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Saturable First-Pass Metabolism of Sulfisoxazole N^1-Acetyl in Rats

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Abstract □ Saturable metabolism of sulfisoxazole N^1-acetyl in the rat during the initial pass of the drug from the intestinal lumen through the liver following oral administration of the drug (saturable first-pass metabolism) was investigated. The fraction of the total amount of drug recovered from the urine as the N^4-conjugate decreased as the dose of orally administered sulfisoxazole acetyl was increased. No dose dependency of the N^4-conjugated fraction was apparent following the intravenous administration of sulfisoxazole acetyl or the oral administration of sulfisoxazole at the same dose levels.

Keyphrases □ Sulfisoxazole acetyl—saturable first-pass metabolism, rats □ Metabolism, saturable first pass—sulfisoxazole acetyl, rats

Evidence of saturable metabolism of drugs in humans during the initial pass of the drug from the intestinal lumen through the liver following oral administration (saturable first-pass metabolism) has been reported for various drugs including propranolol (1), levodopa (2), lidocaine (3), and aspirin (4). Recent studies indicate that the aromatic amino group of certain drugs may undergo first-pass conjugation. For example, aminosalicylic acid is more extensively acetylated following oral administration than following intravenous administration, and the extent of acetylation following oral administration is dose dependent (5, 6).

This type of dose dependency may have great clinical implications, since small changes in the dose given, or in the rate or extent of absorption, may result in large, unexpected changes in the systemic availability of the drug. This study investigated the saturable first-pass conjugation of the aromatic amino group of sulfisoxazole N^1-acetyl (sulfisoxazole acetyl) and sulfisoxazole over a dose range where the extent of conjugation following intravenous administration of sulfisoxazole acetyl is constant.

EXPERIMENTAL

Materials—Sulfisoxazole N^1-acetyl[1], sulfisoxazole[2], propantheline bromide[3], polysorbate 80[4], polyethylene glycol 400 USP[5], ammonium sulfamate[6], reagent grade sodium nitrite[7], and N-(1-naphthyl)ethylenediamine dihydrochloride[8] were used as received. All other reagents were reagent grade.

Dosage Forms—Solutions for oral administration contained 2, 5, or 20 mg of sulfisoxazole acetyl or sulfisoxazole/ml of polysorbate 80. Solutions for intravenous administration contained 5, 12.5, or 50 mg of sulfisoxazole acetyl/ml of polyethylene glycol 400 containing 10% water. A solution of propantheline bromide for intraperitoneal administration contained 2.5 mg of the drug/ml of water.

***In Vivo* Urinary Excretion**—Male Sprague–Dawley rats[9], 200–350 g, were fasted 16 hr prior to and 12 hr following the initia-

[1] Donated by Hoffmann–La Roche, Nutley, N.J.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Pfaltz and Bauer, Flushing, N.Y.
[4] Atlas Chemical Industries, Wilmington, Del.
[5] Ruger Chemical Co., Irvington, N.J.
[6] MCB Manufacturing Chemists, Norwood, Ohio.
[7] J. T. Baker, Phillipsburg, N.J.
[8] Eastman Organic Chemicals, Rochester, N.Y.
[9] Hilltop Lab Animals, Chatsworth, Calif.

Table I—Urinary Recovery of Sulfisoxazole following Oral Administration of 100 mg/kg of Sulfisoxazole Acetyl Suspended in Various Lipids and Water

Vehicle	Cumulative Percent[a] of Dose Recovered at 96 hr			Fraction[c] Conjugated
	Free Drug	N^4-Conjugate[b]	Total	
Water (with 0.5% methylcellulose)	72.72 ± 7.90	5.21	77.93 ± 18.00	0.067
Polysorbate 80[d]	99.27 ± 14.54[e]	12.52	111.79 ± 22.08[e]	0.112
Hexadecane	73.85 ± 3.55	11.25	85.10 ± 9.66	0.132
Triolein	82.29 ± 6.69[e]	13.62	95.91 ± 14.94	0.142
Trioctanoin	84.54 ± 2.22[e]	15.90	100.44 ± 4.95[e]	0.158
Oleyl alcohol[f]	77.14 ± 5.51	25.11	104.56 ± 2.93[e]	0.262

[a] On a molar basis; average of six animals ± *SD* for each vehicle. [b] Difference between total and free drug. [c] Ratio of N^4-conjugate to total. [d] Solution. [e] Significantly different from the water vehicle as determined by the Student *t* test ($p < 0.05$). [f] Urinary recovery at 192 hr to achieve complete drug recovery.

tion of each absorption experiment. Free access to water was allowed throughout the experiments. All studies were initiated at the same time of the day to eliminate circadian variation.

All animals were lightly anesthetized with ether during the administration of oral and intravenous doses. Each oral sulfisoxazole acetyl or sulfisoxazole dosage form was administered *via* gastric tube at dose levels of 10, 25, and 100 mg/kg. The oral dose volume employed was 5.0 ml/kg. Each intravenous dosage form of sulfisoxazole acetyl was administered *via* the dorsal penile vein at dose levels of 10, 25, and 100 mg/kg. The dose volume for all intravenous dosage forms was 2.0 ml/kg.

As in previous studies (7), the amount of sulfisoxazole acetyl and sulfisoxazole delivered by the oral dosage form delivery system[10] was assayed in triplicate by UV spectroscopy (278 and 268 nm, respectively) to calculate the actual dose of drug administered. When it was used, propantheline bromide was injected at a dose of 5 mg/kg ip 1 hr before the administration of 100 mg/kg po of sulfisoxazole acetyl in polysorbate 80.

The animals were subsequently placed in individual metabolism cages[11] in a room with a timed light exposure of 9 hr of light alternated with 15 hr of darkness. Urine was collected at 12, 24, 48, 72, and 96 hr. All urine samples were immediately frozen and stored at −20° until assayed.

Analytical Methods—The colorimetric method of Bratton and Marshall (8) was used to assay for both free (non-N^4-conjugated) and total (N^4-conjugated and non-N^4-conjugated) sulfisoxazole in the urine. As reported previously (7), sulfisoxazole N^1-acetyl was not detected by TLC of urine following oral or intravenous administration of sulfisoxazole acetyl. The amounts of sulfisoxazole recovered from the urine following administration of sulfisoxazole acetyl were converted on a molar basis to apparent milligrams of

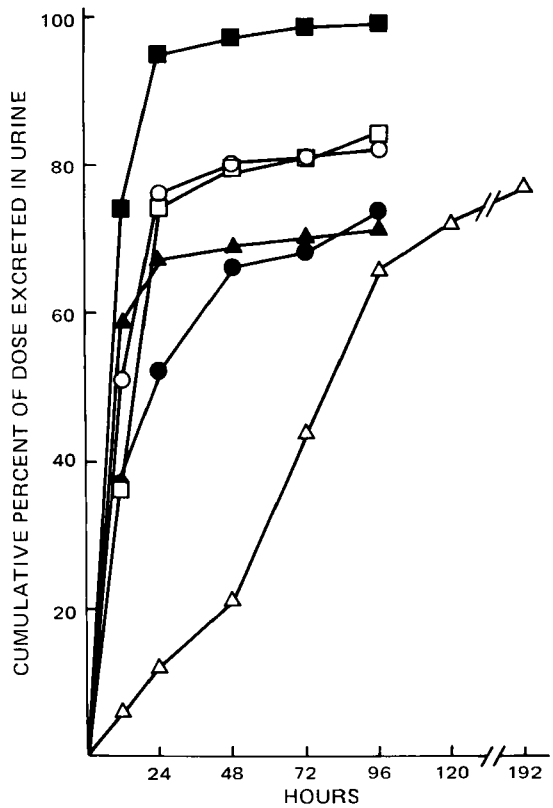

Figure 1—*Cumulative urinary excretion of free sulfisoxazole (expressed as a percent of the administered dose on a molar basis) following oral administration of a 100-mg/kg dose of sulfisoxazole acetyl in lipid vehicles and water. Each point represents the average of six animals. Key: ●, hexadecane; △, oleyl alcohol; ■, polysorbate 80 (solution); □, trioctanoin; ○, triolein; and ▲, water (with 0.5% methylcellulose).*

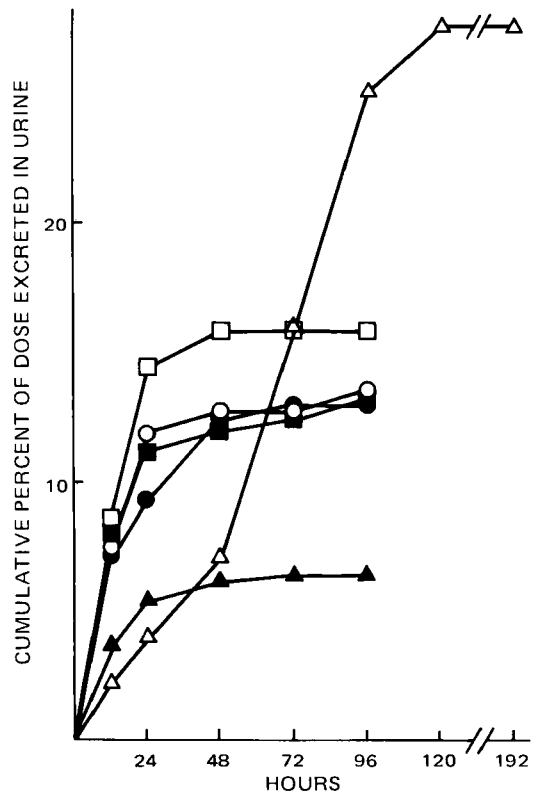

Figure 2—*Cumulative urinary excretion of N^4-conjugated sulfisoxazole (expressed as a percent of the administered dose on a molar basis) following oral administration of a 100-mg/kg dose of sulfisoxazole acetyl in lipid vehicles and water. Each point represents the average of six animals. Key: see Fig. 1.*

[10] Two-milliliter syringe with attached No. 8 French rubber catheter having a blunt tip and a hole in the side 5 mm from the tip.

[11] Model HB-11M with HB-66 food tunnel, Hoeltge, Inc., Cincinnati, Ohio.

Table II—Urinary Recovery of Free[a] Sulfisoxazole following Oral Administration of 100 mg/kg of Sulfisoxazole Acetyl Suspended in Various Lipids and Water

Vehicle	Cumulative Percent[b] of Dose Recovered at 12 hr
Water (with 0.5% methylcellulose)	58.07 ± 8.63
Polysorbate 80[c]	74.09 ± 17.02
Hexadecane	36.96 ± 14.91[d]
Triolein	50.91 ± 6.33
Trioctanoin	35.50 ± 10.71[d]
Oleyl alcohol	6.46 ± 2.49[d]

[a] Non-N^4-conjugated. [b] On a molar basis; average of six animals ± *SD* for each vehicle. [c] Solution. [d] Significantly different from water vehicle as determined by the Student *t* test ($p < 0.05$).

sulfisoxazole acetyl excreted for comparison to the dose of the drug administered to the animal.

Urine was collected from each animal for 24 hr prior to sulfisoxazole acetyl or sulfisoxazole administration and used to determine the assay blank. The assay blank of approximately 0.80 apparent mg of sulfisoxazole acetyl excreted/24 hr was not affected when polysorbate 80 was administered orally (5.0 ml/kg) or when polyethylene glycol 400 containing 10% water was administered intravenously (2.0 ml/kg) without the drug.

RESULTS AND DISCUSSION

The cumulative urinary recoveries of free (non-N^4-conjugated), N^4-conjugated, and total (N^4-conjugated and non-N^4-conjugated) sulfisoxazole following oral administration of sulfisoxazole acetyl (100 mg/kg) in various lipid vehicles and water (7) are plotted against time in Figs. 1, 2, and 3, respectively. The percent of the dose of sulfisoxazole acetyl excreted in the urine as the N^4-conjugate (Fig. 2) is the difference between the percent of the dose excreted in the urine as total sulfisoxazole following oral administration of sulfisoxazole acetyl (Fig. 3) and the percent of the dose excreted in the urine as free sulfisoxazole (Fig. 1) following oral administration of sulfisoxazole acetyl.

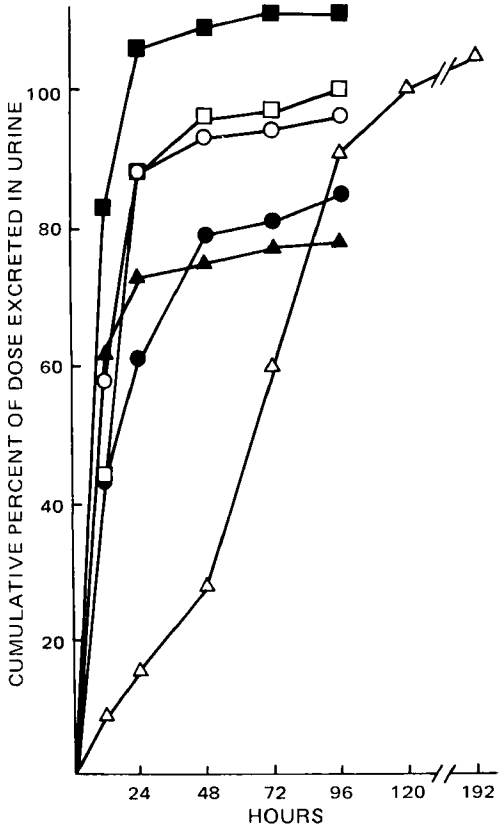

Figure 3—*Cumulative urinary excretion of total (free and N⁴-conjugated) sulfisoxazole (expressed as a percent of the administered dose on a molar basis) following oral administration of a 100-mg/kg dose of sulfisoxazole acetyl in lipid vehicles and water. Each point represents the average of six animals. Key: see Fig. 1.*

Table III—Urinary Recovery of Sulfisoxazole following Intravenous Administration of 100 mg/kg of Sulfisoxazole Acetyl and Simultaneous Oral Administration of the Indicated Vehicle

Vehicle	Cumulative Percent[a] of Dose Recovered at 96 hr: Free Drug	N^4-Conjugate[b]	Total	Fraction[c] Conjugated
Water (with 0.5% methylcellulose)	96.29	12.62	108.91	0.116
Polysorbate 80	94.49	12.93	107.42	0.120
Hexadecane	100.41	14.21	114.62	0.124
Triolein	99.59	12.53	112.12	0.112
Trioctanoin	99.19	11.81	111.00	0.106
Oleyl alcohol	100.61	12.66	113.27	0.112

[a] On a molar basis; average of two animals for each vehicle. [b] Difference between total and free drug. [c] Ratio of N^4-conjugate to total.

The cumulative urinary recoveries of free, N^4-conjugated, and total sulfisoxazole through 96 hr and the fraction conjugated following oral administration of sulfisoxazole acetyl (100 mg/kg) in various lipid vehicles and water (7) are listed in Table I. The fraction conjugated is the ratio of N^4-conjugated sulfisoxazole to total sulfisoxazole recovered from the urine through 96 hr following oral administration of sulfisoxazole acetyl. This fraction apparently varies with the vehicle in which the sulfisoxazole acetyl is administered. The rates of bioavailability, as indicated by the urinary recoveries of free sulfisoxazole at 12 hr (Table II), also have been shown to be affected by the vehicles (7).

Comparison of the fractions conjugated with the rates of bioavailability as indicated by the urinary recoveries of free sulfisoxazole acetyl at 12 hr results in a statistically significant ($p < 0.05$) negative linear correlation (Fig. 4). A similar correlation is ob-

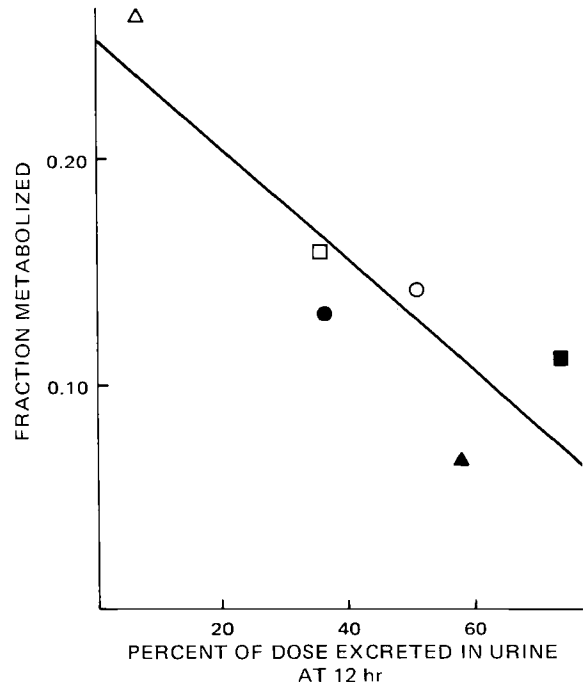

Figure 4—*Correlation of the rate of bioavailability of free sulfisoxazole (expressed as a percent of the administered dose on a molar basis excreted in the urine at 12 hr) with the total N⁴-conjugated metabolite excreted in the urine (expressed as a fraction of the total dose recovered from the urine). Each point represents the average of six animals. Key: see Fig. 1.*

Table IV—Urinary Recovery of Sulfisoxazole following Oral and Intravenous Administration of 10, 25, and 100 mg/kg of Sulfisoxazole Acetyl

Dose, mg/kg	Cumulative Percent[a] of Dose Recovered at 96 hr: Free Drug	N^4-Conjugate[b]	Total	Fraction[c] Conjugated
Oral Administration as Solution in Polysorbate 80				
10	88.68 ± 2.34	26.15	114.83 ± 5.06	0.228
25	85.75 ± 3.67	18.97	104.72 ± 2.79	0.181
100	99.27 ± 14.54	12.52	111.79 ± 22.08	0.112
Intravenous Administration as Solution in Polyethylene Glycol 400 Containing 10% Water				
10	101.15 ± 5.91	9.32	110.49 ± 4.94	0.085
25	99.81 ± 2.89	10.36	110.67 ± 3.29	0.098
100	95.02 ± 8.70	8.42	103.44 ± 9.13	0.081

[a] On a molar basis; average of six animals ± *SD* for each dose. [b] Difference between total and free drug. [c] Ratio of N^4-conjugate to total.

tained when the rate of absorption based on urinary recovery of total sulfisoxazole is used. These correlations suggest saturable first-pass conjugation of the aromatic amino group of sulfisoxazole acetyl during the initial pass of the drug from the GI lumen through the liver following oral administration. The correlation is not attributed to a direct effect of the vehicles, since the fraction conjugated following the intravenous administration of sulfisoxazole acetyl apparently is not affected when the vehicles without sulfisoxazole acetyl are administered orally (Table III).

The cumulative urinary recoveries of free, N^4-conjugated, and total sulfisoxazole and the fraction conjugated following oral and intravenous administration of sulfisoxazole acetyl at dose levels of 10, 25, and 100 mg/kg are listed in Table IV. The fractions conjugated following oral and intravenous administration of sulfisoxazole acetyl at the three dose levels are plotted in Fig. 5. From these data, it is apparent that the extent of conjugation of the drug increased with decreasing dose following oral administration. This behavior is consistent with the hypothesis of saturable first-pass conjugation.

Table V—Urinary Recovery of Sulfisoxazole following Oral Administration of 10, 25, and 100 mg/kg of Sulfisoxazole

Dose, mg/kg	Cumulative Percent[a] of Dose Recovered at 96 hr: Free Drug	N^4-Conjugate[b]	Total	Fraction[c] Conjugated
10	87.01 ± 7.46	12.77	99.78 ± 6.76	0.128
25	81.70 ± 3.48	12.86	94.56 ± 2.79	0.136
100	79.88 ± 4.24	11.41	91.29 ± 5.72	0.125

[a] Average of five animals ± *SD* for each dose. [b] Difference between total and free drug. [c] Ratio of N^4-conjugate to total.

Dose-dependent conjugation of sulfisoxazole acetyl was not observed when the drug was administered intravenously, indicating that the dose-dependent conjugation occurs before the drug reaches the systemic circulation. The extent of conjugation was greater when the drug was administered orally than when it was administered intravenously, giving additional evidence for a first-pass effect. The extent of drug absorption was complete following oral administration of sulfisoxazole acetyl dissolved in polysorbate 80 (Table IV).

To confirm further that the extent of conjugation is related to the rate of bioavailability or the absorption rate of sulfisoxazole acetyl, the effect of reducing the absorption rate by pretreating the animals with propantheline bromide was investigated. Propantheline bromide inhibits the motor activity of the stomach and small intestine and slows the absorption of drugs (9, 10). The extent of conjugation was increased in animals pretreated with propantheline bromide (5 mg/kg) 1 hr prior to the oral administration of sulfisoxazole acetyl in polysorbate 80 compared to the untreated con-

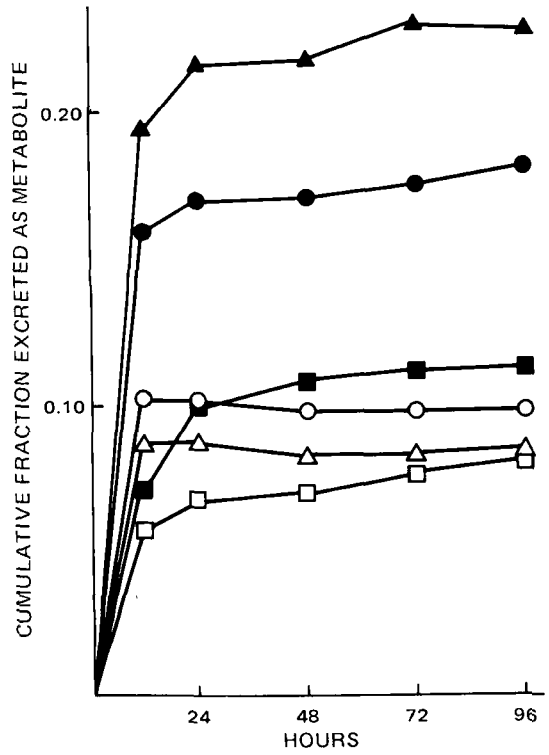

Figure 5—*Effect of the route of administration and dose on the cumulative* N^4*-conjugated metabolite excreted in the urine (expressed as a fraction of the total dose recovered from the urine) following administration of sulfisoxazole acetyl. Each point represents the average of six animals. Key:* △, *10 mg/kg iv;* ○, *25 mg/kg iv;* □, *100 mg/kg iv;* ▲, *10 mg/kg po;* ●, *25 mg/kg po; and* ■, *100 mg/kg po.*

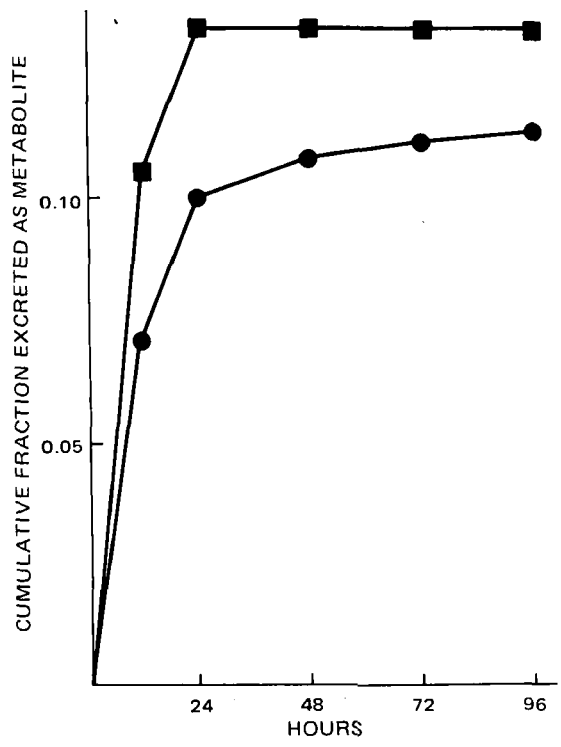

Figure 6—*Effect of propantheline bromide on the cumulative* N^4*-conjugated metabolite excreted in the urine (expressed as a fraction of total dose recovered from the urine) following oral administration of a 100-mg/kg dose of sulfisoxazole acetyl dissolved in polysorbate 80. Each point represents the average of six animals. Key:* ●, *no propantheline pretreatment; and* ■, *5-mg/kg propantheline pretreatment.*

trol group (Fig. 6). However, there was no significant difference between the conjugated fractions of the propantheline bromide-pretreated group and the untreated group.

The cumulative urinary recoveries of free, N^4-conjugated, and total sulfisoxazole and the fraction conjugated following oral administration of sulfisoxazole at dose levels of 10, 25, and 100 mg/kg dissolved in polysorbate 80 are listed in Table V. No difference exists among the total recoveries of sulfisoxazole. In addition, the fraction of sulfisoxazole metabolized following oral administration does not appear to be dose dependent.

This study shows the existence of saturable first-pass metabolism for orally administered sulfisoxazole acetyl in rats at dose levels equivalent to therapeutic maintenance dose levels used in humans (15 mg/kg every 4–6 hr). However, the results also indicate that saturable first-pass metabolism does not occur following the oral administration of similar doses of sulfisoxazole in rats.

Sulfisoxazole acetyl was originally synthesized to obtain an active preparation of sulfisoxazole without a bitter taste, which could be administered as an oral suspension to children (11, 12). Early reports indicate that the poorly water-soluble, lipophilic, sulfisoxazole acetyl is converted to sulfisoxazole in the GI tract, presumably by digestive enzymes (11, 12). The data in this study indicate that sulfisoxazole acetyl may be absorbed intact and presented to the site of N^4-conjugation at a concentration exceeding that of the less lipophilic sulfisoxazole. Thus, following oral administration, sulfisoxazole acetyl saturates the enzymes responsible for N^4-conjugation whereas sulfisoxazole does not.

This theory is supported by the decrease in the fraction metabolized (N^4-conjugated) as the dose of orally administered sulfisoxazole acetyl is increased from 10 to 100 mg/kg, whereas no dose dependency in the fraction metabolized (N^4-conjugated) is apparent following the oral administration of sulfisoxazole over this dose range. Approximately 85% of sulfisoxazole is protein bound in the blood of humans (13). Sulfisoxazole acetyl may be protein bound to a lesser extent than sulfisoxazole at the pH of the blood due to the ionization of sulfisoxazole but not sulfisoxazole acetyl. In general, the degree of protein binding is greater for charged sulfonamides than for uncharged sulfonamides (14).

The rate of uptake of the drug by the liver or another site of metabolism may depend upon its lipophilicity and the concentration of nonprotein-bound drug in the portal blood during the initial pass of the drug from the intestinal lumen through the liver following oral administration. Thus, protein binding of sulfisoxazole may spare it from first-pass metabolism over the dose range studied.

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