Metabolism of Iso-Bile Acids in the Rat*

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Iso-bile acids, the 3β-hydroxy epimers of the normally occurring bile acids, are commonly found in the feces of man and other animals but are not present in the bile. Since isochenodeoxycholic acid and isoursodeoxycholic acid were recently isolated from the feces of patients treated with chenodeoxycholic acid or ursodeoxycholic acid, the intestinal absorption and hepatic metabolism of [24-'4C]isochenodeoxycholic acid, [3α-3H]isoursodeoxycholic acid, and their tauro conjugates were studied in rats with biliary or portal blood diversion. Bile acids were isolated from bile, feces, and portal blood, and their radioactivity and quantity were determined by scintillation spectrometry, thin layer chromatography, and gas-liquid chromatography. After intraduodenal or intracecal infusion of the labeled iso-bile acids, 80 to 90% of the radioactivity was recovered in the bile during 5 h. However, all the label was excreted with the normally occurring 3α-hydroxy bile acid, chenodeoxycholic acid or ursodeoxycholic acid, and only trace amounts of iso-bile acids were detected in the bile. In contrast, when portal blood and feces were examined, label was found only in the 3β-epimer that was infused. Our findings show that isochenodeoxycholic acid, isoursodeoxycholic acid, and their tauro conjugates are well absorbed from both the small and large intestine of the rat and are transported unchanged via the portal blood to the liver. Both iso-bile acids undergo hepatic transformation to their corresponding 3α-hydroxy epimers during hepatic passage. Thus, the absence of iso-bile acids from the bile results from hepatic transformation rather than from impaired absorption or intestinal conversion.

Iso-bile acids, the 3β epimers of 5β-cholanoic acids (Fig. 1a), are excreted in significant amounts in the feces of man and other animals (1, 2). Isocholic acid has been detected in the feces of man and dog (3), but isochenodeoxycholic acid has been found only as a component of human feces (4). Although Kimura (5) has reported the presence of the 3β-epimer of hyodeoxy bile acid in pig bile and gallstones, no other 3β-hydroxy bile acid was detected in the bile of any other species. Hellström and Sjövall (6), in their studies on the turnover of deoxycholic acid in the rabbit, have concluded that the 3β-epimer of deoxycholic acid found in the feces was formed by the action of intestinal microorganisms (7) and that it was not reabsorbed from the intestine.

In recent years chenodeoxycholic acid (Fig. 1b) has been extensively used for the dissolution of gallstones in patients with cholelithiasis. During this treatment relatively large amounts of ursodeoxycholic acid were found in the bile of some subjects (8). It was further demonstrated that UDCA, which differs from CDCA only in the configuration at carbon-7, is effective in the medical therapy of cholesterol cholelithiasis, and might be more potent and safer than CDCA as a gallstone-dissolving agent. Both iso-CDCA and iso-UDCA, which differ from their respective normally occurring bile acids only in the configuration at carbon-3, have been recently isolated in our laboratory from the feces of some patients treated with either CDCA or UDCA, and were considered as potential gallstone-dissolving agents.

The present study was designed to examine the hepatic metabolism and intestinal absorption of both iso-bile acids in the rat animal model, and to test the hypothesis that hepatic enzymes can catalyze the epimerization of the 3β-hydroxyl group of these bile acids.

**EXPERIMENTAL PROCEDURES**

**Materials**

Preparation and Purification of [24-14C]3β,7α-Dihydroxy 5β-cholanic Acid (Iso-CDCA, Specific Activity 2.1 x 10^7 dpm/mg; Fig. 1a)

[24-14C]Iso-CDCA was synthesized in a single-step reaction by a modified procedure of the inversion-esterification method developed by Bose et al. (9). [24-14C]CDCA (100 μCi, New England Nuclear) was diluted with 1.0 g of unlabeled CDCA and methylated with methanolic HCl (10). The 14C-labeled methyl CDCA was dissolved in 50 ml of dry benzene and refluxed with 1.8 g of triphenylphosphine, 0.3 ml of formic acid, and 1.2 ml of diethylcarboxylate for 24–36 h. The completion of the reaction was monitored by TLC as described below. The resulting formoxy derivative of methyl iso-CDCA was purified by chromatography on a column (20 x 2.0 cm) of Florasil (100–200 mesh, Fisher). The column was eluted with increasing amounts of ethyl acetate in benzene, and the methyl 3β-formoxy-5α-hydroxy-5β-cholanic acid was found in the fractions eluted with 3% ethyl acetate in benzene (ν/ν). (Care was taken to complete the chromatography within 5–6 h in order to avoid possible deformation of the iso-bile acid.) The column was monitored by TLC, following mild hydrolysis with 0.5 x KOH in methanol for 20–30 min at room temperature.

**Results**

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Metabolism of Iso-Bile Acids

Biliary Bile Acid Analysis

A. Enzymatic Hydrolysis—The volume of each 1-h bile sample was measured, its radioactivity determined, and 0.5-ml aliquots were deproteinized with 9 volumes of methanol and hydrolyzed with cholyglycine hydrolyase as follows. The dry residue obtained after deproteination was dissolved in 0.4 ml of 0.025 M acetate buffer (pH 5.6) and incubated with 3 mg of cholyglycine hydrolyase (Sigma) in the presence of 2-mercaptoethanol and EDTA according to Nair et al. (17). The reaction was terminated by the addition of 0.5 ml of 6 N HCl. The products were extracted with ethyl acetate (3 X 2 ml), and the combined extracts were washed to neutrality and evaporated to dryness. The residue was dissolved in an appropriate volume of HCl in dry methanol (10) and chromatographed on Silica Gel G plates; solvent system: chloroform:acetone:methanol, 70:20:2.5 (v/v/v; 2X developed). Authentic unlabeled methyl esters of mono-, di-, and trihydroxy bile acids were applied to each side of the plate as markers. The spots were made visible with spray reagent consisting of 5.5% phosphomolybdic acid in isopropanol alcohol, and the distribution of radioactivity was determined after removing individual spots from the TLC plates and measuring their radioactivity in a liquid scintillation counter (Intertechnique SL 4000, Fairfield, NJ) (18). The following RF values of the methyl derivatives were observed: methyl CDCA, 0.63; methyl iso-CDCA, 0.81; methyl UDCA, 0.74; methyl iso-UDCA, 0.69.

B. Alkaline Hydrolysis—Bile aliquots (0.5 ml) were deproteinized, hydrolyzed with 10% aqueous NaOH for 3 h at 116 °C in an autoclave (15 psi), and extracted as described by Evalard and Janssen (19). The free cholic acids were methylated, separated by TLC, and their radioactivity determined as described above.

Fecal Bile Acid Analysis

Feces and intestinal contents were pooled, dried, and extracted with ethanol in a Soxhlet extractor for 48 h, and the radioactivity was recorded. After separation of the acidic steroids from the neutral ones, saponification under pressure, extraction, and methylation (10), the samples were applied to a preparative TLC plate for isolation of bile acid methyl esters as described above for the biliary bile acids. Radioactivity was measured in each bile acid fraction.

Serum Bile Acid Analysis

One volume of serum (0.1-0.5 ml) was diluted with 9 volumes of 0.1 M NaOH in 0.9% NaCl and percolated through a column of Amberlite XAD-2 (Servachrom XAD-2, 100–200 μm, Heidelberg, Germany) (20). The eluate was evaporated followed by deconjugation and extraction of bile acids as described for bile.

Animals and Operative Procedures

All animals in these experiments were 200- to 250-g male Sprague-Dawley rats maintained on laboratory Purina rat chow ad libitum unless otherwise specified. After an overnight fast, each animal was anesthetized with sodium pentobarbital (Fort Dodge Laboratories, Inc., Fort Dodge, IA), and the following operative procedures were performed either singly or in combination. (a) The common bile duct was cannulated above the level of the pancreas, and the polyethylene catheter (outer diameter 0.024 inch) was exteriorized through a stab wound in the lower part of the abdomen. (b) For the purpose of sampling portal blood, a cannula (outer diameter 0.038 inch) was passed into the portal vein via the mesenteric vein and taped in place for the duration of the experiment. (c) For the purpose of infusing test solutions a plastic catheter (outer diameter 0.024 inch) was secured either into the proximal end of the duodenum or directly into the cecum by means of a purse string suture and exteriorized through the lower part of the abdomen. The specific combination of these operative procedures performed in each group is described in detail for each experiment. After surgery the abdomen of each animal was allowed to recover consciousness in a restraining cage, where it remained for the duration of the subsequent experimental period.

Experimental Design

Three types of experiments were carried out. (a) Immediately following biliary diversion, the animals were pulse infused directly into the duodenum or into the cecum with a suspension of 5 mg of labeled iso-CDCA, iso-UDCA, or their respective taurine conjugates. Bile was collected for 24 h at 1-h intervals, and total radioactivity in...
the bile was recorded. The rate of intestinal absorption was evaluated from the proportion of label recovered in the bile compared to the bile was recorded. The rate of intestinal absorption was evaluated from the proportion of label recovered in the bile compared to the bile infused. (b) The second type of experiment was similar to the first, except that the distribution of radioactivity in the individual bile acids was measured. We reasoned that the presence of label in the bile and its association with the same bile acids that were infused would indicate that the tested iso-bile acids were absorbed intact from the intestine. On the other hand, if the label is not associated with the same iso-bile acids that were infused, they must have been transported either in the intestine or during hepatic passage. In this case, a third type of experiment is indicated in order to locate the site of transformation. (c) Immediately following cannulation of the portal vein, the animals are pulse infused with a suspension of the labeled iso-bile acids, and portal blood samples are collected intermittently during a 3-h period. At the completion of the experiment, feces and intestinal contents are pooled, and the radioactivity of the individual bile acids is measured in portal blood and feces. The presence of label in portal blood and its association with the same bile acids that were infused would indicate that these iso-bile acids were transported unchanged via the portal blood, and any change observed previously in the biliary bile acids can be attributed to hepatic transformation. Furthermore, the distribution of label in the fecal bile acids would indicate the role of intestinal bacteria in the metabolism of iso-bile acids.

RESULTS

Validation of Analytical Methods

In order to establish the efficiency of the hydrolysis of the tauro-conjugated iso-bile acids and the recovery of the corresponding free acids from bile, feces, and serum the following experiments were carried out. 5 mg of [24-1C]tauroiso-CDCA or 5 mg of [3a-3H]tauroiso-UDCA were added to an aliquot of bile, feces, or serum and were deconjugated either with cho-lyglycine hydrolase or under alkaline conditions. The free iso-bile acids were isolated by TLC and their radioactivity determined as described under “Methods.” As shown in Table I the enzymatic procedure as well as the chemical hydrolysis resulted in a higher than 96% recovery of both iso-bile acids from rat serum. Similar results were obtained when the labeled tauro-conjugated iso-bile acids were added to an aliquot of bile (0.5 ml) or feces (0.5 g). The recovery of both 14C-labeled and 3H-labeled compounds amounted to >95% from bile and >94% from feces. No radioactive CDCA, UDCA, or other compounds could be detected.

Identification of the Labeled Products Isolated from Portal Blood, Bile, and Feces Following the Administration of 14C-labeled or 3H-labeled Iso-Bile Acids

Samples of portal blood, bile, or feces were collected following intraduodenal or intracecal pulse infusions of [24-1C]iso-CDCA or [3a-3H]iso-UDCA. Known aliquots were saponified and extracted as described under “Methods,” and the radioactive products were separated and identified by a combination of TLC and GLC-MS as follows. The bile acids were applied to a TLC plate (Silica Gel G, 25-mm) without the addition of carriers and developed with benzene:dioxan:glacial acetic acid, 75:25:2 (v/v/v). Authentic unlabeled bile acids (10 µg each) were applied to each side of the plate as markers, and were made visible with spray reagent that consisted of 3.5% phosphomolybdic acid in isopropanol. Retardation factor values of the reference compounds used were: CDCA, 0.25; iso-CDCA, 0.38; UDCA, 0.29; and iso-UDCA, 0.32. The pertinent bands were eluted with methanol and analyzed as the Me3Si-ether methyl derivatives by GLC and mass spectrometry (16), as illustrated below for iso-CDCA and iso-UDCA obtained from portal blood or feces and for CDCA and UDCA isolated from bile. The retention times determined by GLC, relative to 5α-cholestan (3.2 min) were: CDCA, 2.54; iso-CDCA, 2.03; UDCA, 3.35; and iso-UDCA, 3.07. The mass spectra are shown in Table II and were identical with those of the corresponding authentic reference compounds. In addition to CDCA and its 3β-epimer isolated from bile, 2–3% of the total radioactivity was present in α-muricholic acid, and 6–10% in β-muricholic acid following labeled iso-CDCA administration. The muricholic acids were identified by a combination of TLC and GLC-MS as follows. The biliary bile acids were applied to a 25-mm Silica Gel G plate as the methyl ester derivatives, without the addition of carriers, and developed in acetone:benzene, 2:3 (v/v); Rf values: methyl α-muricholate, 0.19; methyl β-muricholate, 0.23. After sution with methanol, the identity of the pertinent bands, corresponding to known reference compounds, was confirmed by GLC mass spectrometry (21).

Radioactive Purity of the 14C-labeled and 3H-labeled Products

To establish the radioactive purity of the 3β-hydroxy bile acids isolated from portal blood and feces, an aliquot from the pertinent band (see above) was diluted with a known amount of the corresponding unlabeled bile acid, and the specific radioactivity was determined by GLC and scintillation counting. The radioactive purity was established by reverse isotope dilution of the free bile acids and their methyl derivatives as shown in Table III for [24-14C]iso-CDCA and [3a-3H]iso-UDCA isolated from portal blood. The specific radioactivity of both iso-bile acids remained constant within the precision of measurement (±5%). The specific radioactivity of the fecal

<table>
<thead>
<tr>
<th><strong>Table II</strong></th>
<th><strong>Bile acid derivatives analyzed by mass spectrometry</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fragment</strong></td>
<td><strong>m/z</strong></td>
</tr>
<tr>
<td>M*</td>
<td>550</td>
</tr>
<tr>
<td>M-15</td>
<td>535</td>
</tr>
<tr>
<td>M-90</td>
<td>460</td>
</tr>
<tr>
<td>M-(90 + 15)</td>
<td>445</td>
</tr>
<tr>
<td>M-(2 x 90)</td>
<td>370</td>
</tr>
<tr>
<td>M-(2 x 90 + 15)</td>
<td>355</td>
</tr>
<tr>
<td>M-(2 x 90 + 31)</td>
<td>338</td>
</tr>
<tr>
<td>B-ring scission</td>
<td>262</td>
</tr>
<tr>
<td>M-(2 x 90 + 115 side chain)</td>
<td>235</td>
</tr>
</tbody>
</table>

The bile acids were analyzed as the Me3Si-ether methyl derivatives (16) (see under “Experimental Procedure”).
iso-bile acids was determined in the same manner and was found to be constant throughout the multiple crystallizations. Furthermore, in order to establish the location of the isotope in the \([\text{H}]\)iso-UDCA, it was oxidized selectively at C-3 with silver carbonate on Celite as described by Dayal et al. (11). No radioactivity was detected in the resulting 3-keto-7β-hydroxy-5β-chenodeoxycholic acid which indicated that the \(\text{H}\) was localized solely at C-3.

Similarly, the radioactivity purity of the biosynthetic 3α-hydroxy bile acids, isolated from bile, was established by reverse isotope dilution techniques. After addition of carrier, \([24-\text{I}^{14}\text{C}]\)CDCA was crystallized from ethyl acetate-heptane, and its methyl ester derivative was further subjected to column chromatography on Florasil, as described under “Experimental Procedures” for the preparation of iso-CDCA [methyl CDCA was eluted with 5% ethyl acetate in benzene (v/v)]. Likewise, following dilution with the corresponding bile acid, the biosynthetic [3β-\text{H}]UDCA, as well as its methyl ester derivative, were crystallized from ethyl acetate. The specific radioactivity of both products remained constant throughout the procedure.

**TABLE III**

<table>
<thead>
<tr>
<th>Labeled bile acids isolated</th>
<th>Specific radioactivity of iso-bile acids</th>
<th>dpm/μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([24-\text{I}^{14}\text{C}])iso-CDCA</td>
<td></td>
</tr>
<tr>
<td>From preparative TLC</td>
<td>82,450</td>
<td>1,099,300</td>
</tr>
<tr>
<td>After addition of carrier</td>
<td>430</td>
<td>1,370</td>
</tr>
<tr>
<td>1st crystallization</td>
<td>410</td>
<td>1,345</td>
</tr>
<tr>
<td>2nd crystallization</td>
<td>442</td>
<td>1,410</td>
</tr>
<tr>
<td>After methylation</td>
<td>420</td>
<td>1,329</td>
</tr>
<tr>
<td>1st crystallization</td>
<td>465</td>
<td>1,380</td>
</tr>
<tr>
<td>2nd crystallization</td>
<td>425</td>
<td>1,365</td>
</tr>
</tbody>
</table>

\(\text{[24-\text{I}^{14}\text{C}]iso-CDCA and [3α-\text{H}]iso-UDCA were extracted from portal blood, separated by TLC, and the specific radioactivity determined by GLC and scintillation counting (see under “Experimental Procedure”). The identity of the products was confirmed by GLC-MS before the addition of carrier, and the location of the isotope in the ([H]iso-UDCA was verified by selective oxidation at C-3 (11).}

\(\text{An aliquot containing 0.025 μmol of [24-\text{I}^{14}\text{C}]iso-CDCA was diluted with 5.0 μmol of unlabeled iso-CDCA.}

\(\text{An aliquot containing 0.025 μmol of [3α-\text{H}]iso-UDCA was diluted with 20.0 μmol of unlabeled iso-UDCA.}

\(\text{Crystallized from ethyl acetate-hexane.}

\(\text{The isolated iso-CDCA or iso-UDCA was methylated, separated by TLC, and the specific radioactivity determined by GLC and scintillation counting as described under “Methods.”}

\(\text{Crystallized from acetone-ethyl acetate.}

**TABLE IV**

<table>
<thead>
<tr>
<th>Bile acid infused</th>
<th>Label found in</th>
<th>Bile acid isolated from</th>
<th>dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bile</td>
<td>Portal blood</td>
</tr>
<tr>
<td>([24-\text{I}^{14}\text{C}])iso-CDCA</td>
<td>955 ± 245</td>
<td>35 ± 2</td>
<td>16 ± 0.5</td>
</tr>
<tr>
<td>([3α-\text{H}])iso-UDCA</td>
<td>7500 ± 372</td>
<td>80 ± 5</td>
<td>22 ± 1.1</td>
</tr>
</tbody>
</table>

\(\text{The identity of the various bile acids isolated from bile, portal blood, and feces was confirmed by GLC-MS, and the radioactive purity was established by reverse isotope dilution techniques, as described in Table III. The location of the \(\text{H}\) in the labeled UDCA and iso-UDCA was demonstrated by selective oxidation at C-3 as described under “Results.”}

\(\text{Bile samples were also analyzed for the presence of sulfated labeled bile acids (30). No sulfates could be detected.}

\(\text{In addition to CDCA or UDCA and their 3α-epimers isolated from feces, approximately 10% of the total radioactivity was detected in isolithocholic acid. This monohydroxy-isobile acid was isolated by TLC as its methyl derivative in an identical manner as described for iso-bile acids; \(R_t\) = 0.82. The identity and radioactive purity of isolithocholic acid was confirmed as described under “Results.”}

\(\text{Following the administration of [24-\text{I}^{14}\text{C}]iso-CDCA, two additional labeled bile acids were detected in the bile: \(\alpha\)-muricholic acid (2-3%) and \(\beta\)-muricholic acid (6-10%) (see under “Results”). No labeled \(\alpha\)-muricholic acid could be detected following [3α-\text{H}]iso-UDCA infusion.}

\(\text{([24-\text{I}^{14}\text{C}]CDCA, 955 ± 42; [3β-\text{H}]UDCA, 2100 ± 98 dpm/μmol). In addition, the retention of tritium at C-3 of the labeled UDCA following hepatic epimerization was verified by selective oxidation as described above. Furthermore, the biosynthetic labeled muricholic acids exhibited no depression of the specific radioactivity following dilution with the appropriate authentic bile acids as previously described (22, 23).}

A. **The Rate of Intestinal Absorption**—The rate of absorption of \([24-\text{I}^{14}\text{C}]\)iso-CDCA or \([3α-\text{H}]\)iso-UDCA injected below the surface of 1.0 ml of isotonic saline to form a suspension. The radioactivity in the individual bile acids was determined as described under “Methods.” The data in the table represent the average of five rats ± S.D.

\(\text{FIG. 2. Intestinal absorption. Percentage of total radioactivity recovered in bile following intraduodenal pulse infusion of a suspension of 5 mg of [24-\text{I}^{14}\text{C}]iso-CDCA (specific activity: 2.1 × 10^6 dpm/μg) (●), or [3α-\text{H}]iso-UDCA (specific activity 2.8 × 10^6 dpm/μg) (○).}

B. **Distribution of Radioactivity in Bile Acids Isolated from Bile**—The distribution of radioactivity in the individual bile acids isolated from bile of rats with biliary diversion is illustrated in Table IV and Fig. 3a. Following intraduodenal pulse infusion of [24-\text{I}^{14}\text{C}]iso-CDCA or [3α-\text{H}]iso-UDCA, virtually all of the radioactivity excreted in the bile was recovered in the normally occurring 3α-hydroxy epimer, CDCA or UDCA, and only trace amounts of the 3β-hydroxy epimers which were administered could be detected (Table IV). Similarly, when the taurine conjugates of these iso-bile acids were infused, the label recovered from the bile was consistently associated with the commonly occurring 3α-epimers and not with the iso-bile acids that were infused (Fig. 3a). These results indicate that the administered iso-bile acids underwent...
Metabolism of Iso-Bile Acids

It has been suggested that iso-bile acids, epimeric at carbon-3 (Fig. 1), are formed from their 3α-hydroxy isomers by the action of intestinal microorganisms and are not reabsorbed from the intestine (6, 7). As outlined in the introduction, however, there are few definitive data to support this thesis, which was based upon the finding that radioactive isodeoxycholic acid could be isolated only from the fecal extract of a rabbit following the administration of [24,4-14C]deoxycholic acid (2). The bile of this rabbit did not contain any 3β,12α-dihydroxy-5β-cholanoic acid, which was attributed to its not being reabsorbed from the intestine. In contrast, data presented in the present study demonstrate that iso-CDCA and iso-UDCA are well absorbed from the small and large intestine of the rat (Fig. 2). Virtually all the radioactivity that was recovered in the bile was accounted for during the first 5-6 h following intraduodenal or intracecal pulse infusion of both iso-bile acids.

It is well known that the taurine conjugates of the 3α-hydroxy bile acids are quantitatively hydrolyzed either under drastic alkaline conditions (19) or by cholyglycine hydrolase (17). Therefore, we validated these two methods of deconjugation (Fig. 3a) for the 3β-hydroxy bile acids, iso-CDCA and iso-UDCA, with special emphasis on the retention of configurational state at carbon-3. As shown under "Results," over 97.5, 94, or 96% of the radioactivity was extracted from bile, feces, or serum, respectively, and almost all the label was associated with either iso-CDCA or iso-UDCA following alkaline hydrolysis. Virtually no label could be detected in CDCA or UDCA, indicating that this saponification is efficient and does not lead to epimerization of the 3β-hydroxyl group to the respective 3α-epimer. Similarly, cholyglycine hydrolase was found to be effective in the deconjugation of both iso-bile acids, which demonstrated that the 3β-configuration did not affect the efficiency of the enzymatic hydrolysis.

The identity and radioactive purity of the iso-bile acids isolated from portal blood are summarized in Table III. The purity of a compound was considered established whenever (a) it exhibited a single peak by GLC before the addition of carrier; (b) its mass spectrum was identical with that of the pure authentic compound; and (c) its specific radioactivity was constant, after dilution with the corresponding bile acid and multiple crystallizations. Each of the 14C-labeled and 3H-labeled iso-bile acids isolated from portal blood and feces exhibited a single peak by GLC (see under "Results"). The mass spectra were identical with those of the corresponding authentic 3β-hydroxy epimers and could be distinguished from the commonly occurring 3α-hydroxy epimers at m/z 460 (M-90, 370 (M-2 x 90) + 115 side chain) for CDCA and iso-CDCA and at m/z 535 (M-15), 370 (M-2 x 90), 255 (M-2 x 90 + 115 side chain), and 243 (C-3 to C-7) for UDCA and iso-UDCA (see Table II). The specific radioactivities remained constant after TLC purification, dilution with the corresponding authentic compounds, and multiple crystallizations of the free acids and their methyl derivatives. The identity and radioactive purity of [24,4-14C]CDCA and [3β,12α-3H]BDA isolated from bile was likewise confirmed.

Although iso-CDCA and iso-UDCA are less soluble in aqueous solutions than the corresponding 3α-isomers, they were efficiently absorbed by the small and large intestine as demonstrated in Fig. 2. Virtually all the radioactivity recovered from portal blood was associated with the original iso-bile acid that was infused, and almost no label was detected in the corresponding 3α-epimer (Table IV). These results indicate that iso-bile acids are absorbed intact and are not transformed by the intestine. In contrast, 98% of the radioactivity in bile was present in iso-CDCA and only 2% remained as iso-UDCA. Similarly, over 97% of the radioactivity in the bile following the infusion of [3α,·3H]iso-UDCA was recovered as UDCA, and only 3% was associated with the 3β-epimer that was infused. It was, therefore, concluded that the 3β-hydroxyl group in both iso-bile acids was transformed to the respective 3α-isomer during hepatic passage. Tauro conjugation of iso-CDCA and iso-UDCA did not affect their intestinal absorption and hepatic transformation (Fig. 3, a and b). Ninety-five percent of the label in portal blood was still associated with the taurine-bile acids that were infused, while the radioactivity in bile was present in CDCA or UDCA, indicating that conjugation does not prevent hepatic transformation.

Little is known about the mechanism involved in the formation of iso-CDCA or iso-UDCA from their 3α-epimers by intestinal bacteria. It is possible, however, that this transformation is analogous to the bacterial epimerization at carbon-7 involving either a keto-bile acid or unsaturated 5β-cholanoic acids as intermediates (24-26). Likewise the precise biochemical pathway and intermediates participating in the hepatic inversion of the hydroxyl group at carbon-3 of the iso-bile acids have not been elucidated. Liver alcohol dehydrogenase, or one of its iso-enzymes, has been shown to be active as a 3β-hydroxy-5β-steroid dehydrogenase, and the resulting 3-oxo-compound was further reduced to form metabolites with a 3α-hydroxyl group (27). Similarly, epimerization at carbon-7 was shown to proceed via a keto intermediate (28, 29). In the present experiments, however, when [3α,·3H]iso-UDCA was administered to rats with biliary diversion, the biosynthetic 3α-epimer isolated from bile was labeled at C-3 as was confirmed by selective oxidation (see under "Results"). This would not be possible if 3-keto-3β-hydroxy-5β-cholanoic acid

![Fig. 3. Distribution of radioactivity in bile acids isolated from (a), bile, or (b), portal blood, following pulse infusion with [24,4-14C]tauroiso-CDCA (I), or [3α,·3H]tauroiso-UDCA (II). The solid bars represent the percentage of radioactivity present in tauro-CDCA and UDCA (3α-epimers); the clear bars represent the taurine conjugates of iso-CDCA and iso-UDCA (3β-epimers). The data in the figure represent the average of five rats.](http://www.jbc.org)
was an intermediate. Thus, an alternate pathway is suggested that involves either direct epimerization of the 3β-hydroxyl group or the formation of a Δ1 or Δ3 unsaturated intermediate.

Although there is scant documentation of the presence of iso-bile acids in human feces, the increasing use of CDCA and UDCA and the demonstration of their convertibility into iso-bile acids mandates a fuller investigation of the metabolism and biological significance of these bile acids in man.

In summary, the results of the present study demonstrate that (a) iso-bile acids are efficiently absorbed from the intestine and are transported unchanged by the portal blood, (b) iso-bile acids undergo hepatic transformation to the normally occurring bile acids, and (c) taurine conjugation does not prevent this transformation during hepatic passage. Thus, the absence of iso-bile acids from bile results from hepatic transformation rather than impaired absorption or intestinal isomerization.

Acknowledgment—We are grateful to Dr. G. Stephen Tint for his contribution in determining the mass spectra of the biosynthetic bile acids.

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