Metabolites of All-trans-retinoic Acid in Bile: Identification of All-trans- and 13-cis-retinoyl Glucuronides*

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The metabolites of all-trans-[3H]retinoic acid were studied in the rat bile. In the bile duct cannulated rat 80 to 86% of the dose is excreted into the bile within 24 h after the intravenous administration of either a physiological or pharmacological dose of all-trans-[3H]retinoic acid. Polar metabolites of retinoic acid predominated (73 to 96% of bile 3H) in the bile at all times; some free retinoic acid (0.2 to 4.8% of bile 3H) is excreted into the bile during the initial 6-h period after dosing. Retinoyl glucuronides are excreted into the bile for 12 h after dosing; their excretion is highest during the first 2 h. More retinoyl glucuronide is excreted from the pharmacological dose (10% of the radiolabel dose) than from the physiological dose (4.4% of the dose) of all-trans-retinoic acid. The retinoyl glucuronides were separated and were shown to consist of all-trans- and 13-cis-retinoyl glucuronides in a ratio of 1.5 to 1.0. Since the starting material was 99% all-trans-retinoic acid and the methods used do not cause isomerization, it is concluded that cis-trans isomerization of retinoic acid is a physiologically relevant reaction.

Studies on the metabolism of retinoic acid are of considerable importance because of the possibility that this physiologically occurring retinoid may be the active form of vitamin A, and because it has therapeutic potential in the carcinogenesis process (1, 2). Recent studies suggest that the rapid disappearance of administered retinoic acid (3-6) is associated with the appearance of many metabolites in the plasma and bile of rats (7, 8). One of the identified biliary metabolites of retinoic acid is retinoyl glucuronide (7, 9); it accounts for 12% of biliary metabolites of retinoic acid after a pharmacological dose (7). These studies have also suggested that the retinoyl glucuronide present in bile is a mixture of all-trans and 13-cis isomers.

The present report demonstrates that 13-cis- and all-trans-retinoyl glucuronides are in vivo metabolites of all-trans-retinoic acid and are found in the bile after a physiological as well as after a pharmacological dose of all-trans-retinoic acid.

**EXPERIMENTAL PROCEDURES**

Radiochemicals, Their Purification and Preparation of Doses—

All-trans-[11,12-3H]retinoic acid, specific activity 31 Ci/mmol (a gift from Hoffmann-La Roche, Inc., Nutley, NJ) and all-trans-[10-3H]retinoic acid, specific activity 1.13 Ci/mmol (a gift from the National Cancer Institute) were examined by high performance liquid chromatography prior to use, using a Zorbax-ODS analytical column (0.46 cm inside diameter × 25 cm; DuPont Analytical Instruments, Wilmington, DE) that was developed with methanol:water, containing 0.01 M ammonium acetate and that resolves all-trans-retinoic acid from its isomers and other impurities. Depending on the impurities present in the radiolabeled samples, the compounds were purified as follows. If there were no isomers of retinoic acid present, the material was purified on a Lipidex 5000 (Packard Instruments, Inc., Downers Grove, IL) column that was developed with methanol:water (86:32, v/v) containing 0.01 M ammonium acetate, immediately prior to use. This chromatographic procedure separates all-trans-retinoic acid from 5,6-epoxyretinoic acid and other polar and less polar impurities but does not resolve retinoic acid isomers. If the radioactive sample contained retinoic acid isomers, it was purified immediately prior to use by HPLC on an analytical reverse-phase Bondapak C18 column (0.4 cm inside diameter × 30 cm; Waters Associates, Milford, MA) that was developed with methanol:water (86:32, v/v) containing 0.01 M ammonium acetate and that separates all-trans-retinoic acid from its isomers and other impurities. Unlabeled all-trans-retinoic acid (Eastman Organic Chemicals, Rochester, NY) did not require purification.

For the studies dealing with the isolation and identification of 13-cis-retinoyl glucuronide the rates were dosed with purified all-trans-[10-3H]retinoic acid that was mixed with unlabeled all-trans-retinoic acid to a specific activity of 59 × 10^6 dpm/µg and 2.22 mg of this dose was injected into stock rats as described (9). For the studies involving a comparison of biliary metabolites of retinoic acid using physiological and pharmacological levels of retinoic acid, the doses were prepared as follows: purified all-trans-[11,12-3H]retinoic acid was mixed with unlabeled all-trans-retinoic acid to a specific activity of 56 × 10^6 dpm/µg and 2.22 mg of this dose injected into stock rats as described (9). Vitamin A-deficient rats were injected intraperitoneally with 12.7 µg of purified all-trans-[10-3H]retinoic acid, specific activity 7.09 × 10^6 dpm/µg, dissolved in 0.1 ml of dimethylsulfoxide.

Chemicals and Solvents—All chemicals and solvents were of reagent grade or of HPLC grade.

TWEEN 40 (polyoxyethylene sorbitan monopalmitate), butylated hydroxytoluene, n-propyl gallate, bovine serum albumin, and β-d-glucuronidase (from bovine liver, Type B-19), dimethylsulfoxide, and ethylenediaminetetraacetic acid were purchased from Sigma.

All-trans-5,6-epoxyretinoic acid and 13-cis-retinoic acid (gifts from the National Cancer Institute) were purified on an analytical reversed phase Zorbax-ODS column using a solvent system identical with that described for Fig. 3. The purified HPLC standards were stored under nitrogen at −70 °C.

Preparation of Animals and Bile Duct Cannulation—Vitamin A-deficient rats were prepared as described previously (10) and used in the experiments when they had reached a weight plateau (at about 8 weeks of age). Male stock rats, about 8 weeks old, were obtained from the Holtzman Co. (Madison, WI) shortly before use.

Rats were lightly anesthetized with ether and injected intraperitoneally with phenobarbital (25 mg/kg of body weight). The bile duct was then cannulated with polyethylene tubing. After bile flow was...
established, the stock rats were injected intraperitoneally with all-trans-retinoic acid, and then their abdominal incision closed with sutures and surgical clips, leaving the bile duct cannula exposed to the outside. The surgical procedure was similar for the vitamin A-deficient rats, except that the dose was injected intraperitoneally. The rats were then placed in restraining cages and allowed 10% dextrose solution ad libitum during the period of bile collection (24 h). The rats were housed in a room equipped with amber lights. The bile was collected under N2 over ice in glass vials that contained 100 µg each of n-propyl gallate and butylated hydroxytoluene. At each collection time, aliquots of bile were removed for determination of radioactivity and the collected bile was stored under -20°C until further analysis.

Extraction of Bile: Chromatography of Extracts—Bile was extracted with 1-butanol as described (9) except that n-propyl gallate, and EDTA (100 µg/ml) were added to the aqueous phase, butanol contained BHT (50 µg/ml), and the extraction was done under N2. The final butanol extract was evaporated under reduced pressure and the metabolites redissolved in methanol for chromatography.

Sephadex LH-20 (particle size 25 to 100 µm, Pharmacia Fine Chemicals, Inc., Piscataway, NJ) was equilibrated with chloroform-hexanemethanol (65:35:6, v/v/v) and then packed into a glass column (2 x 55 cm). The bile extract was applied onto the column in a small volume of methanol (1:1, v/v), and the column developed with the above solvent until 80 ml had been collected; thereafter, the elution was continued with acetonmethanol (1:1, v/v). All solvents contained BHT (50 µg/ml).

Anion exchange chromatography was carried out on a DEAE-cellulose (Cellex-D) column (2 cm inside diameter x 30 cm, packed to a height of 4 cm) that had been converted to anion form. The samples were applied in a small volume of ethanol and the column was first developed with ethanol, followed by acidified (0.3% HCl) ethanol.

For the analysis of fresh bile, 20- to 90-µl aliquots of bile were deproteinized with 5 volumes of cold ethanol, the precipitate removed by centrifugation, and the supernatant evaporated to a small volume and applied directly onto HPLC columns.

HPLC was done on a Beckman Model 332 Gradient Liquid Chromatograph (Beckman Instruments, Inc., Lincolnwood, IL) that was equipped with Waters Associates Model 440 Absorbance Detector fixed at 340 nm and a Waters Associates Model U6K Universal Injector. The analytical columns used were Bondapak C18 (Waters Associates; 0.4 cm inside diameter x 25 cm) The semipreparative column was a Partisil M9 10 ODS-2 (0.4 cm inside diameter x 25 cm, Whatman). Chromatography was done at ambient temperatures in a room equipped with amber lighting. The flow rate was 2 ml/min for the analytical columns and 4 ml/min for the semipreparative column.

Other Procedures—Incubation with β-D-glucuronidase was essentially as described previously (7) except that after incubation the mixture was deproteinized with ethanol as described for fresh bile above.

All operations described here were carried out under amber light; samples were collected on ice, protected from oxidation, and stored at -70°C. Radioactivity was determined in a Packard Tri-Carb Model 3255 scintillation counter equipped with an automatic external standardization system. Samples for counting were dissolved in 3a70B complete counting solution (Research Products International Corp., Elk Grove Village, IL). Ultraviolet spectra were obtained with a Beckman Model 24 recording UV-visible spectrophotometer. Mass spectra were recorded in an A.E.I. MS-9 mass spectrometer equipped with a DS-50 data acquisition system (Associated Electrical Industries, Manchester, England, and Data General Corp., Southboro, MA) using direct probe sample introduction and electron impact ionization (70 eV).

RESULTS

Isolation and Identification of 13-cis-Retinoyl Glucuronide—Bile collected from cannulated stock rats 6 h after an intraperitoneal injection of 3.1 mg of all-trans-[10-3H]retinoic acid (specific activity 59 x 10^4 dpm/µg) was extracted and chromatographed on Sephadex LH-20 (Fig. 1A) as described under "Experimental Procedures." This chromatographic procedure results in a partial purification of the biliary metabolites of retinoic acid. Fractions 15 to 28, 105 to 130, and 140 to 180 contained a yellow-green material. A small amount of radioactivity eluted with all-trans-retinoic acid standard (fraction 40), a fraction that also contains 5,6-epoxyretinoyl acids. Most of the radioactivity (86 to 97%) eluted as a single peak in fractions 110 to 130. These fractions were pooled, the solvent evaporated under water pump vacuum and applied to a Partisil M9 PXS 10/25 Whatman semi-preparative HPLC reverse-phase column (Whatman Partisil M9, 10/25). The elution was isocratic with a mixture of methanol:water (70:30, v/v), containing 0.1 M ammonium acetate, and a flow rate of 4 ml/min; 1.1-ml fractions were collected (B). The elution positions of UV standards are indicated by arrows as follows: 5,6-epoxyretinoid acid (1), 13-cis-retinoyl acid (2); the elution position of the 13-cis-retinoyl glucuronide is indicated by E, and that of all-trans-retinoyl glucuronide is indicated by G.

In studies not shown here, the major metabolite peak obtained from the Sephadex LH-20 column (Fig. 1A) was chromatographed on a DEAECellex-D anion exchange column. Almost all of the radioactivity (98%) was retained by this column and could be eluted with acidified solvent, revealing the charged nature of these metabolites.

In a previous paper (7), we reported the isolation and characterization of a mixture of all-trans- and 13-cis-retinoyl glucuronides from rat bile. The mass spectrum of these glucuronides is shown in Fig. 2; it does not distinguish between the isomers.

The individual retinoyl glucuronides (peak E, 13-cis-retinoyl glucuronide, and peak G, all-trans-retinoyl glucuronide) were collected from the reverse-phase semipreparative col-

FIG. 1. Chromatography of biliary metabolites of all-trans-retinoic acid. Butanol extracts of bile were first chromatographed on a Sephadex LH-20 column using a solvent mixture consisting of chloroform-hexanemethanol (65:35:6, v/v/v) containing 50 µg of BHT/ml. A solvent mixture consisting of acetonmethanol (1:1, v/v) containing 50 µg of BHT/ml was started at 80 ml (arrow, A). The major radioactive peak from the column in A was pooled, evaporated to a small volume, and aliquots of it chromatographed on an HPLC reverse-phase semipreparative column (Whatman Partisil M9). The elution isocratic with a mixture of methanol:water (70:30, v/v), containing 0.1 M ammonium acetate, and a flow rate of 4 ml/min; 1.1-ml fractions were collected (B). The elution positions of UV standards are indicated by arrows as follows: 5,6-epoxyretinoyl acid (1), 13-cis-retinoyl acid (2); the elution position of the 13-cis-retinoyl glucuronide is indicated by E, and that of all-trans-retinoyl glucuronide is indicated by G.
of methanol:water (68:32, v/v) containing 0.01 M ammonium acetate as described under "Experimental Procedures." The extracts from the incubation mixture were chromatographed by HPLC on a Zorbax-ODS analytical column using a solvent mixture containing 60% methanol and 40% water containing 0.01 M ammonium acetate. The flow rate was 2 ml/min; 1.1-mI fractions were collected. A, chromatography of peak G (all-trans-retinoyl glucuronide); B, chromatography of peak E (13-cis-retinoyl glucuronide). Arrows indicate the elution positions of UV standards: 5,6-epoxy-retinoic acid (1), 13-cis-retinoic acid (2), all-trans-retinoic acid (3). The elution positions of peaks E and G are also indicated by arrows.

Fig. 3. Purification of separated retinoyl glucuronides by HPLC on analytical columns. The individual retinoyl glucuronides obtained from chromatography in Fig. 1B were rechromatographed on a Zorbax-ODS analytical column using gradient elution technique. The solvent was a mixture of methanol and water containing 0.01 M ammonium acetate; the flow rate was 2 ml/min; 1.1-ml fractions were collected. A, chromatography of peak G (all-trans-retinoyl glucuronide); B, chromatography of peak E (13-cis-retinoyl glucuronide). Arrows indicate the elution positions of UV standards: 5,6-epoxy-retinoic acid (1), 13-cis-retinoic acid (2), all-trans-retinoic acid (3). The elution positions of peaks E and G are also indicated by arrows.

Fig. 4. Ultraviolet absorption spectra of all-trans-retinoyl glucuronide (A) and 13-cis-retinoyl glucuronide (B).
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Fig. 5. Structures of retinoyl glucuronides: all-trans-retinoyl glucuronide, A; 13-cis-retinoyl glucuronide, B.

Although there is no guarantee that an unknown metabolite will behave like the standard during a recovery test, a control experiment is necessary to evaluate the feasibility of the various isolation procedures to be employed.

When labeled all-trans-retinoic acid was added to the bile collected from untreated rats and the bile processed as described under "Experimental Procedures," the overall recovery of radioactivity was 86%. Of this radioactivity, 77% was all-trans-retinoic acid, 11% was 13-cis-retinoic acid, 0.8% was 5,6-epoxyretinoic acid, while the remaining radioactivity was distributed among various polar and nonpolar compounds.

In most of our studies that deal with specific metabolites and the quantitation of these metabolites, we routinely apply fresh, deproteinized bile directly onto HPLC columns, as this procedure reduces the isolation steps to a minimum and yields very reproducible HPLC profiles. When using this procedure to evaluate the recovery of purified, labeled all-trans-retinoic acid added to fresh bile from nondosed rats, 94% of the radioactivity was recovered as all-trans-retinoic acid; 0.1% of the radioactivity co-migrated with 13-cis-retinoic acid, 2% with 5,6-epoxyretinoic acid, while 1% and 3% of the radioactivity, respectively, was associated with the very polar and nonpolar eluates. The chromatography of all-trans-retinoic acid injected directly onto the HPLC column did not cause any isomerization or decomposition.

Since the lengthy butanol extraction procedure is associated with some isomerization and decomposition of all-trans-retinoic acid, we used this method only for the generation of specific metabolites; while for the quantitation of the various biliary metabolites of retinoic acid, we examined fresh bile directly.

Comparison of Biliary Metabolites Produced from Pharmacological or Physiological Doses of [3H]Retinoic Acid—The bile ducts of stock rats were cannulated, the animals injected intraperitoneally with 2.22 mg of all-trans-[11,12-3H]retinoic acid, specific activity $56 \times 10^3$ dpm/μg and the bile collected as described under "Experimental Procedures."

In a parallel experiment, the bile ducts of vitamin A-deficient rats were cannulated and the rats injected intraperitoneally with 12.7 μg of all-trans-[10-3H]retinoic acid, specific activity $7.1 \times 10^6$ dpm/μg. The bile was collected at 2-h intervals, aliquots removed for determination of radioactivity, and the remaining bile stored at -20 °C for further chromatography.

Fig. 7 illustrates that the excretion pattern of radioactivity in bile is very similar for both of the retinoic acid dose levels used. The maximum excretion of radiolabel occurs between 2 to 4 h after the administration of all-trans-retinoic acid and had decreased considerably by 8 h. At 6 h, 60 to 70% of the dosed radioactivity had already appeared in the bile, and at

![Fig. 7. Biliary excretion of radioactivity following the administration of all-trans-[3H]retinoic acid. Cumulative per cent (A) and per cent of dose radioactivity per 2-h intervals (B) excreted into the bile of bile duct-cannulated rats after the intravenous administration of all-trans-[11,12-3H]retinoic acid (2.2 mg) to vitamin A-sufficient rats (×--×) and all-trans-[10-3H]retinoic acid (12.7 μg) to vitamin A-deficient rats (○--○).](http://www.jbc.org/)
24 h, the bile contained 80 to 85% of dose radioactivity.

Tables I and II show the distribution of the bile radioactivity in the various chromatographic fractions. It is evident that polar metabolites of retinoic acid predominate in the bile at all time, although some free retinoic acid is excreted during the first 4 h after dosing (Tables I and II).

An example of an incubation of fresh bile with β-D-glucuronidase is shown in Fig. 8. The upper panel (A) represents the biliary metabolite profile obtained after an incubation of fresh bile with boiling β-D-glucuronidase. The bile for this experiment was an aliquot from the first 2-h collection period after the injection of all-trans-retinoic acid, and it contains some free 13-cis- and all-trans-retinoic acids. At this time, there is a considerable amount of radioactivity (614% of column \( ^3 \mathrm{H} \)) in the area of retinoic glucuronides (peaks E and G). After an incubation with fresh β-D-glucuronidase (Fig. 8, lower panel, B), 80% of the radioactivity that had previously been associated with the retinoic glucuronides was hydrolyzed. This resulted in a corresponding increase of radioactivity in the elution areas of 13-cis- and all-trans-retinoic acid.

In subsequent studies, the biliary retinoyl glucuronides were resolved using an HPLC system similar to that described in Fig. 3; thereafter, they were individually quantitated.

The rate of excretion of retinoyl glucuronides is illustrated in Fig. 9. A relatively larger amount of retinoyl glucuronide is excreted from the pharmacological dose during each time period as compared to the amount excreted after a physiological dose of retinoic acid. At 24 h after the dose, about 10% of the dose radiolabel is excreted in the bile in the form of retinoyl glucuronides from the 2.22-mg dose, while only 4.4% of the radiolabel from the 12.7-μg dose of retinoic acid is in the form of retinoyl glucuronides. Retinoyl glucuronide excretion is maximum during the first 2 h after dosing, when 21% of dose radioactivity is in the retinoyl glucuronide fraction at the 2.22-mg dose level and 13.9% at the physiological dose level (Tables I and II).

Table III presents the biliary excretion of retinoyl glucuronides as a function of time after dose and as a function of bile flow. With the pharmacological dose of all-trans-retinoic acid (which was 220 times that of the physiological dose), this 200:1 relationship holds also for the amount of retinoyl glucuronides excreted during the first 6 h, when expressed as a function of

### Table I

**Distribution of bile radioactivity in the various chromatographic fractions after the injection of a pharmacological dose of all-trans-[^3]H]retinoic acid**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Time period after dosing during which bile was collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2 h</td>
</tr>
<tr>
<td>% total bile ( ^3 \mathrm{H} )</td>
<td></td>
</tr>
<tr>
<td>Polar metabolites*</td>
<td>73.3</td>
</tr>
<tr>
<td>Retinoyl glucuronides*</td>
<td>21.0</td>
</tr>
<tr>
<td>Retinoic acids*</td>
<td>2.3</td>
</tr>
<tr>
<td>Methanol-eluted fraction*</td>
<td>4.4</td>
</tr>
</tbody>
</table>

*Deproteinized aliquots of bile were chromatographed on the HPLC system described in Fig. 3. The fractions were collected and their radioactivity determined. Each value represents the averages of bile assays from two rats.

### Table II

**Distribution of bile radioactivity in the various chromatographic fractions after the injection of a physiological dose of all-trans-[^3]H]retinoic acid**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Time period after dosing during which bile was collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2 h</td>
</tr>
<tr>
<td>% total bile ( ^3 \mathrm{H} )</td>
<td></td>
</tr>
<tr>
<td>Polar metabolites*</td>
<td>76.1</td>
</tr>
<tr>
<td>Retinoyl glucuronides*</td>
<td>13.9</td>
</tr>
<tr>
<td>Retinoic acids*</td>
<td>4.8</td>
</tr>
<tr>
<td>Methanol-eluted fraction*</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*Deproteinized aliquots of bile were chromatographed as described in Fig. 3, the fractions collected and their radioactivity determined. Each value is the average from assays of bile samples from two to six rats.

*Pooled fractions 1 to 60, as in Fig. 3.

* Combined all-trans and 13-cis isomers.

*Pooled fractions 120 to 140, as in Fig. 3.
after HPLC of fresh bile samples revealed that with the physiological dose of all-trans-[1-12C]retinoic acid (2.2 mg) into vitamin A-deficient rats. Our results also point out the fact that retinoyl glucuronide represents only 10% of the dose radioactivity per 2-h interval.

**TABLE III**

Excretion of retinoyl glucuronides into bile

The experimental details are described in Tables I and II. The calculations were based on the assumption that the retinoyl 3-glucuronide has the same specific radioactivity of the injected all-trans-[3H]retinoic acid.

<table>
<thead>
<tr>
<th>Time after dose (h)</th>
<th>Dose of all-trans-retinoic acid (2.22 mg)</th>
<th>Dose of all-trans-retinoic acid (12.7 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>38.9 µg/ml bile 37.0 µg/h</td>
<td>0.125 µg/ml bile 0.075 µg/h</td>
</tr>
<tr>
<td>2-4</td>
<td>27.6 µg/ml bile 34.5 µg/h</td>
<td>0.220 µg/ml bile 0.128 µg/h</td>
</tr>
<tr>
<td>4-6</td>
<td>15.7 µg/ml bile 16.5 µg/h</td>
<td>0.070 µg/ml bile 0.040 µg/h</td>
</tr>
<tr>
<td>6-8</td>
<td>0.041 µg/ml bile 0.018 µg/h</td>
<td></td>
</tr>
<tr>
<td>8-10</td>
<td>1.7 µg/ml bile 1.3 µg/h</td>
<td>0.026 µg/ml bile 0.012 µg/h</td>
</tr>
<tr>
<td>10-12</td>
<td>0.008 µg/ml bile 0.003 µg/h</td>
<td></td>
</tr>
<tr>
<td>12-14</td>
<td>0.005 µg/ml bile 0.002 µg/h</td>
<td></td>
</tr>
<tr>
<td>14-16</td>
<td>0.000 µg/ml bile 0.000 µg/h</td>
<td></td>
</tr>
<tr>
<td>16-24</td>
<td>0.000 µg/ml bile 0.000 µg/h</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 9.** Biliary excretion of retinoyl glucuronides (RG) following the administration of all-trans-[10-3H]retinoic acid. Cumulative per cent (A) and per cent of dose radioactivity per 2-h intervals (B) excreted into bile. ○—○ after an injection of all-trans-[10-3H]retinoic acid (12.7 µg) into vitamin A-deficient rats. □—□ after an injection of all-trans-[11,12-3H]retinoic acid (2.2 mg) into vitamin A-sufficient (stock) rats.

The most important observation in the present report is the biliary excretion of both 13-cis- and all-trans-retinoyl glucuronides as metabolites of all-trans-retinoic acid. Of the total biliary retinoyl glucuronides, 40% are in the form of 13-cis-retinoyl glucuronides. Furthermore, our control studies (11) indicate that formation of 13-cis-retinoyl glucuronide from all-trans-retinoyl glucuronide does not occur during our isolation procedure. We conclude that 13-cis- and all-trans-retinoyl glucuronides are formed in vivo from all-trans-retinoic acid. Our results therefore suggest that the in vivo isomerization of all-trans-retinoyl acid to 13-cis-retinoyl acid in the liver is a first step in the metabolism of all-trans-retinoic acid. It is possible, but not very likely, that the isomerization of all-trans-retinoyl acid takes place on the rather bulky retinoyl glucuronide. Further studies involving the isomerization of all-trans-retinoyl acid in liver will provide a definitive answer to these questions.

The physiological significance of retinoyl glucuronides in the bile is not clear. The biological activity of the purified retinoyl glucuronides must be first determined to evaluate the previously suggested idea of enterohepatic circulation of the retinoyl glucuronides as a possible in vivo mechanism for the temporary preservation of retinoic acid.

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