Gemfibrozil (GEM), a human pharmaceutical agent, causes hepatomegaly and hepatic peroxisome proliferation in rats, which have been associated with hepatocarcinogenesis. Hamsters are less susceptible than rats to peroxisome proliferation, and no hepatotoxicity has been reported in humans using gemfibrozil. The relationship between hepatic peroxisome proliferation in rodents and human cancer risk is unclear. We investigated the metabolism and excretion of [14C]gemfibrozil in male and female Sprague-Dawley rats and Syrian golden hamsters to better understand species differences in gemfibrozil-induced toxicity. Bile-duct cannulated rats and hamsters excreted 99% and 7 to 20% of a single i.v. gemfibrozil dose in bile, respectively. Cumulative urinary and fecal excretion of gemfibrozil-derived radioactivity after a single oral dose (30 or 2000 mg/kg) were dependent on species and, in rats, on dose. Hamsters excreted 90% of the dose in urine. Rats excreted 55 to 60% of the dose in feces after the low dose and 55 to 70% in urine after the high dose, suggesting possible saturation of biliary excretion. Repeated administration of the low dose to male rats did not alter the routes of excretion compared to a single dose. Major metabolites present in urine and bile were the glucuronide conjugates of gemfibrozil, the 4'-ring hydroxylated metabolite, and the meta-benzoic acid metabolite. The extensive urinary excretion of radioactivity by hamsters and enterohepatic recycling in rats suggests that rats were exposed to a much higher effective dose of gemfibrozil, which may in part explain the previously reported species differences in gemfibrozil-induced toxicity.
established the degree to which GEM undergoes enterohepatic recycling in hamsters. The objective of the current study was to investigate metabolism and elimination as possible explanations for the observed species differences in GEM-induced hepatotoxicity.

**Materials and Methods**

**Chemicals.** GEM (≥99% pure), β-glucuronidase from *Helix pomatia* (89,400 units/ml glucuronidase and 3300 units/ml sulfatase), and β-glucuronidase from *Escherichia coli* (Type VII; 2000 units/ml glucuronidase in 0.1 M sodium acetate buffer, pH 6.8) were purchased from Sigma Chemical Company (St. Louis, MO). [14C]GEM, randomly radiolabeled with carbon-14 on the ring methyl groups (40.0 mCi/mmol), was received from Wizard Laboratories (West Sacramento, CA). Citric acid, monohydrate, and trifluoroacetic acid (TFA) were purchased from J.T. Baker (Phillipsburg, NJ). Soluene-350 tissue solubilizer and Ultima Gold scintillation cocktail were purchased from Packard Instrument Company, Inc. (Meriden, CT). Emulphor EL-620 was provided by Rhone-Poulenc (Cranberry, NJ). Methylcellulose (40,000 centipoise) and acetonitrile [high-pressure liquid chromatography (HPLC) grade] were purchased from Fisher Scientific (Atlanta, GA). GEM metabolite standards were kindly provided by Parke-Davis Pharmaceutical Research (Ann Arbor, MI).

**Animals.** All studies were conducted in accordance with federal guidelines for the care and use of laboratory animals and were approved by the Research Triangle Institute Institutional Animal Care and Use Committee. Male and female Hsd:Sprague-Dawley rats (10–12 weeks old) were purchased from Harlan Sprague-Dawley (Frederick, MD; Dublin, VA; Madison, WI). Male and female Syrian golden hamsters (Lak:LVG(SYR)BR) (10–12 weeks old) were purchased from Charles River Laboratories (Kingston, NY). Animals were held in quarantine for at least 1 week before use in a study and were allowed feed (Certified Purina Rodent Chow 5002, Purina Mills Inc., St. Louis, MO) and tap water ad libitum. Animal quarters were maintained at 69–76°F and 40 to 70% relative humidity. Light/darkness was cycled at 12-h intervals. In oral administration studies, animals were housed individually in all-glass metabolism chambers that provided for separate collection of urine, feces, CO₂ and expired volatile organics. Animals were acclimated to the metabolism chambers one day before dosing.

**Dose Preparation.** Oral dose formulations were prepared in 0.5% aqueous methylcellulose in a dose volume of 5 ml/kg at target doses of 30 and 2000 mg of GEM/kg. Intravenous dose formulations were prepared in 1:1:8 Emulphor/ethanol/water and administered in a dose volume of 1 ml/kg at a target dose of 3 mg GEM/kg.

**Oral Administration and Excreta Collection.** In the single-dose studies, all animals received 10 to 22 μCi [14C]GEM. In the low-single oral dose study, administered doses (mean ± S.D.; N = 4) were 29.6 ± 0.2 (male rats), 29.1 ± 0.2 (female rats), and 30.2 ± 0.1 (male and female hamsters) mg of GEM/kg. In the high single-dose study, administered doses were 1840 ± 7 (male rats), 1745 ± 25 (female rats), 1873 ± 12 (male hamsters), and 1886 ± 7 (female hamsters) mg of GEM/kg. In the multiple low-oral dose study, male rats (N = 4) were orally administered approximately 30 mg of GEM/kg/day for 11 days, with radiolabeled doses (15 μCi/rat) administered on days 1, 5, and 9. Animals were housed individually in all-glass metabolism chambers. In the single-dose studies, urine and feces were collected over dry ice in timed fractions ending at 6 (urine only), 12, 24, 48, and 72 h after dosing. In the repeated-dose study, urine and feces were collected over dry ice in timed fractions ending at 4 (urine only), 8 (urine only), 24, 48, and 72 h after administration of the radiolabeled doses on days 1, 5, and 9, with an additional 96-h collection after dosing on days 1 and 5. Urine was collected into receptacles containing 400 to 500 mg of solid citric acid to stabilize any acyl glucuronides excreted in urine. Radiolabeled volatile organics and carbon dioxide in exhaled breath were collected in ethanol and sodium hydroxide traps, respectively. In both the single-dose and repeated-dose studies, breath traps were changed at 6, 12, 24, 48, 72, and 96 h (after dose days 1 and 5 in the repeated-dose study only).
Intravenous Administration and Bile Collection. The bile ducts of animals (N = 4 per group) in the i.v. studies were surgically cannulated before dosing. Rats were anesthetized orally (35 mg/kg) and i.p. (45 mg/kg), and hamsters were anesthetized i.p. only (ca. 100 mg/kg) with sodium pentobarbital. In rats the bile duct was cut, cannulated with PE-10 tubing, and secured with silk sutures. In hamsters, the bile duct was first ligated just below the gall bladder to prevent bile from being stored in the gall bladder. Then a small cut was made in the duct into which the PE-10 tubing was inserted and secured with silk sutures. The canulas were exteriorized and the abdominal incision closed with sutures. Throughout the study, animals were placed on heating pads to maintain body temperature. A state of anesthesia was maintained by i.p. injections of sodium pentobarbital as needed. After a single i.v. dose (ca. 100 mg/kg) sodium pentobarbital was administered by the tail vein and hamsters (cephalic vein), bile was collected at 0.5-h (rats) or 1-h (hamsters) intervals for up to 4 h into tared vials containing 4 to 5 mg citric acid.

Liquid Scintillation Spectrometry (LSS). Samples were assayed for total radioactivity by LSS either directly (urine, bile, ethanol trapping solution, and sodium hydroxide trapping solution) or after solubilization in Soluene-350 (feces). Control samples of urine, feces, breath, and bile were collected before dose administration and analyzed for radiochemical content to determine background counts.

HPLC. All chromatographic analyses were conducted using two Waters model 510 pumps (Milford, MA), a Rheodyne model 7125 injector (Alltech Associates, Inc.; Deerfield, IL), a UV detector set at 270 nm (Spectroflow 757 or Applied Biosystems 757 Absorbance Detector; Aston, PA) and a flow-through radioactivity detector (Ramona LS or β-RAM with 500-μl glass cell; IN/US Instruments, Tampa, FL).

Composite urine and bile samples were prepared as follows. Urine collected during intervals ending at 6, 12, and 24 h after dosing were pooled for individual animals to make a 24-h composite for each animal. Pooled daily composites were prepared by pooling 24-h urine composites (day 1), 48-h urine samples (day 2), and 72-h urine samples (day 3) from four animals per dose group for both sexes of each species. The daily composites were analyzed by HPLC to evaluate the radiolabeled urinary metabolite profiles. For rats, hourly bile samples were pooled for each animal (i.e., pooled 0.5- and 1-h samples, 1.5- and 2-h samples, and so on for each animal) to prepare hourly composites for each animal. Hour 1, hour 2, hour 3, and hour 4 bile composites were prepared by pooling 1-, 2-, 3-, and 4-h bile samples (hamsters) or hourly composites (rats) from four animals (three for female hamsters) per sex per species group. Urine and bile composites were analyzed for GEM and radiolabeled metabolites with a Zorbax XDB-C8 analytical column (5-μm particle size, 250 × 4.6 mm; MAC-MOD Analytical Inc., Chadds Ford, PA) and a mobile phase consisting of 0.1% TFA in acetonitrile (Solvent A, pH 2) and 0.1% TFA in water (Solvent B, pH 2) at a flow rate of 1.5 ml/min. Elution was accomplished by the following gradient program: 30% Solvent A for 5 min, changed linearly to 100% Solvent A in 10 min, and then held at 100% Solvent A for 5 min. Radiolabeled metabolites in each sample were quantitated by fraction collection and analysis for total radioactivity by LSS.

Urinary metabolites were isolated using a semipreparative Zorbax XDB-C8 (5 μm particle size, 250 × 9.4 mm; MAC-MOD Analytical Inc.; Chadds Ford, PA) with 0.1% TFA in acetonitrile (solvent A, pH 2) and 0.1% TFA in water (solvent B, pH 2) as the mobile phase at a flow rate of 5.0 ml/min. Elution was accomplished using the following gradient program: 30% solvent A for 15 min, changed linearly to 100% solvent A in 10 min, and then held at 100% solvent A for 10 min. Isolated radiolabeled metabolites were concentrated (Meyer N-Evp-Analytical Evaporator; Organization Associates Inc., South Berlin, MA), and further purified by C18 Bond Elut solid phase extraction cartridges (500 mg/6 ml; Varian; Harbor City, CA). Purified metabolites were identified by mass and NMR spectrometry (B. F. Thomas et al., 1999).

Purified metabolites that had been identified as glucuronide or sulfate conjugates (Thomas et al., 1999) were cleaved to their aconjugates and compared with metabolite standards provided by Parkes-Davis and [14C]GEM based on chromatographic retention times using the Zorbax analytical column and mobile phase described above and the following gradient program: 35% solvent A for 15 min, changed linearly to 65% solvent A in 15 min, changed linearly to 100% solvent A in 2 min, and then held at 100% for 8 min. The flow rate was 1.5 ml/min for 30 min and then increased linearly to 2 ml/min at 32 min into the run.

Enzymatic and Base Hydrolysis. For enzymatic hydrolysis, either 20 μl of enzyme (β-glucuronidase from H. pomatia; 89,400 units/ml glucuronidase and 3300 units/ml sulfatase) plus 20 to 80 μl of 0.1 M sodium acetate buffer (pH 5) or 125 to 200 μl of enzyme solution (β-glucuronidase from E. coli, Type VII, 2000 units/ml glucuronidase in 0.1 M sodium acetate buffer, pH 6.8) was added to amber 1/2-drums vials that contained 150 to 350 μl of purified metabolites in acetonitrile. Controls were prepared with purified metabolites and enzyme that had been heat deactivated (boiled for 10 min) and flash frozen. Incubates were maintained at 37°C overnight in a reciprocating shaker and then analyzed by HPLC. For base hydrolysis, 20 μl of 5 M NaOH was added to 100 to 300 μl of purified metabolites in acetonitrile. The incubates

### TABLE 1

<table>
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<th>Sex and Dose Level</th>
<th>Overall Recovery in Urine</th>
<th>Chromatographic Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>Male rat</strong></td>
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</tr>
<tr>
<td>30 mg/kg</td>
<td>2.639</td>
<td>0.186</td>
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<td>(29.9)</td>
<td>(2.1)</td>
<td>(1.7)</td>
</tr>
<tr>
<td>2000 mg/kg</td>
<td>1223</td>
<td>239^a</td>
</tr>
<tr>
<td>(53.6)</td>
<td>(10.5)</td>
<td>(6.6)</td>
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<tr>
<td><strong>Female rat</strong></td>
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<td>30 mg/kg</td>
<td>1.893</td>
<td>0.054</td>
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<tr>
<td>(32.6)</td>
<td>(0.9)</td>
<td>(1.1)</td>
</tr>
<tr>
<td>2000 mg/kg</td>
<td>1043</td>
<td>52^a</td>
</tr>
<tr>
<td>(67.7)</td>
<td>(3.4)</td>
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<tr>
<td><strong>Male hamster</strong></td>
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</tr>
<tr>
<td>30 mg/kg</td>
<td>3.230</td>
<td>0.116</td>
</tr>
<tr>
<td>(90.2)</td>
<td>(3.2)</td>
<td>(11.5)</td>
</tr>
<tr>
<td>2000 mg/kg</td>
<td>2000</td>
<td>247</td>
</tr>
<tr>
<td>(VII)</td>
<td>(87.3)</td>
<td>(7.7)</td>
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</table>

^a Results are expressed in milligram equivalents and percent dose excreted; percent dose in parentheses.

^b Peaks are listed in order of elution with metabolite A being the most polar. See Figs. 4 and 7 for metabolite structures.

^c Metabolite not detected.

^d Composed of at least two components. Combined results of the two are listed.
were maintained at 37°C for 1 to 2 h and then acidified with 20 to 50 μL of 5 M TFA before analysis by HPLC.

**Statistical Analyses.** The mean values of the excretion of radioactivity (percent dose) in urine, feces, and bile among sex, species, and dose groups were compared by orthogonal contrasts with the GLM Procedure (SAS Software, Version 6.12; SAS Institute, Cary, NC). A p value of .05 was used to determine statistical significance.

**Results**

**Urinary and Fecal Excretion after a Single Oral Dose.** Cumulative urinary and fecal excretion of [14C]GEM-derived radioactivity after a single oral dose are shown in Fig. 1. Radioactivity exhaled as volatile organics and carbon dioxide was negligible in all studies. The rates and routes of excretion of radioactivity were similar for male and female rats in the low oral dose studies (Fig. 1, A and B), and no significant sex-related difference in the cumulative urinary or fecal excretion of radioactivity was observed. Averages of 59 and 56% of the delivered low dose were excreted in feces and averages of approximately 30 and 33% excreted in urine, by male and female rats, respectively. The absolute mass of metabolites excreted in urine by rats in the low oral dose study was approximately 2 to 3 mg-equivalents (Table 1). As with rats, there was little difference in the routes and rates of excretion of radioactivity for male and female hamsters in the low oral dose study (Fig. 1, panels C and D). Similar to rats, no significant sex-related difference in the cumulative urinary or fecal excretion of radioactivity was observed in hamsters. In contrast to rats, hamsters excreted the majority of the dose in urine (ca. 90%), with only approximately 4% excreted in feces, and GEM-derived radioactivity was excreted more rapidly by hamsters than by rats (Fig. 1). The absolute mass of metabolites excreted in urine by hamsters in the low oral dose study was approximately 3 to 4 mg-equivalents (Table 1).

As in the low-dose study, no significant sex-related difference in the cumulative urinary or fecal excretion of radioactivity was observed in rats in the high-dose study. Averages of 55 and 70% of the delivered dose were excreted in urine, and averages of 34 and 17% excreted in feces, by male and female rats, respectively (Fig. 1). There was a high degree of variability in fecal and urinary excretion of radioactivity by male rats in the high-dose group. The rate of excretion (percent dose excreted per hour) decreased by approximately 50% in the first 24 h after dosing, and the primary route of excretion shifted from fecal to urinary elimination as the dose increased in rats. Dose-related differences were observed in the cumulative urinary and fecal excretion of radioactivity in male and female rats. The absolute mass of metabolites excreted in urine by rats in the high-dose study was approximately 1000 to 1200 mg-equivalents (Table 1).

No sex- or dose-related differences were observed in the cumulative urinary and fecal excretion of radioactivity in hamsters (Fig. 1). The rate of excretion of radioactivity by hamsters, however, decreased as the dose increased, yet the rate of excretion by hamsters in the high-dose group was 1.7-times more rapid than the rate of excretion by rats at the high dose. The absolute mass of metabolites excreted in urine by hamsters in the high oral dose study was approximately 800 to 950 mg-equivalents (Table 1). As in the low-dose study, significant species-related differences were observed in the cumulative urinary and fecal excretion of radioactivity were observed.

**Urinary and Fecal Excretion after Multiple Low Oral Doses.** Cumulative excretion results from the multiple low-oral-dose study in male rats, based on the percentage of the administered dose excreted after each radiolabeled dose (day 1, 5, or 9), are shown in Fig. 2. Repeated doses of GEM to male rats did not alter the route of excretion when compared to the single-dose study. Approximately 80% of each radiolabeled dose was excreted, with approximately 26% excreted in urine and 54% in feces, which was comparable to excretion by male rats after a single low oral dose (Fig. 1). The only clinical observation noted was a slightly higher fecal output compared with male rats in the single dose study.

**Biliary Excretion after a Single i.v. Dose.** Cumulative biliary excretion of radioactivity (percent dose) from animals in the i.v. dose study was species-dependent (Fig. 3). No sex-related difference in biliary excretion of [14C]GEM-derived radioactivity was observed in rats. By 1.5 h after dosing, >90% of the dose was recovered in the bile of rats, and essentially all of the administered radioactivity (ca. 3000–4000 mg-equivalents) was recovered in bile by 4 h (Fig. 3; Table 2). In contrast to rats, a significant sex-related difference was observed in the biliary excretion of [14C]GEM-derived radioactivity by hamsters; approximately 20% (ca. 315 mg-equivalents) and 8% (ca. 100 mg-equivalents) of the delivered dose were recovered in the bile of male and female hamsters, respectively (Fig. 3; Table 2). The majority of radioactivity in hamster bile was recovered in the first hour after dosing for both males and females. The mass of bile excreted per unit of time was relatively constant over the 4-h collec-
tion period in both rats (ca. 70–76 mg bile/min/kg) and hamsters (ca. 44–53 mg bile/min/kg).

Characterization of Urinary and Biliary Metabolites. Representative chromatograms of day 2 urine composites are shown in Fig. 4 (low oral dose) and Fig. 5 (high oral dose), and representative chromatograms of hour 2 bile composites are shown in Fig. 6. At least eight urinary and five biliary metabolites were present. Peak identities as labeled in Figs. 4 to 6 are as follows: A = 5'-methyl ether glucuronide of the dihydroxylated (both 2'- and 5'-methyl groups oxidized) metabolite (hamster only); A2 = acyl glucuronide of the 4'-ring hydroxylated metabolite (rat only); B = dihydroxylated metabolite (both 2'- and 5'-methyl groups oxidized); C = mixture of the 5'-acid, 4'-alcohol metabolite and the sulfate conjugate of the 4'-ring hydroxylated metabolite; L = ether glucuronide of the 4'-ring hydroxylated metabolite; D = definitive structure has not been determined, but appears to be derived from GEM rather than an impurity; E = acyl glucuronide of the 5'-methyl-hydroxylated metabolite; F = acyl glucuronides of the meta-benzoic acid metabolite (one conjugated at the meta-benzoic acid group, and another at the 1'-position); I = acyl glucuronide of GEM; K = GEM. The peak eluting between peaks F and I in Figs. 4 and 6 was characterized by mass and NMR spectrometry and appears to be derived from an impurity in the radiolabeled material, not from GEM. A proposed metabolic scheme for GEM is shown in Fig. 7.

Cumulative urinary excretion of individual metabolites as percent dose and milligram equivalents excreted in 72 h are provided in Table 1. Regardless of oral dose level, male and female rats excreted approximately 10 and 20% of the dose as the acyl glucuronide of GEM in urine, respectively (Table 1). The acyl glucuronide of GEM present in urine accounted for approximately 1 mg-equivalent after the low dose (male and female rat), and approximately 190 and 347 mg-equivalents after the high dose in male and female rat urine, respectively (Table 2). As the dose increased, increases were observed in urinary excretion of glucuronide conjugates of the meta-benzoic acid metabolite.

### Table 2

<table>
<thead>
<tr>
<th>Sex and Species</th>
<th>Overall Recovery in Bile</th>
<th>Chromatographic Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Male rat</td>
<td>4155</td>
<td>147</td>
</tr>
<tr>
<td>(98.9)</td>
<td>(3.5)</td>
<td>(ND)</td>
</tr>
<tr>
<td>Female rat</td>
<td>2900</td>
<td>48</td>
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<tr>
<td>(99.3)</td>
<td>(1.6)</td>
<td>(ND)</td>
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<tr>
<td>Male hamster</td>
<td>315</td>
<td>12</td>
</tr>
<tr>
<td>(20.2)</td>
<td>(0.8)</td>
<td>(0.9)</td>
</tr>
<tr>
<td>Female hamster</td>
<td>105</td>
<td>2</td>
</tr>
<tr>
<td>(8.5)</td>
<td>(0.2)</td>
<td>(0.4)</td>
</tr>
</tbody>
</table>

* Results are expressed in microgram equivalents and percent dose excreted; percent dose in parentheses.
* Peaks are listed in order of elution with metabolite A being the most polar. See Fig. 4 and 7 for metabolite structures.
* Metabolite not detected.

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**Fig. 4.** Radiochromatograms of urine collected 24 to 48 h after a single oral dose of 30 mg [14C]GEM/kg to rats and hamsters.

Peak identities in Figs. 4 to 6 are as follows: A = 5'-methyl ether glucuronide of the dihydroxylated (both 2'- and 5'-methyl groups oxidized) metabolite (hamster only); A2 = acyl glucuronide of the 4'-ring hydroxylated metabolite (rat only); B = dihydroxylated metabolite (both 2'- and 5'-methyl groups oxidized); C = mixture of the 5'-acid, 4'-alcohol metabolite and the sulfate conjugate of the 4'-ring hydroxylated metabolite; L = ether glucuronide of the 4'-ring hydroxylated metabolite; D = definitive structure has not been determined, but appears to be derived from GEM rather than an impurity; E = acyl glucuronide of the 5'-methyl-hydroxylated metabolite; F = acyl glucuronides of the meta-benzoic acid metabolite (one conjugated at the meta-benzoic acid group, and another at the 1'-position); I = acyl glucuronide of GEM; K = GEM. See Fig. 7 for structures (for elucidation of metabolite structures see Thomas et al., 1999).
acid metabolite in male and female rats (from ca. 3 to 15% of the dose).

In contrast to rats, urinary metabolites in male hamsters were glucuronide conjugates of the meta-benzoic acid metabolite (ca. 35% of the dose), the acyl glucuronide of GEM (10–15% of the dose), and the dihydroxylated metabolite (ca. 10–15% of the dose). The pattern of metabolite excretion in urine varied with oral dose level in female hamsters. Female hamsters excreted approximately 20 and 40% of the administered dose in urine as glucuronide conjugates of the meta-benzoic acid metabolite after the low and high oral dose, respectively, and approximately 40 and 15% of the dose as the acyl glucuronide of GEM after the low and high oral dose, respectively.

After a single i.v. dose, rats excreted approximately 70 to 75% of the dose as the acyl glucuronide of GEM and 10 to 15% of the dose as glucuronide conjugates of the meta-benzoic acid metabolite in bile by 2 h after administration. Rats excreted < 0.5% of the i.v. dose as GEM in bile. The little radioactivity that was excreted in bile by male hamsters after an i.v. dose of GEM was present as the acyl glucuronide of GEM and glucuronide conjugates of the meta-benzoic acid metabolite in approximately equal amounts (ca. 5% of the dose).
Female hamsters excreted approximately 2 and 4% of the i.v. dose as the acyl glucuronide of GEM and glucuronide conjugates of the meta-benzoic acid metabolite in bile, respectively.

**Discussion**

The hypolipidemic agent GEM induces hepatomegaly and hepatic peroxisome proliferation in rats, but there is no evidence of these effects in humans (Reddy, 1980; Fitzgerald et al., 1981; Lalwani et al., 1983; Gray and de la Iglesia, 1984; Todd and Ward, 1988; Sausen et al., 1995). Although peroxisome proliferation has been associated with hepatocarcinogenesis in rats, the meaning of hepatic peroxisome proliferation in rodents as it relates to cancer in humans is not yet understood (Rao and Reddy, 1991; Latruffe, 1997). Compared with rats, hamsters are generally less susceptible to the peroxisome proliferative effects of GEM (Gray and de la Iglesia, 1984). Previous toxicokinetic studies showed that orally administered GEM was more bioavailable in rats (>80%) than in hamsters (<20%), and that the elimination half-life of GEM is longer for rats (5–10 h) than hamsters (≤1 h) (Grizzle et al., 1995). In the current studies, we investigated the metabolism and disposition of GEM in rats and hamsters to aid in the understanding and interpretation of observed species differences in GEM toxicokinetics and toxicity (e.g., peroxisome proliferation).

The cumulative excretion of orally administered radioactivity in rats and hamsters was species-dependent and, in rats, dose-dependent. Fecal excretion of GEM-derived radioactivity by rats in the low-dose study is consistent with previously published results (Okerholm et al., 1976; Curtis et al., 1985; Sallustio et al., 1996). Repeated administration of the low oral GEM dose to male rats did not alter the route of excretion, and no new metabolites were present in the radiochromatograms of urine compared to the single-dose study (not shown). At the high oral dose (2000 mg/kg), the primary route of excretion by rats shifted from feces to urine, which suggests that biliary transport mechanisms may be saturated at the high oral dose. Once the rats were dosed, an excessive amount of water was dispensed from the water sipper tubes attached to the metabolism cages of both male and female rats. Feces from several rats in the high-dose group, especially males, appeared to be encased in gel-like capsules.

In contrast to rats, hamsters excreted the majority of the administered radioactivity in urine regardless of dose. The urinary excretion of GEM-derived radioactivity reported by Okerholm et al. (1976) for humans (ca. 66%) is intermediate between rats (ca. 30%) and hamsters (ca. 90%). Hamsters also excreted dose-derived radioactivity more rapidly than did rats. The rate of excretion of radioactivity (percent dose per hour) by hamsters, however, decreased as dose increased. Hamsters appeared to tolerate the high dose better than did rats; however, at necropsy, pale kidneys were observed in several hamsters.

An extraordinary species difference in the biliary excretion of i.v.-administered GEM was observed in rats and hamsters. Male and female rats excreted essentially all of the dose-derived radioactivity in bile by 2 h after administration. Male hamsters, on the other hand, excreted approximately 20% of the dose in bile, with females excreting about half that of males. This is consistent with the higher fecal excretion of [14C]GEM-derived radioactivity by rats compared with hamsters after an oral dose, and with the longer elimination half-life reported for rats compared with hamsters (Grizzle et al., 1995).

A proposed metabolic scheme for GEM is shown in Fig. 7. It has been reported that the free meta-benzoic acid metabolite of GEM was present in the urine of rats and humans treated with GEM (Okerholm et al., 1976; Curtis et al., 1985; Nakagawa et al., 1991; Sallustio and...
Fairchild, 1995). In the current studies, however, the dihydroxylated metabolite and the acid alcohol metabolite were the only free (i.e., unconjugated) phase I metabolites observed in urine or bile from treated animals. The use of sophisticated mass and NMR spectrometric techniques in the current studies permitted the identification of 10 GEM metabolites isolated from urine. This work included the first determination of the position of the glucuronic acid moiety on the various glucuronidated metabolites (Thomas et al., 1999). Chromatographic analyses of the metabolites subjected to β-glucuronidase/sulfatase enzyme incubation and base hydrolysis were in accord with mass spectrometry and NMR identifications, and the retention times of the cleaved aglycones were consistent with those of the metabolite standards obtained from Parke-Davis or the parent GEM. Two previously unreported metabolites were identified in which both ring methyl groups were oxidized, the dihydroxylated metabolite and the 5′-acid-4′-alcohol metabolite (Fig. 7). The 4′-ring hydroxylated metabolite was present in urine as both glucuronide and sulfate conjugates.

The majority of an i.v. dose to rats was excreted in bile as the acyl glucuronide of GEM. In contrast to rats, 5% or less of the dose was excreted in hamster bile as this metabolite. Active biliary transport systems such as the canalicular multispecific organic anion transporter and P-glycoproteins play a role in the biliary excretion of xenobiotics, including glucuronides, in rats (Koboyashi et al., 1991; Vore, 1993; Oude Elferink and Jansen, 1994; Shimamura et al., 1994; Oude Elferink et al., 1995; Yamazaki et al., 1996; Jedlitschky et al., 1997; Suchy et al., 1997; Takenaka et al., 1997; Vore et al., 1997). One or more of these transporters may be responsible for the efficient biliary excretion of glucuronidated GEM metabolites in rats. The literature contains no descriptions of biliary transport systems in the hamster. The results for GEM reported here suggest that the biliary excretion of GEM and its metabolites in hamsters may be occurring by a mechanism different from that in rats. Another possible explanation for the observed species difference in biliary excretion is that the specificity of the biliary transport systems in hamsters is different from that in rats.

Urinary metabolite profiles varied with species, sex, and dose (Figs. 4 and 5). Not only did hamsters excrete more of the dose in urine than did rats, hamsters excreted more radioactivity as oxidative metabolites. The majority of the oral dose administered to male hamsters was excreted in urine as conjugates of oxidative GEM metabolites, primarily as glucuronides of the meta-benzoic acid metabolite. For female hamsters, glucuronide conjugates of the meta-benzoic acid were the major metabolites in urine after the high dose, and the acyl glucuronide of GEM was the major metabolite after the low dose. The rapid urinary excretion of oxidative metabolites is consistent with the short biological half-life reported for GEM in hamsters (Grizzle et al., 1995).

Male rats were similar to female hamsters in the pattern of urinary metabolites, the major metabolites in male rat urine were the acyl glucuronide of GEM (low dose) and the glucuronide conjugates of the meta-benzoic acid (high dose). The major metabolite in female rat urine after the low oral dose was the acyl glucuronide of GEM. It should be noted that in male and female rats, urinary excretion of the acyl glucuronide of GEM was not dependent on dose, and females excreted approximately twice as much of this conjugate than did males. In both male and female rats, however, there was a dose-dependent increase in the number and extent of free and conjugated oxidative metabolites (e.g., meta-benzoic acid and 4′-ring hydroxylated metabolites) excreted in urine. The increase in urinary excretion of oxidative metabolites after the high oral dose, in conjunction with the extraordinary biliary excretion of the acyl glucuronide of GEM after an i.v. dose, suggest that formation and/or biliary transport of the acyl glucuronide of GEM may be saturated in rats after a high oral dose. Both of these would result in an increase in the proportion of the high oral dose that is available for oxidative metabolism and subsequent renal elimination.

These studies demonstrate that enterohepatic circulation does not play a significant role in the elimination of GEM in hamsters because of the extensive and rapid urinary excretion. The urinary excretion of GEM-derived radioactivity by humans is intermediate between the low excretion by rats and the high excretion by hamsters. Biliary excretion of the acyl glucuronide of GEM and enterohepatic recycling play a major role in GEM disposition in rats. The acyl glucuronide of GEM has been shown to bind to plasma and tissue proteins (Sallustio and Foster, 1995) and to cause damage to nuclear proteins and DNA (Sallustio et al., 1997). Therefore, increased exposure of the liver to the acyl glucuronide of GEM via enterohepatic recycling in rats may be a significant factor in the observed peroxisome proliferation and/or hepatocarcinogenesis in rats, which is not observed in hamsters or humans.

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References


