Metoclopramide Metabolism and Determination by High-Pressure Liquid Chromatography

LINA TENG ˣ, ROBERT B. BRUCE, and L. KAY DUNNING

Abstract □ A high-pressure liquid chromatographic method suitable for determining plasma metoclopramide levels at normal (10–20 mg) doses is described. Eight metabolites as well as metoclopramide were isolated and identified in rat, dog, and human urine. The only common metabolite in these species is 2-[(4-amino-5-chloro-2-methoxybenzoyl)-amino]acetic acid. *N*-Deethylation is a major pathway for metoclopramide metabolism in the lower animals but not in humans. Metoclopramide is excreted mainly unchanged or as its conjugates by humans.

Keyphrases □ Metoclopramide—high-pressure liquid chromatographic analysis, various metabolites isolated and identified, rat, dog, and human urine □ Metabolites, various—of metoclopramide, isolated and identified, high-pressure liquid chromatographic analyses, rat, dog, and human urine □ High-pressure liquid chromatography—analyses, metoclopramide and various metabolites in rat, dog, and human urine □ Antiemetic agents—metoclopramide and various metabolites, high-pressure liquid chromatographic analyses in rat, dog, and human urine

Metoclopramide is an antiemetic and antispasmodic agent presently undergoing clinical testing in the United States. Results previously were reported on its metabolism in lower animals (1–3). Plasma levels were followed in lower animals (2) after relatively large doses by use of a colorimetric procedure and TLC–colorimetry. However, human plasma levels following normal (10–20 mg) doses have not been reported.

This study confirms some metabolites found in lower animals and reports additional metabolites not previously found in animals and humans following usual clinical doses. An analytical method suitable for determining plasma levels at clinical doses is also reported.

EXPERIMENTAL

Animals—Three female Sprague–Dawley rats[1], 200–225 g, were given metoclopramide solution orally at 100 mg/kg. Each rat received 20.16 mg (12.5 μCi). One male beagle dog[2], 10.8 kg, received metoclopramide solution orally at 20 mg/kg. The dog received 215.7 mg in 50.0 ml of water (21.3 μCi). The animals were housed in individual metal metabolism cages with free access to food and water.

Blood samples from the dog were collected in oxalate-containing tubes and analyzed immediately for radioactivity. The urinary and fecal excrements were collected at 24-hr intervals for 3 or 4 days. The urine samples were frozen until analysis, and the fecal samples were freeze dried.

Human Subjects—Two healthy male volunteers who had given informed written consent each received an oral capsule containing ^{14}C-metoclopramide (10.0 mg, 45.0 μCi). Blood, urine, and feces were collected at intervals and kept frozen until analysis.

Materials—Metoclopramide[3] was used as received. The radiochemical purity of carbonyl-labeled ^{14}C-metoclopramide[4] (4.5 μCi/mg) was higher than 98% as determined by TLC in three solvent systems.

Enzyme[5] was used as purchased. The resin[6] was washed with methanol, acetone, and water before use.

Identification of Metabolites—The metabolites were purified by preparative TLC, and the purified metabolites were identified by mass spectrometry. Comparisons of IR and NMR spectra were used when enough material was available. The mass spectra[7] were taken with a direct probe method. NMR spectrometry[8] was carried out in chloroform-*d* solution. IR[9] spectra were run as thin films or potassium bromide pellets.

TLC—Aliquots or extracts of urine were spotted on precoated silica gel GF plates[10] and developed with the following solvent systems: A, chloroform–methanol–concentrated ammonium hydroxide (70:30:1); B, 2 *N* ammonium hydroxide–ethanol–ethyl acetate (3:3:8); C, ethyl acetate–acetic acid–water–ethanol (25:12:8:5); D, chloroform–methanol–concentrated ammonium hydroxide (70:30:2); E, chloroform–methanol–concentrated ammonium hydroxide (90:10:1); and F, 1-butanol–ethyl acetate–acetic acid–water (1:1:1:1).

Radioassay—Aliquots of solution were put in glass liquid scintillation counting vials and counted in a liquid scintillation spectrometer[11] using KTT phosphor. The freeze-dried fecal samples were combusted[12] and counted in a scintillation spectrometer. KTT phosphor was prepared by mixing 2,5-diphenyloxazole (8.25 g), 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (0.25 g), isooctylphenoxypolyethoxyethanol (500 ml), and toluene (1000 ml).

Column Chromatography—Raw urine samples were put through a resin[6] column (bed volume = one-half of the sample volume) and washed with water (two times bed volume); the drug-related compounds were eluted with methanol (three times bed volume).

Enzyme Hydrolysis—Raw urine was adjusted to pH 5.0 with acetic acid. The methanol eluate from column chromatography was evaporated to dryness, and the residue was taken up in 0.2 *M* acetate buffer (pH 5.0). The buffered solution was incubated with enzyme at 37° overnight[13].

Analysis of Plasma—The analysis method developed for the study of metoclopramide pharmacokinetics is based on HPLC with a UV detector at 280 nm. Since the UV spectrum of metoclopramide in water indicates a minimum of absorbance at 250 nm with maxima at 272 and 308 nm, the usual detector employed at 254 nm is not suitable and the 280-nm wavelength was used.

The following liquid chromatographic parameters were used: column, 15 cm × 0.5 mm silica gel M131; flow rate, 2.0 ml/min; temperature, ambient; and solvent, methanol–chloroform–concentrated ammonium hydroxide (30:70:0.5).

Procedure—A 5.0-ml sample of plasma (whole blood and urine also were analyzed) was mixed with 9.0 ml of water and 1.0 ml of 500 ng/ml of internal standard. Concentrated ammonium hydroxide, 2 ml, was added, and the mixture was extracted twice with 10.0-ml portions of chloroform. Layers were separated by centrifugation, and the chloroform extracts were combined.

The chloroform phase was extracted with 5 ml of 0.5 *N* HCl, and the organic phase was discarded after centrifugation. The aqueous layer was made alkaline, and the metoclopramide was extracted back into 5.0 ml of chloroform. Then the chloroform layer was transferred to another tube, evaporated under nitrogen to dryness, and dissolved in 100 μl of chloroform; 25 μl was injected into the liquid chromatograph[14].

The internal standard was prepared by the reaction of 4-amino-5-chloro-2-methoxy-*N*-(aminoethyl)benzamide with *n*-propyl bromide by refluxing overnight in ethanol. The ethanol was evaporated, and the residue was taken up in water, made basic, and extracted with chloroform. On evaporation, an oily residue crystallized. This residue was recrystallized from benzene to give 4-amino-5-chloro-*N*-[2-(propylamino)ethyl]-

[1] Charles River Animal Laboratory, Wilmington, Mass.
[2] Hazelton Research Laboratory, Cumberland County, Va.
[3] Laboratories Delagrange, Paris Cedex 07, France.
[4] A gift from Merck Sharp and Dohme, Rahway, N.J.
[5] Glusulase, Endo Laboratories, Garden City, N.Y.
[6] XAD-2, Rohm & Haas, Philadelphia, Pa.
[7] Hitachi RMU-6H mass spectrometer, Perkin-Elmer, Norwalk, Conn.
[8] Varian A60 NMR spectrometer, Varian, Palo Alto, Calif.
[9] Model 621 IR spectrometer, Perkin-Elmer, Norwalk, Conn.
[10] Analtech, Inc., Newark, Del.
[11] Tri-Carb model 3385, Packard Instrument Co., Downers Grove, Ill.
[12] Model 305 sample oxidizer. Packard Instrument Co., Downers Grove, Ill.
[13] Thelco model 83 incubator, Precision Scientific Co., Chicago, Ill.
[14] Model 3500 liquid chromatograph with model 225 dual-channel UV absorbance detector, Spectra-Physics, Santa Clara, Calif.

Table I—Excretion of ^{14}C-Metoclopramide (Percent) in Rats, Dogs, and Humans

	Rat[a]		Dog		Human[b]	
Hours	Urine	Feces	Urine	Feces	Urine	Feces
0–24	71.9	5.7	65.3	—	77.8	2.0
24–48	8.5	4.4	6.9	18.3	5.7	1.0
48–72	1.0	1.7	1.0	1.0	0.8	1.6
72–96	N.C.[c]	N.C.	N.C.	N.C.	—	0.7
Subtotal	81.4	11.8	73.2	19.3	84.3	5.3
	93.2		92.5		89.6	

[a] Average of three rats. [b] Average of two human subjects. [c] N.C. = not collected.

2-methoxybenzamide. This compound shows the same solubility properties as metoclopramide and is well separated from both metoclopramide and the monodeethylated metabolite on chromatography, acting as an ideal internal standard. The retention times of metoclopramide and the internal standard were 2.8 and 4.5 min, respectively. The monodeethylated compound appeared at 7.5 min, and the dideethylated metabolite appeared at 5.7 min.

RESULTS

Excretion—Table I shows the radioactivity recovery from rats, dogs, and humans. The majority of the radioactivity was eliminated in the urine in the first 24 hr after dosing.

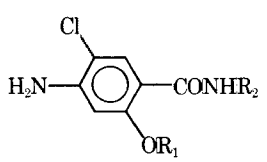
Metabolites in Rat Urine—The methanol eluate from column chromatography showed three major radioactive zones. There was no qualitative or quantitative difference in the raw urine and enzyme-hydrolyzed urine. The metabolites identified in the 0–24-hr rat urine accounted for over 60% of the total dose. The metabolites are listed according to descending R_f values.

Zone RA—The radioactivity in this zone accounted for 1–2% of the dose. Rechromatography with System E resolved this zone into several minor zones, two of which were identified.

Zone RA1—Mass spectrum of this metabolite showed a molecular ion at 200, indicating an even number of nitrogen atoms in the molecule. The base peak at *m/e* 184 indicated that there was no change on the ring substituents. This compound was assigned as 4-amino-5-chloro-2-methoxybenzamide (II).

Zone RA2—Two metabolites were in this zone, and one showed a molecular ion at *m/e* 244. The even molecular weight indicated an even number of nitrogen atoms. The base peak was at 184, indicating no change on the ring. Acetylation of this metabolite with acetic anhydride and pyridine gave a derivative of M + 328 with the base peak shifted to *m/e* 226. The metabolite picked up two acetyl groupings, one on the ring amino group and the other on the side chain. Another significant peak of the acetyl derivative at 268 was probably due to the loss of acetic acid. From these mass spectral data, Structure III, 4-amino-5-chloro-*N*-(2-hydroxyethyl)-2-methoxybenzamide, was assigned to this metabolite. The second component, M + 285, was not identified until the same metabolite was found later in dog urine.

Zone RB—IR, NMR, and mass spectra of this compound matched those of metoclopramide. Recrystallization of the compound twice from benzene gave a crystalline powder, mp 142–144° [lit. (1) mp 146.5°]. Therefore, this component was unchanged metoclopramide (I). Metoclopramide did not show a molecular ion under the present experimental



I: $R_1 = CH_3$, $R_2 = CH_2CH_2N(C_2H_5)_2$
II: $R_1 = CH_3$, $R_2 = H$
III: $R_1 = CH_3$, $R_2 = CH_2CH_2OH$
IV: $R_1 = H$, $R_2 = CH_2CH_2N(C_2H_5)_2$
V: $R_1 = CH_3$, $R_2 = CH_2CH_2NHC_2H_5$
VI*a*: $R_1 = CH_3$, $R_2 = CH_2COOH$
VI*b*: $R_1 = CH_3$, $R_2 = CH_2COOCH_3$
VI*c*: $R_1 = CH_3$, $R_2 = CH_2COOC_2H_5$
VII: $R_1 = CH_3$, $R_2 = CH{=}CH_2$
VIII: $R_1 = CH_3$, $R_2 = CH_2CH_2NH(COCH_3)$
IX: $R_1 = H$, $R_2 = CH_2CH_2NHC_2H_5$

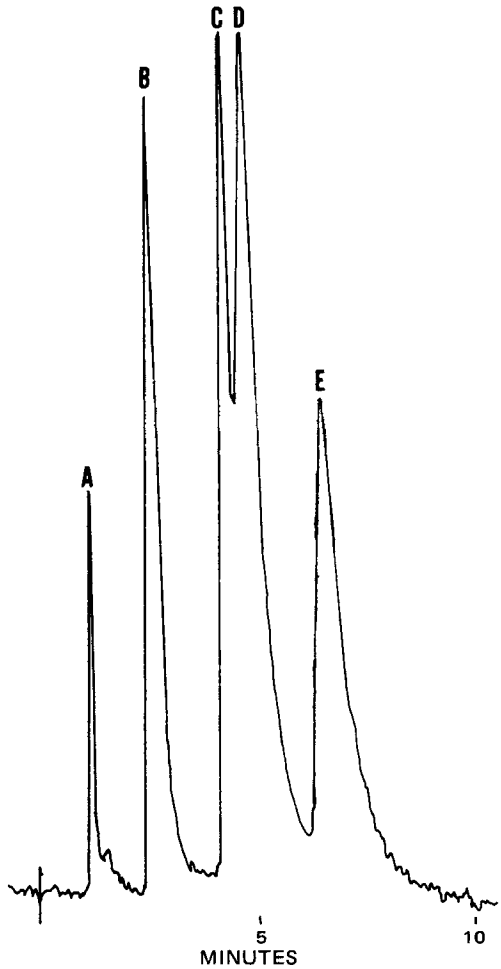


Figure 1—*Chromatogram of metoclopramide (B), internal standard (C), dideethylated metoclopramide (D), and monodeethylated metoclopramide (E). Peak A is the solvent.*

conditions. The base peak for this compound was 86 with strong peaks at 227, 201, 184, and 99.

Zone RC—On rechromatography with System E, this zone resolved into two components; one had a molecular weight of 285 by mass spectrometry. The base peak at 86 along with a strong peak at 99 suggested the existence of a $CH_2CH_2N(CH_2CH_3)_2$ grouping. The presence of a peak at 170 and the absence of a 184 peak suggested demethylation of the methoxy group on the ring. Not enough sample was available for derivatization. Structure IV, 4-amino-5-chloro-*N*-(2-hydroxy)-*N*-[2-(diethylamino)ethyl]benzamide, was assigned for this metabolite. The second metabolite in this zone was not identified.

Zone RD—On rechromatography with System F, this zone resolved into two compounds; one was identified as 4-amino-5-chloro-*N*-[2-(ethylamino)ethyl]-2-methoxybenzamide (V) by the following findings. The metabolite did not show a molecular ion. The base peak at *m/e* 58 along with other peaks at 213, 201, 184, and 71 was identical to the mass spectrum of the authentic sample. Comparison of the IR spectra of the metabolite and the authentic compound confirmed the identity further. The second metabolite was not identified.

Zone RE—Only one metabolite was found, and its mass spectrum showed only two peaks at *m/e* 184 and 44. The peak at *m/e* 44 (CO_2) indicated that the metabolite was probably an acid. The peak at *m/e* 184 indicated no change on ring substituents. Methylation of the metabolite with ethereal diazomethane gave a compound with a molecular weight of 272 and a strong peak at *m/e* 184. Ethylation of the metabolite with ethanol and hydrogen chloride gas gave an ethyl ester with a molecular weight of 286. This compound was assigned as 2-[(4-amino-5-chloro-2-methoxybenzoyl)amino]acetic acid (VI*a*). Its methyl and ethyl esters are VI*b* and VI*c*, respectively.

Metabolites in Dog Urine—TLC of the methanol eluate from the column revealed only two major radioactive zones. The metabolites were identified by TLC and mass spectrometry. The following metabolites

Table II—Results of Recovery of Known Amounts of Metoclopramide Added to Control Plasma (n = 6)

Metoclopramide, ng/ml		
Added	Found (Mean)	*SD*
10	9.6	±1.77
20	21.1	±1.89
60	61.3	±2.71
100	93.3	±5.93

were found in the 0–24-hr urine and amounted to about 60% of the dose.

Zone DA—This zone resolved into several minor zones with System E.

Zone DA1—This zone had a molecular ion at *m/e* 272. The metabolite had the same chromatographic behavior as 2-[(4-amino-5-chloro-2-methoxybenzoyl)amino]acetic acid methyl ester (VI*b*). This compound is possibly an artifact formed by the reaction of methanol and the acidic metabolite of Zone DF during workup.

Zone DA2—The molecular weight of this metabolite was established as 226 by mass spectrometry. Two other significant peaks were at *m/e* 200 and 184; unfortunately, not enough material was available for derivatization. This metabolite was tentatively assigned as 4-amino-5-chloro-*N*-ethenyl-2-methoxybenzamide (VII).

Zone DA3—This zone was identical to Zone RA1 in rat urine.

Zone DA4—The molecular weight of this metabolite was determined as 285 by mass spectrometry, indicating an odd number of nitrogen atoms. The base peak at *m/e* 184 indicated no change on the ring substituents. Acetylation of the metabolite gave a derivative M + 327, 42 mass units higher than the metabolite. The base peak shifted to *m/e* 226 from 184, indicating the only acetylation site to be NH_2 on the ring. The other nitrogen atoms were probably amide nitrogen or tertiary amine nitrogen. The mass spectrum of this acetylated metabolite was identical to that of the acetyl derivative of 4-amino-*N*-(2-aminoethyl)-5-chloro-2-methoxybenzamide. It was concluded that this metabolite was *N*-[2-(acetylamino)ethyl]-4-amino-5-chloro-2-methoxybenzamide (VIII).

Zone DA5—This metabolite was identical to the metabolite of Zone RA2 in rat urine.

Zone DB—This zone contained unchanged metoclopramide as shown by mass spectrometry and TLC.

Zone DD—This zone contained only one drug-related compound. The mass spectrum and TLC were identical to those of V.

Zone DE—This zone resolved into two drug-related compounds; one showed a weak molecular ion at *m/e* 257. The base peak at *m/e* 58 and another peak at *m/e* 71 suggested *N*-deethylation on the side chain. A strong peak at *m/e* 170 and others at *m/e* 200, 199, and 187 strongly indicated demethylation on the ring. From these data, 4-amino-5-chloro-*N*-[2-(ethylamino)ethyl]-2-hydroxybenzamide (IX) was proposed for this metabolite.

Zone DF—The only metabolite in this zone was identical to the metabolite of Zone RE in rat urine. Structure VI was proposed for it.

Metabolites in Human Urine—There were three significant radioactive spots in human urine. These three metabolites, found in the 0–24-hr urine, amounted to 60% of the dose.

Zone HA—This radioactive zone was shown to be unchanged metoclopramide by mass spectrometry and TLC comparisons in several solvent systems.

Zone HB—Due to large amounts of impurity, no meaningful spectral data were obtained initially for this metabolite. After treatment with diazomethane, a single radioactive derivative was isolated in the pure state. The methylated compound had M + 272 and the base peak was at *m/e* 184. This methyl ester was identical to the methyl ester of Zone RE in rat urine and Zone DF in dog urine. Therefore, the metabolite was VI*a*.

Zone HC—The intensity of this zone differed markedly before and after enzyme hydrolysis of the urine. This spot accounted for about 50% of the urinary radioactivity before enzyme hydrolysis, about 18% after hydrolysis, and practically none after acid hydrolysis. Hydrolysis of this spot gave unchanged metoclopramide. This zone was concluded to be the glucuronide and/or sulfate of metoclopramide.

HPLC Method—This method is sensitive to the determination of as little as 10 ng of metoclopramide/ml of plasma or blood when 5-ml samples are used. This sensitivity is sufficient for following kinetics of 10- or 20-mg doses. The method also appears to be specific under the conditions described, and I is well separated from expected metabolites (Fig. 1). However, no metabolite was detected in plasma or blood. The accuracy and precision of the procedure are indicated by the results shown in Table II. These results were obtained by analyzing samples of control plasma containing known quantities of added metoclopramide.

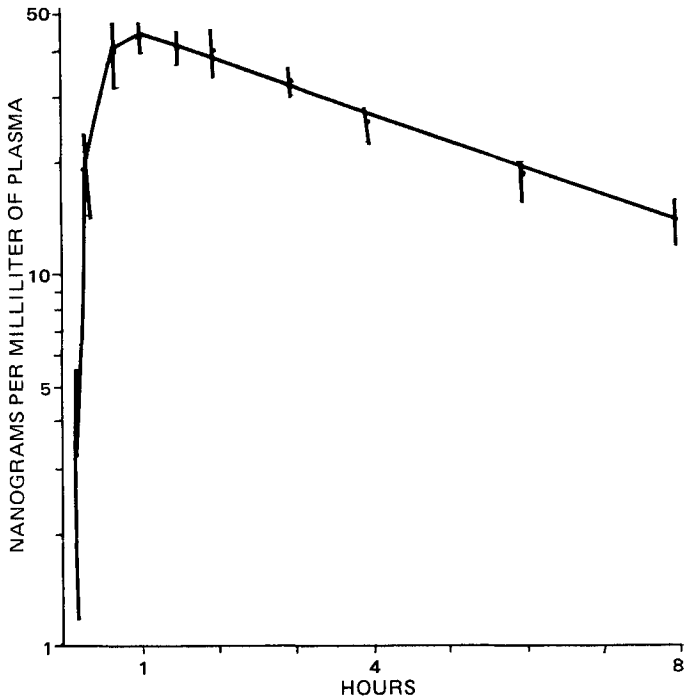


Figure 2—*Average blood levels of six normal male subjects who received oral doses of 10 mg of metoclopramide in solution. Vertical lines denote standard errors.*

A preliminary study was conducted with two normal male subjects to determine whether the method was sufficiently sensitive to determine blood levels of metoclopramide at an oral dose of 20 mg. The results are shown in Table III for the 8 hr during which the samples were taken. These results indicate that the necessary sensitivity was obtained. The results of a second study in which an oral dose of 10 mg was administered to each of six subjects as a liquid also indicated that sufficient sensitivity is obtained by this method (Fig. 2).

Blood levels were also obtained in the radioactive metoclopramide studies with humans. Both the total radioactivity of blood and unchanged metoclopramide levels are shown in Fig. 3. The levels were considerably higher as determined by radioactivity, even in the initial samples, indicating rapid drug metabolism.

DISCUSSION

Earlier investigators (1–4) showed that the major urinary metabolite of metoclopramide in rabbits, dogs, and rats is monodeethylated metoclopramide (V). This finding was confirmed in the present studies. There was no indication of the presence of a dideethylated drug in the urine of the three species studied (3). Neither the mono- nor dideethylated derivative could be detected in human urine.

Rabbits, according to Arita *et al.* (1), excrete metoclopramide in part as sulfate and glucuronide conjugates. This has also been found to be true in humans but not in dogs or rats. In humans, about one-half of the total radioactivity excreted was as a metoclopramide conjugate, indicating that this is a major route of excretion. The other metabolite found in humans

Table III—Plasma Metoclopramide Levels for Two Male Subjects who Received a 20-mg Oral Dose

Hours after Dose	Metoclopramide, ng/ml	
	Subject 1	Subject 2
0.25	0	0
0.50	63.0	28.6
1.0	72.1	87.2
1.5	71.1	81.0
2.0	66.7	81.1
3.0	53.0	76.8
4.0	42.1	58.0
6.0	33.3	35.0
8.0	20.3	22.6

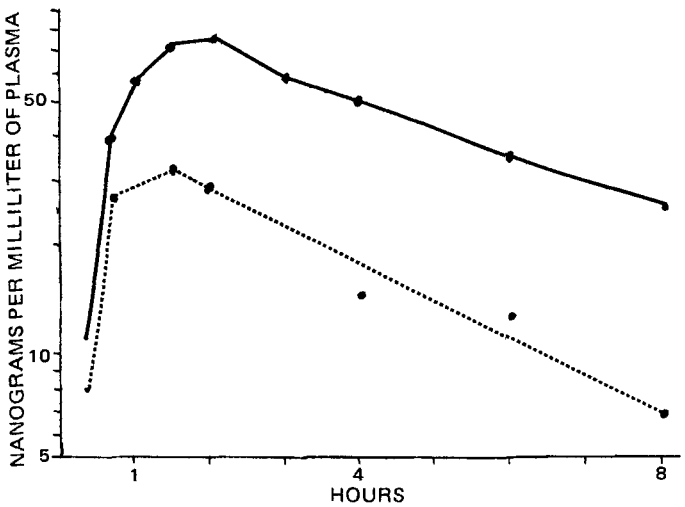


Figure 3—*Average plasma levels of total radioactivity, calculated as unchanged drug, and unchanged metoclopramide (by HPLC) of two subjects who received 10 mg of ^{14}C-metoclopramide. Key: —, radioactivity; and - - -, unchanged drug.*

(VI) also was observed in dog and rat urine, but it had not been observed previously.

A number of other metabolites were isolated and identifed from the urine of dogs and rats. An *O*-demethylated metabolite was earlier identified in rat urine by Hucker *et al.* (5). Two metabolites, IV and IX, were identified in the present study. No evidence of the aromatic amine oxidation could be found in these studies, as was noted by Arita *et al.* (1) for rabbits.

Previous studies showed that metoclopramide is rapidly and well absorbed in lower animals. The results in these studies indicate that this is also true in humans. About 78% was excreted in the urine (Table I) in the first 24 hr. Maximum blood levels following oral doses were found within the first 2 hr. The half-life of the drug for the 3–8-hr period following dosing was 4.00 hr (*SEM* 0.23) for the six subjects who received 10-mg doses (Fig. 2). The maximum concentration in the blood for these subjects was approximately 40 ng/ml; subjects that received 20 mg in the preliminary study gave twofold higher blood levels (Table III), indicating that the blood level is proportional to dose in this range. Further information concerning pharmacokinetics will appear in another report.

A comparison of the curves in Fig. 3 shows that a considerable amount of the radioactive material in the blood cannot be accounted for as unchanged metoclopramide. Because such a large portion of the drug was excreted as conjugates, samples were analyzed both before and following enzyme[5] hydrolysis of plasma samples from one subject. However, the results indicated that such conjugates were not present in appreciable concentrations. This difference must, therefore, be due to some unidentified metabolite.

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High-Speed Liquid Chromatographic Analysis of Dapsone and Related Compounds

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Abstract □ A nonaqueous solvent absorptive support system and an aqueous solvent reversed-phase support liquid chromatographic system for analysis of dapsone and related compounds were investigated. The absorptive support system was more suitable for analysis of dapsone in raw materials, formulations, and tissue residues. The suitability was judged by the relative selectivity, efficiency, precision, and sensitivity of the systems. The adsorptive support system was used for the analysis of trace amounts of raw material impurities and dapsone metabolites. Coupling fluorometric detection to the chromatographic system yielded a 10-pg on-column detection limit for dapsone; the UV detection limit was 250 pg.

Keyphrases □ Dapsone and related compounds—high-speed liquid chromatographic analyses, bulk drug, pharmaceutical formulations, and biological fluids □ High-speed liquid chromatography—analyses, dapsone and related compounds, bulk drug, pharmaceutical formulations, and biological fluids □ Leprostatic antibacterials—dapsone and related compounds, high-speed liquid chromatographic analyses, bulk drug, pharmaceutical formulations, and biological fluids

Dapsone (4,4′-sulfonylbisbenzeneamine) (I), a potent antileprotic drug (1–3), has been commercially available since 1949. Various analytical techniques have been investigated for its analysis in pharmaceutical preparations and biological fluids.

Colorimetry and titrimetry, the first techniques to be used, were reviewed previously (4). Although these methods are sufficiently sensitive for the analysis of pharmaceutical preparations, they are not sufficiently selective for the determination of chemical stability or for metabolite studies. The current USP method for the analysis of dapsone in tablet formulations is a sodium nitrite titration (5). This method is not specific for dapsone, however, since other arylamino sulfones present as decomposition products and synthetic impurities (4) interfere.

Later, fluorometric methods were developed; they are more sensitive and specific and, thus, are especially suitable for biological fluid metabolite studies (4, 6). However, these methods are not very accurate because of quenching and other complications, and they also involve complicated extraction and cleanup steps.

These limitations led to the development of TLC (7),