automatic well counter. Aliquots were decolorized for beta counting by the dropwise addition of hydrogen peroxide. They were then mixed with a commercial scintillation mixture‡ and, after stabilization, counted in an automatic beta counter. When calculating the results, correction was made for the detection of Se-75 by the beta counter.

Tissue distribution and whole-body retention was measured in groups of intact animals over a period of 10 days, and for compound G through 41 days using a small-animal counter. This consisted of two horizontally opposed NaI(Tl) scintillation detectors contained within a chamber shielded with 2 in. of lead. The distance between the two crystals was adjusted according to the count rate, and the animals were restrained in a plastic tube placed between them. Measurement of gastrointestinal transit time was made in the rats using a non-absorbable marker, I-131 polyvinyl pyrrolidone (PVP). At the end of the experiment the animals were killed and activities in the tissues counted using an automatic gamma well counter.

RESULTS

The recovery of Se-75 radioactivity from the bile of preparations with biliary cannulae, following oral administration of each of the test compounds, is given in Table 3. The results are expressed as a percentage of the recovery of C-14 cholic acid. Compounds F, G, and H in particular show high recoveries following oral administration. Table 4 gives details of the excretion in bile of Se-75 following intravenous administration of compounds C, F, G, and H to rats with cannulated bile ducts. The rates of appearance of Se-75 and C-14 radioactivity, following the oral administration of a mixture of compound G and C-24 cholic acid, are shown in Fig. 2. The tissue distribution of Se-75 radioactivity following oral administration of compound G (SeHCAT) is shown in Table 5.

The rats' retention data for each compound, as determined by whole-body counting, can be shown by graphical analysis to approximate to a function of the form: $\text{Retention}_{(t)} = ae^{-xt} + be^{-yt} + C$. The half-life of

TABLE 4. EXCRETION OF Se-75 RADIOACTIVITY IN BILE FOLLOWING I.V. ADMINISTRATION OF COMPOUNDS C, F, G, AND H TO RATS WITH CANNULATED BILE DUCTS

Compound	% Dose excreted in 2 hr	Peak (% dose/min)	Time after injection of peak (min)
C	96.5	7.8	5
F	99.1	10.3	4.5
G	99.3	15.3	4
H	97.6	10.7	4.5

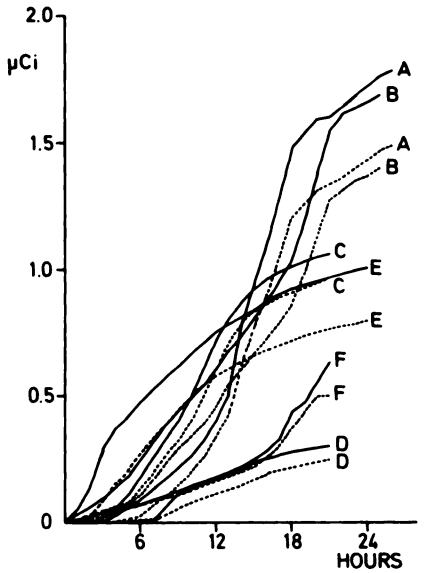

FIG. 2. Cumulative excretion in bile from cannulated rats (designated A, B, C, D, E, F) following oral simultaneous administration of C-14 cholic acid (solid lines) and SeHCAT (dotted lines). Administered activities were the same for each animal.

TABLE 5A. TISSUE DISTRIBUTION OF ^{75}SeHCAT IN RATS*

Organ	24 hr	48 hr	7 days
Brain	0.0076 ± 0.0014	0.0065 ± 0.0044	0.013 ± 0.004
Skin	0.033 ± 0.019	0.034 ± 0.020	0.023 ± 0.012
Lung	0.085 ± 0.056	0.049 ± 0.034	0.056 ± 0.025
Fat	0.017 ± 0.007	0.024 ± 0.019	0.010 ± 0.006
Muscle	0.023 ± 0.008	0.018 ± 0.007	0.013 ± 0.006
Heart	0.047 ± 0.008	0.037 ± 0.024	0.039 ± 0.025
Liver	0.398 ± 0.064	0.338 ± 0.069	0.186 ± 0.008
Spleen	0.040 ± 0.005	0.045 ± 0.021	0.063 ± 0.031
Blood	0.061 ± 0.014	0.049 ± 0.032	0.054 ± 0.024
Kidney	0.191 ± 0.018	0.159 ± 0.110	0.198 ± 0.110

TABLE 5B. CUMULATIVE EXCRETION OF ^{75}SeHCAT BY RATS AFTER 10 DAYS

Urine	0.58 ± 0.22%
Feces	97.2 ± 0.1%

* Expressed as % of administered dose/g wet weight of tissue ± s.d. (n = 5).

each component and the value of C are shown for each of the compounds in Table 6. A whole-body retention curve for compound G is shown in Fig. 3.

DISCUSSION AND CONCLUSIONS

In general, pathways of absorption are more specific than those of excretion. Many substances are rapidly excreted by the liver into the bile following i.v. administration—for example Tc-99m HIDA derivatives or rose bengal—but few compounds are reabsorbed and fewer still reabsorbed and re-excreted unchanged. Thus although there are many gamma-labeled tracers suitable for studying the excretory function of the liver, hitherto there have been none suitable for studying the complete enterohepatic circulation. Of the eight substances in the present study, only one, Compound A, differs radically from cholic acid. All of the others were absorbed from the gut and excreted into the bile to a significant extent, the patterns of appearance in the bile in each case resembling that of the reference compound, cholic acid. The recoveries of these compounds from the bile, relative to the reference compound, varied between 20 and 100%, being greater than 80% for compounds F, G, and H. These last three were rapidly and wholly excreted into bile following intravenous administration (Table 4), the rates of clearance being similar to that for cholic acid. The similarity of these compounds to cholic acid—both in the patterns of appearance in the bile following oral administration and in the rates of clearance by the liver

TABLE 6. ANALYSIS OF RAT WHOLE-BODY RETENTION DATA

Compound	$t_{1/2}$ (days) (ae^{-xt})	$t_{1/2}$ (days) (be^{-yt})	C(%)
A	0.30	1.31	4.0
B	0.33	1.12	6.0
C	0.275	1.82	0.80
D	0.30	1.07	3.36
E	0.45	1.28	0.58
F	—	1.3	1.5
G	—	1.8	>0.2*
H	0.55	2.8	1.2

* Monitoring of Compound G over an extended period of 41 days revealed a slower clearing component (2% of dose with $t_{1/2}$ of 5–10 days) and C >0.2%.

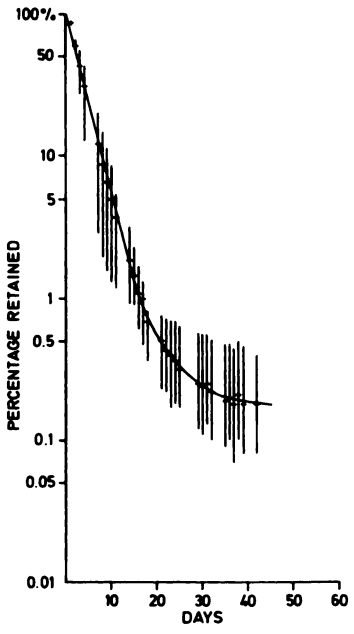

FIG. 3. Whole-body retention of SeHCAT in rats. Horizontal bars indicate mean values, vertical lines observed range. Five animals per point.

following intravenous administration—indicates that the rates of intestinal absorption must be similar.

Tissue distribution studies (data given only for Compound G) indicate that the compounds are confined almost entirely to the enterohepatic circulation and are eliminated in the feces. The whole-body excretion of all of the compounds can be described as the sum of a series of exponentials, and Table 6 gives the half-times for each component. The half-times of the fast components were in all cases longer than that of the unabsorbed marker I-131 polyvinyl pyrrolidone (about 0.17 days). However, half-times obtained by "stripping" of exponentials represent the true half-times of the separate compartments only if these are in parallel. If they are in series (as other body compartments are to the enterohepatic circulation), there is no simple relationship between the results obtained by curve-stripping and the true rate constants for the individual compartments. It is noteworthy that for compounds F and G the fast-clearing component, which is equivalent to that of a nonabsorbed tracer, is absent and that about 98% of Compound G (SeHCAT) is monoexponentially excreted by rats, with a half-time of about 2 days (Fig. 3). Certainly the whole-body clearance curve for G is consistent with the constant fractional elimination of a nonmetabolized compound from a single compartment, i.e., the enterohepatic circulation.

In choosing a Se-75 labeled bile acid analog for investigation of ileal dysfunction, it was considered that a primary bile acid would be most appropriate and that an analog based on cholic acid would be preferable to one based on chenodeoxycholic acid. 7-α-dehydroxylation of the former yields deoxycholic acid, which subsequently behaves much as the primary bile acids do, whereas the latter yields lithocholic acid, a bile acid having somewhat different properties. Compound G (SeHCAT) was chosen for more intensive investigation in clinical studies. This choice was made for the following reasons.

1. A taurine conjugate was required in order to minimize nonionic passive diffusion and thereby maximize active ileal absorption.

2. Compound G, if it were deconjugated, would give rise to the free acid F, having better absorption characteristics than the free acid C.

3. Compounds C, E, and H presented a certain problem in that each probably consisted of a mixture of the 20R and 20S isomers.

4. Owing to steric hindrance at the 20-carbon atom, Compounds C, E, and H were more difficult to prepare.

The behavior of SeHCAT in rats sufficiently mimics that of natural conjugated bile acids to justify its further investigation.

FOOTNOTES

* Merck 60 F254 TLC plates.

† From the Animal Facility of the Western General Hospital or the Radiochemical Center, Amersham.

‡ Instagel, Packard Co.

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