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BILIARY AND RENAL EXCRETION, HEPATIC METABOLISM, AND HEPATIC SUBCELLULAR DISTRIBUTION OF METRONIDAZOLE IN THE RAT*

NICHOLAS F. LARUSSO,†‡ DONALD G. LINDMARK and MIKLÓS MÜLLER
The Rockefeller University, New York, NY 10021, U.S.A.

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Abstract—We studied the biliary and renal excretion, hepatic metabolism, and hepatic subcellular distribution of [^{14}C]metronidazole in bile fistula rats. An average of 71.1 per cent of an intraduodenal or intravenous dose of [^{14}C]metronidazole was excreted in 24 hr, 23.9 per cent in bile and 47.6 per cent in urine. Renal pedicle ligation caused a 150 per cent increase in biliary excretion of label, whereas phenobarbital pretreatment had no effect. The majority of label in bile and urine was associated with a polar derivative, tentatively identified by thin-layer chromatography and enzymatic hydrolysis as the monoglucuronide conjugate of metronidazole. After intraduodenal administration of purified conjugated [^{14}C]metronidazole to rats with ligated renal pedicles,only a small amount of label (12.6 per cent of dose in 24 hr) appeared in bile. Growth inhibition studies showed the glucuronide conjugate to be devoid of antimicrobial activity against a metronidazole-sensitive organism, *Tritrichomonas foetus*. Uptake studies indicated that these organisms were incapable of concentrating conjugated metronidazole. Fractionation of rat liver homogenates by differential centrifugation after intravenous [^{14}C]metronidazole showed that 90 per cent of label present in liver was in the non-particulate fraction. Our results in rats indicate that metronidazole undergoes an enterohepatic circulation and that the liver plays a major role in the metabolism and excretion of this compound.

Little information exists on the hepatic metabolism and biliary excretion of metronidazole [Flagyl, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], an agent used widely in the treatment of bacterial and parasitic infections [1]. On the one hand, its low molecular weight (mol. wt = 171), trivial protein binding [2, 3], and relatively high renal clearance [4–6] argue against significant biliary excretion. On the other hand, the large and persistent hepatic localization of [^{14}C]metronidazole demonstrated by radioautography in mice [7], the identification of oxidized and conjugated metabolites of metronidazole in human urine [8–10], and the detection of metronidazole in rat bile after oral [11] or intravenous [12] administration, all suggest a major role for the liver in the metabolism and excretion of this compound.

In studies reported here, we have measured the biliary and renal excretion of [^{14}C]metronidazole during 24 hr in bile fistula rats under a variety of conditions. We also characterized the biliary and urinary metabolites of metronidazole, and described its hepatic subcellular distribution. Finally, we isolated and purified the major biliary metabolite of metronidazole, and investigated its enterohepatic circulation in rats, as well as its uptake by and cytocidal activity against a sensitive protozoon. Our results indicate that a significant amount of metronidazole (25 per cent of the administered dose) undergoes an enterohepatic circulation, and that its major biliary metabolite is a glucuronide conjugate. Our data also suggest that hepatocyte intracellular transport and biliary excretion of metronidazole involve non-vesicular mechanisms. Finally, our results show that conjugation of metronidazole to glucuronic acid abolishes its antimicrobial activity by preventing uptake by susceptible organisms. The results have been reported in part in abstract form [13].

MATERIALS AND METHODS

Materials. Metronidazole [1-(2-hydroxyethyl) 2-methyl-5-nitroimidazole] was kindly supplied by G. D Searle & Co. (San Juan, PR). Two metronidazole derivatives, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 1-acetic acid-2-methyl-5-nitroimidazole, and [^{14}C]metronidazole [1-(2-[U-^{14}C]-hydroxyethyl)-2-methyl-5-nitroimidazole] provided by Searle Laboratories (Chicago, IL). [^{14}C]metronidazole had a specific activity of 17 μCi/mg and was 98 per cent pure by thin-layer chromatography (t.l.c.). A highly polar derivative of [^{14}C]metronidazole tentatively identified as its glucuronide conjugate was isolated by thin-layer chromatography of bile from rats previously given [^{14}C]metronidazole intravenously. The material, purified by preparative thin-layer chromatography, showed greater than 98 per cent radiochemical purity by t.l.c.

β-Glucuronidase (type H-2, from *Helix pomatia*)

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† Dr. LaRusso is a Mayo Foundation Scholar and a Teaching and Research Scholar of the American College of Physicians.

‡ Address reprint requests to: Nicholas F. LaRusso, M.D., Gastroenterology Unit, St. Marys Hospital, Mayo Clinic and Foundation, Rochester, MN 55901, U.S.A.

and sulfatase (type V) were purchased from Sigma Chemical Co., St. Louis, MO. Sprague–Dawley male rats (150–350 g body weight) were used in all studies.

General experimental procedure. A total bile fistula was prepared using ether anesthesia at 7.00 a.m. in rats kept from solid food for the previous 24 hr. The rats were put in Bollman restraining cages prepared by the Engineering Department of the Rockefeller University and modified to allow quantitative urine collections separate from faeces. [^{14}C]metronidazole or its glucuronide conjugate was administered as a bolus intravenously via a tail vein or intraduodenally by transmucosal needle-puncture of the duodenum. Bile was collected through a fine catheter in the common bile duct for 24 hr into preweighed tubes at 1- to 2-hr intervals for 12 hr, and then at 8- to 12-hr intervals. Volumes were determined by the weight difference in tubes before and after bile collection, assuming a specific gravity for bile of 1.0. Urine was collected into graduated test tubes placed beneath a funnel attached to the bottom of the restraining cage. During the study period, nothing was given by mouth, but saline (0.9% NaCl) was administered through a tail vein at 1.7 ml/hr.

Excretion studies. The amount of label excreted during 24 hr in the bile and/or urine of five groups of rats was measured (Table 1). Group 1 was given (i.v.) 1.5 μCi [^{14}C]metronidazole diluted in normal saline with 0.33 to 20.0 mg of cold metronidazole. Group 2 was given approximately the same doses intraduodenally. Since no differences were evident in the excretion rates of ^{14}C in bile over the dose range administered, a standard dose of 0.33 mg of cold metronidazole was used to dilute the 1.5 μCi of [^{14}C]metronidazole given in all subsequent studies. Group 3 was pretreated for 5 days with 75 mg/kg body weight of intraperitoneal phenobarbital before intravenous administration of [^{14}C]metronidazole. Group 4 received intravenous [^{14}C]metronidazole after bilateral renal pedicle (renal artery, vein and ureter) ligation, and bile was collected for 24 hr. Finally, [^{14}C]metronidazole glucuronide, prepared as described, was administered intraduodenally to rats with renal pedicle ligation (Group 5), and bile collected as above.

Determination of ^{14}C. ^{14}C was determined by liquid scintillation counting using external standardization for quench correction in a Packard 3255 liquid scintillation spectrometer after 100–500 μl aliquots of bile and urine were mixed with 10 ml of Bray's solution [14].

Total body ^{14}C remaining at the end of the study period in rats of Group 1 was also determined [15]. Animals were sacrificed and placed whole into beakers containing 10% KOH for approximately 2 weeks. Twelve ml of the resulting suspension was mixed with 4 ml H_2O in duplicate, and 1 ml of 30% H_2O_2 added to 1 ml of this mixture. The samples were then heated at 80° for 5 hr, left overnight at room temperature, and ^{14}C was determined as above.

Chromatographic analysis of body fluids. A thin-layer chromatographic system was developed which conveniently separated metronidazole from its two available polar derivatives (i.e. the 1-acetic acid and 2-hydroxymethyl compounds). The solvent consisted of methanol–heptane–chloroform–ammonium hydroxide (26:15:59:3, v/v). Samples of bile and urine were chromatographed on silica gel plates (Eastman Chromatogram Sheet, silica gel with fluorescent indicator) in this system before and after treatment with β-glucuronidase or sulfatase. Treatment with a large excess (400 units) of enzyme was done at 37° for 3–6 hr in 150 mM acetate buffer, pH 5.

Subcellular distribution studies. Two hr after the intravenous administration of [^{14}C]metronidazole, two rats were exsanguinated, their livers quickly removed, placed separately in ice-cold sucrose (250 mM), and homogenized with three strokes of a Potter–Elvehjem homogenizer. The homogenate from each rat was then separated into five fractions on a Beckman L2 ultracentrifuge, as described [16]. ^{14}C in each fraction was measured by liquid scintillation counting with external standardization for quench correction. Marker enzymes (cytochrome oxidase for mitochondria; β-*N*-acetylglucosaminidase for lysosomes; esterase for microsomes) [17–20], as well as total protein [21], were also measured in each fraction.

Inhibition and uptake studies. The capacity of biliary metronidazole and its glucuronide conjugate to inhibit the growth of *Tritrichomonas foetus*, an anaerobic protozoon sensitive to metronidazole, was tested using the single-layer agar plate technique in anaerobic jars (GasPak anaerobic system; BBL) as described [22]. Inhibitory concentrations of metronidazole and metronidazole glucuronide were also compared. Finally, the anaerobic uptake by *T. foetus* of metronidazole and its glucuronide conjugate before and after treatment with β-glucuronidase was determined as described [23]. Briefly, cells were collected by centrifugation at room temperature and suspended in a buffered salt solution. Aliquots of the suspension were placed in closed flasks in a shaking water bath at 37°, and the flasks continuously flushed with 5% CO_2 in N_2. Test solutions containing isotopically labeled drug were injected and samples taken periodically. Radioactivity in aliquots of dissolved cells previously washed and isolated by centrifugation was determined by liquid scintillation counting.

Statistical analysis. All statistical analyses were done utilizing an unpaired, two-tailed *t*-test, and data are expressed as mean ± standard deviation. Variability for cumulative ^{14}C-excretion in bile was expressed as the coefficient of variation (mean/standard deviation ×100) for each time point.

RESULTS

Biliary and renal excretion of ^{14}C. Table 1 gives results for the 24-hr excretion of label in bile and urine of rats in Groups 1–5. Groups 1–3 excreted 73–86 per cent of the administered label by these two routes. Another 15.8 ± 6.6 per cent of the administered label remained in the organism as shown by total body dissolution of four rats from Group 1 at the end of the collection period. Thus, over 90 per cent of the administered label was

Table 1. Biliary and renal excretion of ^{14}C

Groups*	N	Route of drug administration	Manipulation	Per cent of administered dose (mean ± S.D.) Bile	Urine	Total
1	7	Intravenous		23.1 ± 7.1	50.0 ± 11.5	73.1 ± 9.6
2	5	Intraduodenal		24.6 ± 4.7	45.1 ± 9.9	69.1 ± 10.5
3	5	Intravenous	Phenobarbital pretreatment	23.7 ± 7.7	61.9 ± 4.3‡	85.5 ± 5.7‡
4	4	Intravenous	RPL†	58.0 ± 10.5‡		
5	3	Intraduodenal	RPL	12.6 ± 6.1‡		

* [^{14}C]metronidazole was administered to Groups 1–4; purified [^{14}C]metronidazole glucuronide was administered to Group 5.

† RPL = renal pedicle ligation.

‡ Significant ($P < 0.05$) difference compared to other groups.

recovered, and approximately 33 per cent of the total label excreted was found in bile. There was no difference in the amount of label excreted in bile and urine by rats after intravenous (Group 1) and intraduodenal (Group 2) administration. However, more label was excreted by rats pretreated with phenobarbital (Group 3) than by untreated rats. This difference reflected greater renal, not biliary, excretion. After intravenous administration of [^{14}C]metronidazole to rats with renal pedicle ligation (Group 4), biliary excretion of label increased by approximately 150 per cent compared to rats with intact renal function (Groups 1–3). Finally, when [^{14}C]metronidazole glucuronide, previously purified from bile, was instilled intraduodenally in rats with renal pedicle ligation (Group 5), only 12.6 per cent of the administered label appeared in bile. This was markedly less than was excreted by rats with renal pedicle ligation given [^{14}C]metronidazole by the same route (Group 4).

Figure 1 shows the time course of biliary excretion of ^{14}C in Groups 1–5. At 8 hr after administration, more than 90 per cent of the total label recovered in bile had been excreted by rats in Groups 1–3. In contrast, only 75 per cent of the label in bile had been excreted in 8 hr by rats with renal pedicle ligation given [^{14}C]metronidazole (Group 4). Finally, biliary excretion of ^{14}C by rats with renal pedicle ligation given purified [^{14}C]metronidazole glucuronide intraduodenally was slower and smaller than in rats given [^{14}C]metronidazole. The average coefficient of variation for all time points for all groups was 30 per cent.

The time course of renal excretion of [^{14}C]metronidazole by animals in Groups 1–3 (data not shown) was similar to biliary secretion although the total amount of ^{14}C excreted, as given in Table 1, was greater.

Thin-layer chromatography of bile and urine. Figure 2 shows results of a representative thin-layer chromatogram of bile taken at 3 hr after intravenous administration of [^{14}C]metronidazole to a rat from Group 1. More than 90 per cent of the label (91.7 ± 5.1, Group 1) is localized to two zones: (1) a fast migrating zone ($R_f = 0.72$) which moved identically to the metronidazole standard and comprised 7 per cent (7.5 ± 2.7, Group 1) of biliary ^{14}C, and (2) a more polar, slowly migrating zone

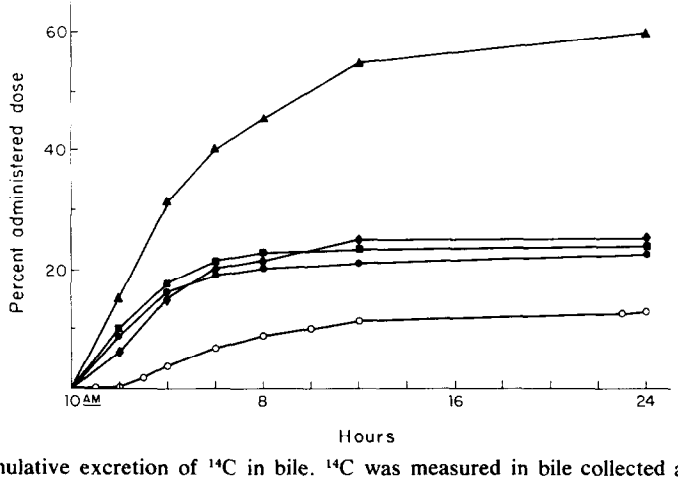

Fig. 1. Cumulative excretion of ^{14}C in bile. ^{14}C was measured in bile collected at times indicated from bile fistula rats under the following conditions: Group 1 (●—●), intravenous [^{14}C]metronidazole; Group 2 (◆—◆), intraduodenal [^{14}C]metronidazole; Group 3 (■—■), intravenous [^{14}C]metronidazole after phenobarbital treatment; Group 4 (▲—▲), intravenous [^{14}C]metronidazole after renal pedicle ligation; and Group 5 (○—○), intraduodenal [^{14}C]metronidazole glucuronide after renal pedicle ligation.

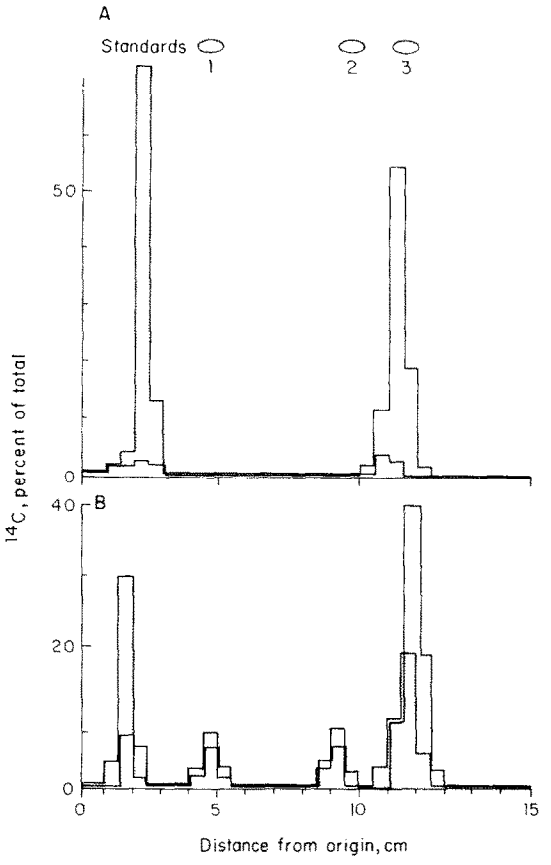

Fig. 2. Thin-layer chromatographic distribution of ^{14}C in bile (A) and urine (B). Chromatography was done before (⊡) and after (□) exposure of bile and urine to β-glucuronidase. Standards used were: 1-acetic acid-2-methyl-5-nitroimidazole (1), 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (2), and metronidazole (3).

($R_f = 0.16$) comprising 85 per cent (84.2 ± 6.2, Group 1) of biliary ^{14}C. Incubation of bile with β-glucuronidase prior to chromatography caused a 90 per cent (89.8 ± 3.4, Group 1) decrease in the size of the slowly migrating zone with an identical increase in the zone corresponding to metronidazole (Fig. 2). Incubation of bile with sulfatase had no effect on the chromatographic distribution of label. Results from chromatography of bile taken from rats of Groups 2, 3 and 4 were similar.

A representative thin-layer chromatogram of urine showed a more complex pattern with more than 90 per cent (92.6 ± 4.4, Group 1) of the ^{14}C distributed among four identifiable peaks (Fig. 2). Approximately 41 per cent (40.9 ± 14.3, Group 1) of the label was localized to a highly polar, slowly migrating zone ($R_f = 0.15$) which showed a marked decrease after incubation of urine with β-glucuronidase. The remaining three peaks migrated identically to the three standards and comprised, in order of decreasing polarity, 10, 5 and 36 per cent (10.7 ± 3.2, 5.0 ± 2.0 and 36.0 ± 12.6) of the total ^{14}C respectively. Incubation with β-glucuronidase caused the zone which migrated identically to metronidazole to approximately double (65.9 ± 7.3) in radioactivity, this increase accounting for the loss of radioactivity from the slowly moving ($R_f = 0.15$) peak. β-Glucuronidase has no effect on the two peaks corresponding to the 1-acetic acid (10.1 ± 3.6) and 2-hydroxymethyl (5.6 ± 2.3) derivatives of metronidazole. Results from chromatography of urine taken from Groups 2 and 3 were similar.

Subcellular distribution of ^{14}C in rat liver. Figure 3 shows mean data for marker enzymes, total protein, and ^{14}C-distribution in rat liver (N = 2). Typical enzyme distribution patterns were obtained, with cytochrome oxidase, *N*-acetyl-β-glucosaminidase and esterase had their highest relative specific activity in the mitrochondrial (M), lysosomal (L) and microsomal (P) fractions, respectively. The typical enzyme distributions, as well as the recoveries, attest to the adequacy of the fractionation procedure. The overwhelming majority of the ^{14}C (90 per cent) was unassociated with any particulate fraction and was localized primarily in the non-sedimentable (S) fraction. The remaining label (10 per cent) was distributed among the four particulate fractions approximately in proportion to their protein content (Fig. 4).

Control experiments (N = 2) in which [^{14}C]metronidazole was added *in vitro* to liver homogenates and subsequent differential centrifugation performed showed results similar to the studies in which the labeled drug was given intravenously (data not shown).

Inhibition and uptake studies. Inhibition of the growth of *T. foetus* by metronidazole, rat bile without label (control), rat bile containing [^{14}C]metronidazole and [^{14}C]metronidazole glucuronide (test), and pure [^{14}C]metronidazole glucuronide isolated from rat bile is shown in Table 2. Metronidazole produced a sizable zone of inhibition (12 mm) at a concentration of 0.5 μg/μl. Bile alone did not inhibit growth, whereas bile containing both free and conjugated drug produced detectable inhibition only when the concentrations of free metronidazole were sufficiently high. The purified conjugate did not inhibit growth of *T. foetus* even at concentrations ten times higher than the inhibitory concentration of metronidazole.

As shown in Fig. 4, purified [^{14}C]metronidazole glucuronide was not taken up by *T. foetus* cells, whereas, after incubation with β-glucuronidase, uptake was similar to that of the metronidazole standard.

DISCUSSION

Excretion and hepatic metabolism of [^{14}C]-*metronidazole.* Our results indicate that, after intravenous or intraduodenal administration of [^{14}C]metronidazole in rats, a significant amount of label appears both in urine (50 per cent of dose) and bile (25 per cent of dose). Ings and McFadzean [12] also reported that about 50 per cent of oral [^{14}C]metronidazole is excreted in the urine of rats over 24 hr. In our studies, the majority of the label in both body fluids is associated with a highly polar derivative of metronidazole, tentatively identified as its glucuronide conjugate. Conjugation of metronidazole to a single glucuronic acid molecule would yield a product with a calculated molecular weight of 347. Hirom *et al.* [24] and others [25, 26] have

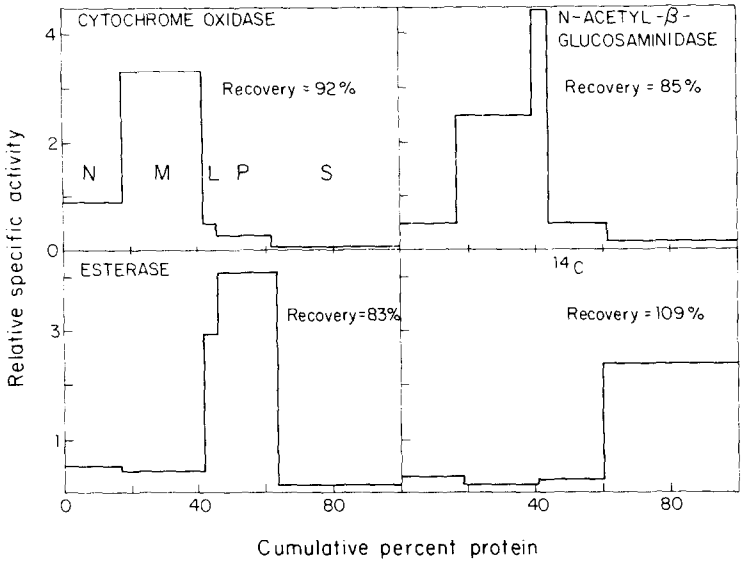

Fig. 3. Distribution patterns of constituents after fractionation of liver by differential centrifugation. Fractions are plotted in order of the average coefficient of sedimentation of their subcellular components, i.e. from left to right, N (nuclear fraction), M (heavy particle or mitrochondrial fraction), L (light particle or lysosomal fraction), P (microsomal fraction), and S (final supernatant fraction). Each fraction is represented separately on the ordinate scale by the relative specific activity of the constituent (percentage of total amount/percentage of total protein), and cumulatively on the abscissa by its percentage protein. Recovery represents a ratio of the sum of enzyme activities or ^{14}C in the individual fractions to values measured on the original liver homogenate.

reported that the molecular weight of the compound is a major determinant of the extent of its biliary and urinary excretion. Their data indicate that compounds with molecular weights between 300 and 500 are extensively excreted by both the kidney and liver, whereas those with molecular weights of less than 300 or more than 500 are excreted predominantly by the kidney or liver, respectively. The results of our studies on the biliary and renal excretion of metronidazole are consistent with their work.

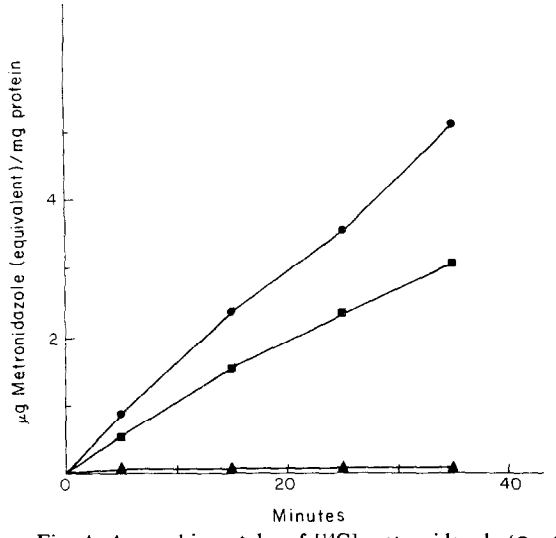

Fig. 4. Anaerobic uptake of [^{14}C] metronidazole (●—●), [^{14}C]metronidazole glucuronide (▲—▲), and β-glucuronidase-treated [^{14}C]metronidazole glucuronide (■—■) by *T. foetus*.

Hirom *et al.* [24] also showed that the biliary excretion of compounds with molecular weights of 300–500 increased greatly when the renal excretory route had been blocked. Our finding that biliary excretion of metronidazole increases by 150 per cent after renal pedicle ligation agrees with their results. Therefore, we interpret our data to indicate that the renal and biliary excretory routes of metronidazole are complementary, at least in the rat. These data also emphasize the importance of characterizing the metabolic alterations of a compound when evaluating the extent of its renal and biliary excretion.

While additional data are necessary to confirm the chemical nature of the major polar derivative of metronidazole in bile, our results utilizing thin-layer chromatography and enzymatic hydrolysis are strongly suggestive that this compound is the glucuronide conjugate of metronidazole. Metronidazole contains a hydroxyethyl group which would be capable of forming an ether linkage with glucuronic acid. While both the acetic acid and hydroxymethyl derivatives of metronidazole present in urine could also be conjugated to glucuronic acid via an ester or ether linkage, respectively, this probably does not occur, at least in the rat. Treatment of bile and urine with β-glucuronidase resulted in the majority of label migrating identically to metronidazole, with no increase in label migrating identically to either of these derivatives. Thus, it appears that the major metabolic alteration of metronidazole which occurs in the liver is the addition of a single glucuronic acid molecule via an ether linkage to the 1-hydroxyethyl group. While our studies provide no evidence for sulfation of metronidazole by the liver, others have reported chromatographic evidence of a sulfate of metronidazole in rat bile [12].

Table 2. Inhibition of growth of *T. foetus*

Substance tested		Zone of growth inhibition (mm)
Metronidazole		
0.5 μg/μl		12
1.0 μg/μl		22
Control bile*		
5–20 μl		0
Test bile†		
[^{14}C]metronidazole	[^{14}C]metronidazole glucuronide	
0.1 μg/μl	0.6 μg/μl	0
0.2 μg/μl	1.2 μg/μl	0
0.4 μg/μl	2.4 μg/μl	4
[^{14}C]metronidazole glucuronide (purified)		
1.2–4.8 μg/μl		0

* Bile from rat not previously given [^{14}C]metronidazole.

† Bile from rat (Group 1) 3 hr after administration of [^{14}C]metronidazole. Concentrations of [^{14}C]metronidazole and [^{14}C]metronidazole glucuronide are calculated values based on radioactivity in bile after chromatographic separation of the two compounds.

Our studies demonstrating the biliary excretion of label after intraduodenal administration of [^{14}C]metronidazole establish the existence of an enterohepatic circulation. Whether the glucuronide conjugate of metronidazole undergoes additional enterohepatic cycles after biliary excretion depends primarily on the extent of its intraluminal hydrolysis. It is well known that glucuronide conjugates are highly acidic compounds that are completely ionized at physiologic pH, and thus are poorly absorbed by the intestine [27]. Therefore, the appearance of label in bile after the intraduodenal administration of purified [^{14}C]metronidazole glucuronide suggests that intraluminal deconjugation with subsequent reabsorption of the aglycone is occurring [28]. Ideally, the extent of such deconjugation and subsequent enterohepatic cycling should be assessed by studying the intestinal absorption of metronidazole glucuronide differentially labeled on both the nitroimidazole and glucuronic acid moieties. Nevertheless, our data are consistent with the following sequence of events: intestinal absorption of metronidazole, hepatic conjugation to glucuronic acid, biliary excretion of metronidazole glucuronide, moderate intraluminal deconjugation and reabsorption of the aglycone, and subsequent reconjugation and re-excretion in bile, i.e. a repetitive enterohepatic cycling.

Since phenobarbital has been reported to increase the biliary excretion of certain exogenous compounds (i.e. dicumarol) that undergo hepatic glucuronidation [29, 30], we studied its effect on the biliary excretion of [^{14}C]metronidazole. Phenobarbital pretreatment, at a dosage known to stimulate UDP-glucuronyl transferase activity [31], did not increase the biliary excretion of the label. However, total excretion of label increased compared to untreated rats because more label was excreted by the kidneys. Others have also reported that phenobarbital may increase the renal excretion of the glucuronide conjugates of certain compounds [32]. The mechanism of this effect is now known.

The failure of phenobarbital to increase the biliary excretion of metronidazole does not eliminate the possibility that other microsomal enzyme inducers (i.e. 3-methylcholanthrene) might have this effect, since there seems to be no correlation between the degree of enzyme induction produced by a compound and its ability to enhance biliary excretion [33].

Our studies provide no direct information on the precise mechanism (i.e. active vs passive) of biliary excretion of metronidazole. The small amount of free metronidazole present in bile (less than 10 per cent of the administered dose after 24 hr) is consistent with a passive transport process. Passive hepatic transport is also consistent with the known physicochemical properties of metronidazole, which behaves as a weak base in aqueous systems, and so is largely non-ionized at physiologic pH. However, transport of the glucuronide conjugate at the canalicular side of the hepatocyte should require an active process, since the ionization state of the conjugate would prevent extensive passive diffusion [27]. Additional studies are necessary to precisely characterize the biliary transport processes of metronidazole and its glucuronide conjugate.

Hepatic subcellular distribution of metronidazole. In order to investigate the possible role of hepatocyte subcellular organelles in the metabolism and biliary excretion of metronidazole, we performed differential centrifugation of rat liver homogenates after intravenous administration of [^{14}C]-metronidazole. Rats were sacrified 2 hr after intravenous administration of [^{14}C]metronidazole, a time when, based on our excretion studies, we judged that hepatic transport of label was probably maximal. Marker enzymes were determined to monitor the accuracy of the fractionation procedure, and to provide a basis for comparing the intracellular distribution of ^{14}C. Such an approach has been used sparingly to investigate the hepatic metabolism of drugs [34], but has been important in developing the concept of subcellular drug "targets" (e.g. lyso-

somotropic agents) [35]. Furthermore, since UDP-glucuronyl transferase appears to be an integral protein of the inner aspect of the endoplasmic reticulum [36, 37], it seemed possible that an association between metronidazole and the microsomal fraction might be demonstrable. Finally, autoradiographic studies showing prolonged localization of label in mouse liver after intravenous administration of [^{14}C]metronidazole suggested that the drug might be associated with a specific hepatocyte organelle [7]. Results of our centrifugation studies show that nearly 90 per cent of the label was localized to the nonparticulate fraction of rat liver homogenates. The remaining 10 per cent was distributed among the four particulate fractions in proportion to the amount of protein present in each fraction. This distribution was clearly different from that of the marker enzymes measured. These data are consistent with the interpretation that the intracellular transport and biliary excretion of metronidazole glucuronide probably involve nonparticulate mechanisms, possibly a cytoplasmic transport protein. Others have reported that the biliary excretion of bilirubin [34] probably does not involve vesicular transport. Whether the biliary excretion of other endogenous or exogenous substances might involve vesicular transport across the hepatocyte is not known.

Antimicrobial properties of the glucuronide conjugate of metronidazole. The results of our growth inhibition studies indicate that the antimicrobial activity present in bile containing both free and conjugated metronidazole is due entirely to the unconjugated compound. Results of our uptake studies showed that purified metronidazole glucuronide was not taken up by the test organisms. However, the ability of these organisms to take up metronidazole, which is a prerequisite for its antimicrobial activity [23], was restored by treatment of the conjugate with β-glucuronidase. Thus, the lack of antimicrobial activity of the glucuronide conjugate is probably due to its failure to be taken up by susceptible organisms, rather than to any chemical alteration of the active site [23, 38, 39]. The loss of biological activity of a compound as a result of glucuronidation has been reported for other antimicrobial agents [40].

While *T. foetus* is known to possess glucuronidase activity, this enzyme is localized within lysosomes, and thus would be unable to hydrolyze the glucuronide conjugate of metronidazole unless it had already been taken up [41]. Our data suggest that *T. foetus* does not extensively hydrolyze metronidazole glucuronide, but gut bacteria possibly could do so. It is relevant that others have reported that metronidazole can be metabolized by certain gut bacteria [42]. Thus, if bacterial hydrolysis of the glucuronide conjugate of metronidazole occurred, the antimicrobial capacity of mletronidazole, which depends on reduction of the nitro group [22, 39, 40], would probably be restored. Alternatively, intraluminal hydrolysis of metronidazole glucuronide by extracellular glucuronidases might occur, resulting in release of the active aglycone. The appearance of label in bile after the intraduodenal instillation of purified [^{14}C]metronidazole glucuronide is consistent with both possibilities. Clearly, additional studies are needed to clarify the fate of the glucuronide conjugate of metronidazole within the gut lumen.

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