Physiological Disposition of a New Diuretic, ¹⁴C-Metolazone, in Dogs

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Abstract ☐ Counting procedures using ¹⁴C were used to follow the concentration of metolazone and its metabolites in the blood, plasma, plasma water, bile, urine, and feces of dogs given single oral or intravenous doses of metolazone. Doses were administered orally from 0.1 to 50 mg./kg. and intravenously from 0.1 to 5 mg./kg. Regardless of the route of administration, a log–log relationship existed between the peak blood level and the dose. Within the blood, the drug was distributed among the fractions according to the approximate proportion: 80% erythrocyte, 15% plasma, and 5% plasma water. The appreciable hemoglobin and albumin protein binding of drug may explain the relatively long half-life, 5–6 hr., of the total counts in circulation from an intravenous dose of 5 mg./kg. Hydroxylated and further products of oxidation accounted for 40–60% of the excreted drug. There did not appear to be any accumulation or preferential retention of drug or metabolites. This was coupled with a recovery of 90–100% of the dose in urine and feces. The rate at which the metabolites were excreted via the urine was equivalent to that of the intact drug. In intravenous studies, excretion of drug occurred primarily in the urine; about 25% of the dose may be excreted in the bile.

Keyphrases ☐ Metolazone, radiolabeled—absorption, distribution after oral and intravenous administration, dogs ☐ Blood level–time relationships—metolazone after oral and intravenous administration, dogs ☐ Plasma protein binding—metolazone after oral and intravenous administration, dogs ☐ Absorption, drug—metolazone after oral and intravenous administration, dogs ☐ Excretion, drug—metolazone and metabolites after oral and intravenous administration, dogs

Metolazone¹ (7-chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-o-tolyl-6-quinazolinesulfonamide) is a new diuretic agent (1, 2) structurally (I) related to quinethazone. This quinazolinone derivative has been shown in both the rat and dog to be a potent, long-acting diuretic and natriuretic agent, exhibiting low kaliuretic activity and possessing virtually no toxic side effects (3, 4).

In the present studies, dogs were given single oral or intravenous doses of the ¹⁴C-labeled drug over a wide dosage range to elucidate several parameters of drug disposition in this species. Among the parameters studied were blood level–time relationships, the biological half-life in plasma, and the extent of binding to plasma proteins and erythrocytes. Several possible routes of drug metabolism were deduced from identi-
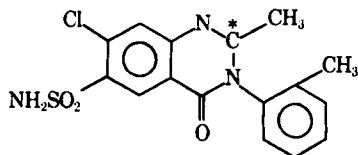
I: metolazone (the asterisk indicates the position of the labeled carbon atom)

fication and quantitation of metolazone and metabolites in urine and feces. The extent of biliary secretion was also examined.

MATERIALS AND METHODS

Purebred female beagle dogs, weighing 7–10 kg., were housed in individual stainless steel metabolism cages. The dogs were fed daily between 2:00 and 4:00 p.m. and allowed water ad libitum. The drug was administered between 8:00 and 9:00 a.m. on the day of the experiment. For intravenous administration, the compound was dissolved in a few drops of acetone and brought to volume with water or physiological saline. For oral administration, exact amounts of the compound were weighed on edible cachets, which were then placed into gelatin capsules.

Metolazone was synthesized with ¹⁴C in the 2-position of the ring system (I) and had a specific activity² of 0.97 mc./mmole. Radioactivity in the blood, urine, feces, and bile was calculated as metolazone from the specific activity. The extent to which these values represent metolazone or its metabolites is unknown in blood and bile. In some experiments with urine and fecal samples, however, certain metabolites were identified and their concentrations were followed through the time course. The biologically derived samples were counted at 2° using a liquid scintillation system³. An external standard was utilized for correction of the quenching effect by relating the counting rate for the isotope in the sample to the increase in the counting rate of the sample when exposed to a γ source (¹³³Ba).

Blood was collected from the jugular vein without stasis in heparinized tubes (Vacutainer). Urine was collected at prescribed
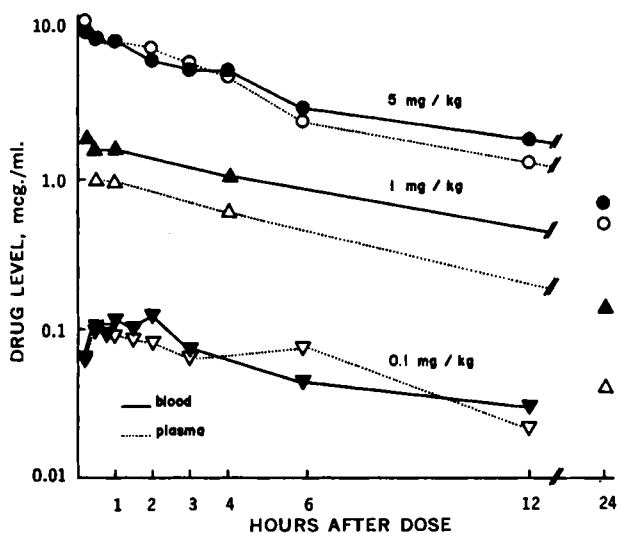

Figure 1—*Relationship of blood and plasma levels of metolazone and/or metabolites as a function of time following intravenous administration to dogs.*
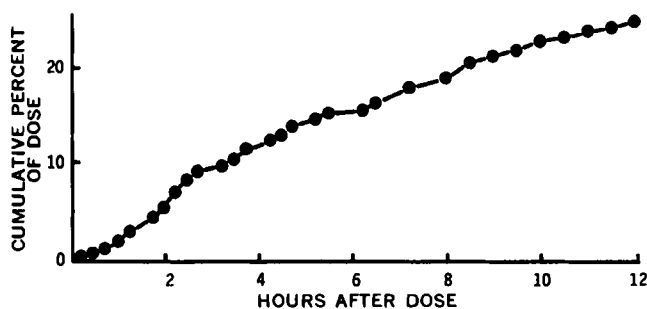
Figure 2—*Cumulative excretion of metolazone and/or metabolites in the bile of a dog given a 2-mg./kg. i.v. dose of the drug. Bile was collected through a polyethylene catheter which had been surgically placed in the common bile duct the preceding day.*

Table I—Half-Lives of ^{14}C-Metolazone in Plasma and Whole Blood of Dogs Receiving Single Intravenous Doses of the Drug[a]

Dose, mg./kg.	Whole Blood, hr.	Plasma, hr.
0.1	5.8	5.8
1.0	6.5	5.3
5.0	6.4	5.3

[a] The rate constant for disappearance was calculated from the disappearance portion of the blood and plasma concentration–time curves by least-squares regression analysis. The half-lives were calculated from the rate constant.

intervals with a metal catheter and, between catheterizations, from a metal pan in the metabolism cages. The volume collected over a given interval included the urine voided into the pan of the metabolism cages as well as that obtained by catheterization. Aliquots of blood and plasma (0.2 ml.) were prepared for liquid scintillation counting according to the method of Mahin and Lofberg (5). One milliliter urine and 1 ml. 95% ethanol were mixed with 15 ml. Bray's solution for liquid scintillation counting (6). All samples were prepared in duplicate, and results are reported as the average values. While substantiating this method, known amounts of labeled metolazone, which had been added to blood, plasma, and urine, were fully recovered (mean $\pm SD = 102.5 \pm 3.2, n = 18$).

The values for metolazone associated with the erythrocyte fraction were determined from the differences in the concentration of the drug in 1 ml. of whole blood and in the plasma thereof as determined from the hematocrit value. To calculate the total amount of drug in the circulation, total blood volume was assumed to be 10% of body weight. Ultrafiltrates for the measurement of drug in the plasma water were prepared by a modification (7) of the method described by Coolidge (8). For liquid scintillation counting, 1 ml. water was added to the aliquot of the ultrafiltrate (which was usually less than 1 ml.) plus 15 ml. Bray's solution.

Feces were collected from the metabolism cages and frozen until analyzed. The thawed feces were homogenized in acetone and extracted by refluxing (soxhlet) overnight. One-milliliter aliquots of the acetone extracts were counted as described for urine samples. To be certain that all radioactivity was extracted, 30-mg. samples of the dried acetone-extracted fecal material were prepared in the same manner as already described for blood and plasma and were found to contain no counts significantly higher than background. Since most of the urine was collected by catheterization at frequent
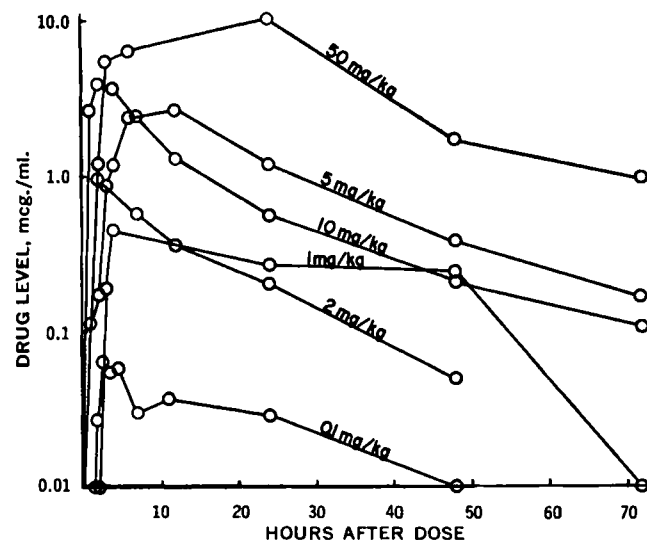
intervals, there was little chance for contamination of the feces by voided urine. In the daytime, fecal material was removed as soon as it was observed in the cage.

To study biliary excretion, two in-dwelling polyethylene catheters were inserted into the common bile duct of a dog under pentobarbital anesthesia. One catheter was directed upward toward the gallbladder; the other was directed in the opposite direction toward the duodenum. The two free ends were united to form a loop outside of the animal which permitted uninterrupted bile flow. The morning following surgery, a 5-mg./kg. dose of ^{14}C-metolazone was given intravenously and the loop was disconnected to allow bile collections from the proximal tube. The distal tube was clamped until the end of the initial 12-hr. collection period. During the 12–24-hr. interval of the experiment, the loop was reconnected to allow biliary flow to the duodenum. Aliquots of 0.1 ml. of the bile were prepared for liquid scintillation counting as described for blood and plasma.

The disappearance rate of drug from whole blood and plasma was determined using least-squares regression analysis (9). A number of predicted metolazone metabolites, which included hydroxylated and further oxidized products (Scheme I), were synthesized[4] to serve as standards for the identification and quantitation of metabolites in urine and feces by a method previously described (10). Briefly, this procedure involves a liquid chromatographic analysis of the chloroform-extractable tagged drug and its metabolites. These materials were extracted into chloroform, concentrated by removal of the solvent under nitrogen, reconstituted with 100 ml. tetrahydrofuran, and injected into a liquid chromatograph[5].

Figure 3—*Levels of metolazone and/or metabolites in whole blood following oral administration of the drug at the doses indicated. Each curve represents the blood levels obtained from a single dog.*
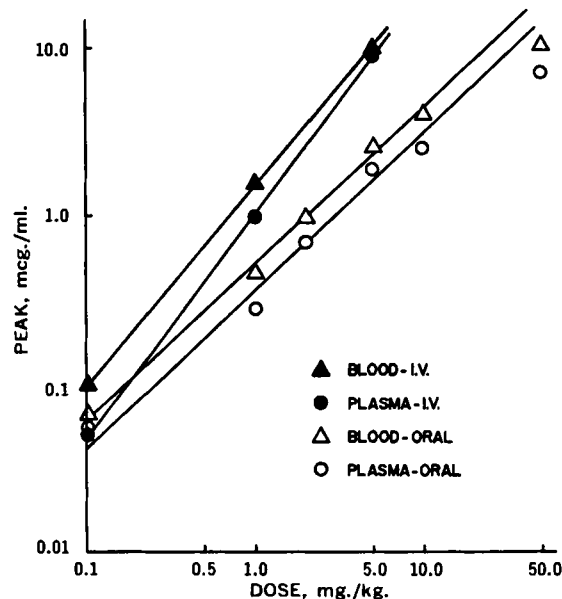

Figure 4—*Relationship between peak blood and plasma levels of metolazone and/or metabolites as a function of dose. Metolazone was administered by both the oral and intravenous routes.*

[4] By the Organic Chemistry Department, Pennwalt Corp.
[5] Waters ALC-100.

Table II—Metolazone and Converted Metolazone in Feces of Dogs Administered 2 mg./kg.[a]

| Hours Postdose | Distribution in Feces | | | | | |
| | Intravenous Percent of Dose | | | Oral Percent of Dose | | |
	Total	Metolazone	Converted Metolazone	Total	Metolazone	Converted Metolazone
12	—	—	—	54.8	51.6	3.2
24	0.6	NM[b]	NM	2.4	1.8	0.6
30	—	—	—	3.0	1.8	1.2
48	17.6	3.0	14.6	3.3	1.9	1.3
72	2.4	0.2	2.2	1.7	NM	NM

[a] Metolazone and metabolites were determined in acetone extracts of the fecal samples by separation by two-dimensional TLC, comparison of the R_f's with known standards, and quantitation by liquid scintillation counting. [b] NM = not measured.

Measurements were obtained by either utilizing peak height proportions or collecting individual fractions and obtaining the disintegrations per minute count. This method led to the identification of metabolites that were extractable into chloroform from the aqueous phase at about pH 5. The remaining counts in the aqueous phase were not identified.

RESULTS

Blood Levels and Excretion following Single Intravenous Doses— The concentrations of total radioactivity expressed as metolazone in the whole blood and plasma after intravenous doses of 0.1, 1.0, and 5.0 mg./kg. are shown in Fig. 1. Linear semilogarithmic plots of drug levels versus time were obtained over the major portion of the curves prepared from these data. A half-life of 5-6 hr. for metolazone in blood and plasma was obtained (Table I). Metolazone was no longer detectable in the blood at the end of 24 hr. for the low doses and 72 hr. for the highest dose. Close to 70% of the label was recovered in the urine of these animals. Fecal material was not analyzed.

Biliary Excretion of Metolazone and Metabolites— The cumulative percent excretion of radioactivity in the bile after a 5-mg./kg. intravenous dose is depicted in Fig. 2. The bile was collected at 15-30-min. intervals. At the end of the initial 12-hr. collection period, approximately 25% of the labeled dose was recovered through the bile duct fistula. Several peaks in both concentration and the total amount of drug excreted over the collection intervals were observed and were interpreted as intermittent contractions of the gallbladder. Initial attempts to chromatograph biliary extracts and to identify unconverted drug and its metabolites were not successful. To determine indirectly the extent of excretion of metabolites in the bile, the distribution of metabolites was determined in the feces of a dog receiving a 2-mg./kg. i.v. dose. About 20% of the label was excreted in the feces in 72 hr., of which about 80% was metabolites (Table II). For an equivalent oral dose, 65% of the dose was excreted in the feces in 72 hr., of which only about 10% was metabolites. These results would indicate that incomplete absorption occurred from the GI tract and that biliary excretion consists primarily of metabolites.

Absorption and Excretion following Single Oral Doses— The blood levels of metolazone and/or metabolites at various time intervals following oral doses of 0.1, 1.0, 2.0, 5.0, 10.0, and 50.0 mg./kg. are shown in Fig. 3. Excellent correlations exist between the

Table III—Relationship between Dose, Peak Blood Levels, and 24-hr. Urinary Excretion of Metolazone

| Dose | | 24 hr. Urinary Excretion | | Peak Blood Level Reached within 24 hr. | |
mg./kg.	Ratio[a]	mg./kg.	Ratio[a]	mcg./ml.	Ratio[a]
0.1	1	0.055	1.0	0.07	1.0
1.0	10	0.26	4.6	0.47	6.7
2.0	20	0.60	10.9	0.97	13.9
5.0	50	2.06	36.8	2.65	37.8
10.0	100	2.08	37.8	4.03	57.6
50.0	500	8.22	145.4	10.22	146.0

[a] Ratio of value at lowest dose level to the indicated value. All animals received the dose by oral administration.

log of the different dose levels studied and both the log of their respective peak blood levels (Fig. 4) and the cumulative 24-hr. urinary drug excretion. A good relationship also exists between peak blood level and cumulative 24-hr. excretion in urine, as shown in Table III. There was a substantial rise in the peak blood level (Figs. 3 and 4) as well as the duration of measurable blood levels at each increment of oral dose up to 50 mg./kg. (Fig. 3). The same relationships held for intravenous doses of 0.1-5 mg./kg. (Figs. 1 and 4).

The cumulative urinary and fecal excretion of the orally administered drug is shown in Table IV. Over the dose range administered, 74-100% of the dose was recovered. With increasing dosage, the percent of the administered dose excreted in the urine decreased, with concomitant increases being found in the feces. This would imply an inverse relationship between bioavailability and dose.

Distribution of Drug in Blood Fractions— The distribution of drug and/or metabolites between erythrocytes and plasma at peak blood levels for oral and intravenous doses is shown in Table V. More
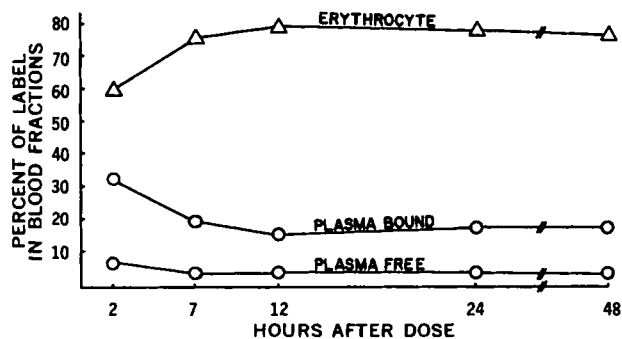
Figure 5—Distribution of metolazone and/or metabolites as a function of time in the blood fractions of a dog given an oral 2-mg./kg. dose of metolazone. The whole blood concentration is considered to be 100%.
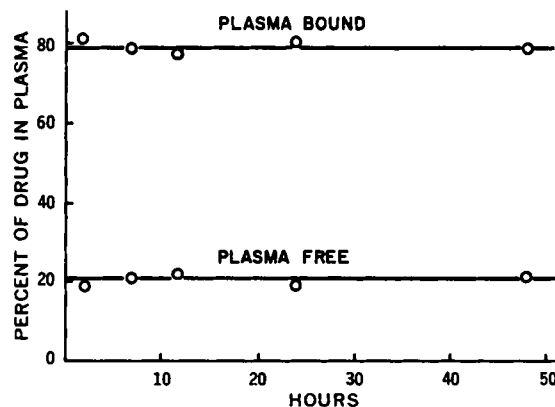
Figure 6—Distribution of metolazone and/or metabolites in the plasma of a dog given an oral 2-mg./kg. dose of metolazone. The whole plasma concentration is considered to be 100%.

Table IV—Recovery and Distribution of Metolazone Radioactivity as Found in Urine and Feces of Dogs after Administration of Various Oral Dose Levels

Dose, mg./kg.	Cumulative Percent of Dose in Urine Hours					Cumulative Percent of Dose in Feces Hours					Total Recovery, %
	12	24	48	72	96	12	24	48	72	96	
0.1	46.4	55.6	65.2	—	—	—	—	—	—	—	—
1.0	—	26.2	43.8	56.3	58.5	—	—	—	24.0	28.5	87.0
2.0	23.2	30.2	34.0	34.8	—	54.8	57.2	63.5	65.1	—	99.9
5.0	26.1	41.1	50.0	51.0	—	0.2	9.7	41.7	—	—	92.7
10.0	17.8	20.8	22.1	22.7	—	—	74.4	74.7	75.3	—	98.0
50.0	—	16.4	24.9	25.1	25.2	—	37.5	47.8	48.5	48.6	73.8

drug was associated with the erythrocyte fraction, as demonstrated by the fact that the erythrocyte to plasma ratio was greater than 1. The only exception was the 5-mg./kg. i.v. dose, in which the ratio was less than 1. This may be a function of the dose and the route of administration.

The distribution of radioactivity as a function of time between the three blood fractions—erythrocytes, plasma proteins, and plasma water—at the 2-mg./kg. oral dose is shown in Fig. 5. Close to 80% of the drug was bound to erythrocytes and about 15% was found in whole plasma, with only 5% or less actually in solution in the plasma water following equilibration of the labeled material between the three blood fractions. The consistency of the distribution within the plasma with time for this dog is shown in Fig. 6. In the plasma fraction, over a concentration range of 18–699 ng./ml., 80% of the drug was bound to proteins. Only 20% appeared free in the water.

The disappearance half-lives of total ^{14}C in the case of the oral doses were similar to the intravenous values, as calculated from results in Fig. 3. The half-life was about 6 hr. with the exception of the dog that received 1 mg./kg. orally and had a half-life of 29 hr. The reason for this result has not been explained.

The volumes of distribution that could be estimated from the plasma drug levels following the intravenous doses approximated a volume equivalent to the total body water. If the amount of drug dissolved in the plasma water was used, the volume of distribution greatly exceeded the total body water, indicating substantial binding of the drug in the body tissues.

Where data were available from both intravenous and oral dosages, the bioavailability of the oral dose was estimated by comparing the area under the plasma concentration–time curve to that of its respective intravenous dose. At the 0.1-mg./kg. dose, the bioavailability of the oral dose was 80% that of the intravenous dose. When the doses were raised to 1 and 5 mg./kg., the bioavailability decreased to 52 and 38%, respectively.

Metabolite Identification—Some predicted metabolites are shown in Scheme I. The distribution of drug and metabolites found in the total 24-hr. urine and fecal samples for dogs receiving oral and intravenous doses is shown in Table VI. The bulk of the metabolites are hydroxylated derivatives of the native drug. As much as 25–50% of the label remained unextractable and in the aqueous phase. This fraction represents more polar derivatives, although this cannot be stated with certainty since the partition coefficients for the known metabolites in this extraction system are not known.

The rate of urinary excretion of unconverted metolazone and of total metabolites in the urine of dogs receiving 5 mg./kg. of metolazone by either the oral or intravenous route is shown in Fig. 7. The rate is expressed as the amount of metolazone to be excreted in the urine as a function of time following drug administration. The amount to be excreted in the urine is defined as the total amount of drug eliminated by this route minus the amount found in the urine up to the time indicated on the abscissa. When administered intravenously, both metabolites and unconverted metolazone were excreted rapidly at equivalent first-order rates for the first 8 hr. After this time, a second slower first-order rate loss was evident. Following oral administration, the rate of excretion of both unconverted metolazone and metabolites was very slow for the first 6–8 hr. Following this slow phase of elimination, a rapid first-order loss of metolazone and metabolites was observed, which appeared to parallel the first phase of elimination following the intravenous
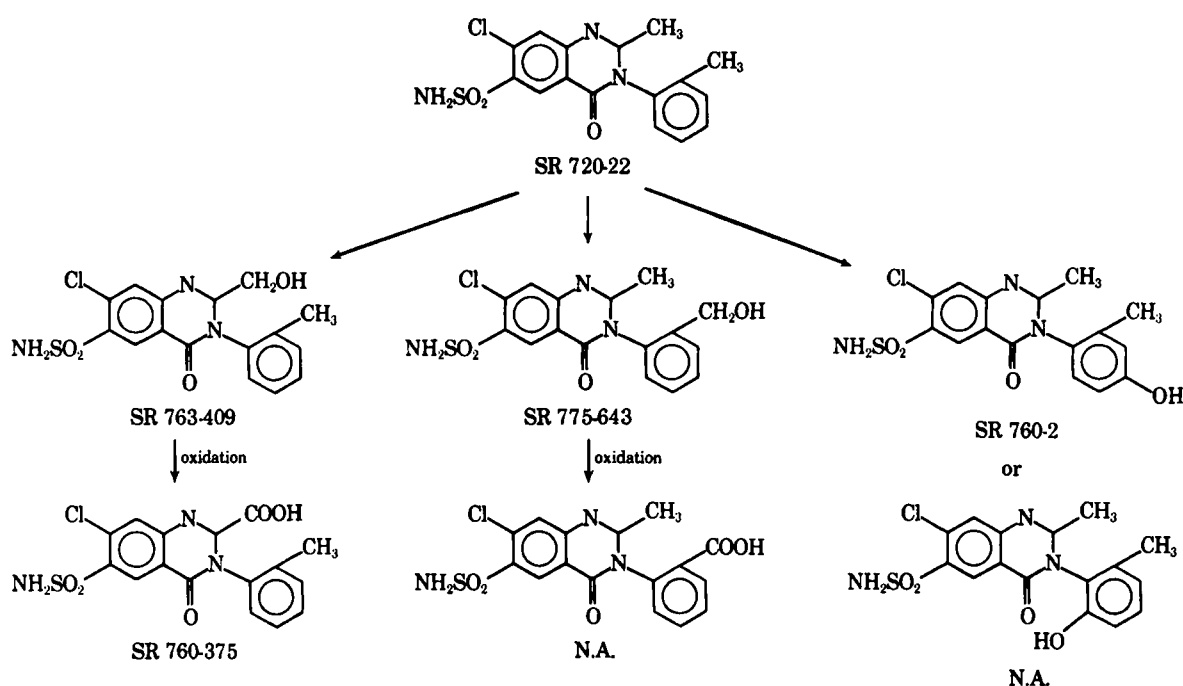
Scheme I—Structures of some possible metabolites of metolazone. N.A. indicates that a suitable standard was not available.

Table V—Effect of Dose and Route of Administration on Drug Distribution between Erythrocytes and Plasma at the Peak Blood Level

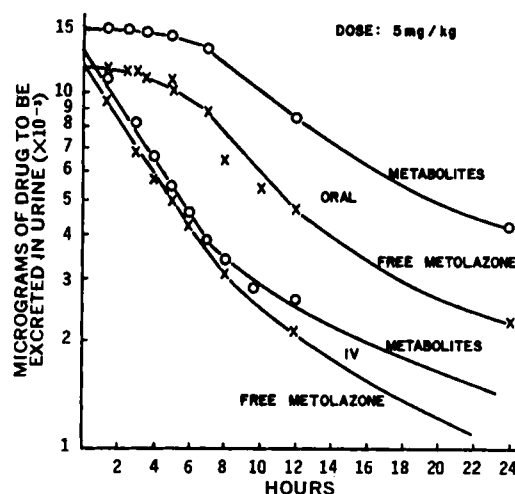| Dose, mg./kg.	Peak Time, hr.	Blood	Erythrocyte	Plasma	Ratio Erythrocyte Plasma
		Percent Dose			
Intravenous Route					
0.1	—	12.3	7.8	4.5	1.7
1.0	—	14.6	9.8	4.8	2.0
5.0	—	18.7	5.8	12.9	0.4
Oral Route					
0.1	2.5	6.4	4.1	2.3	1.8
1.0	4.0	4.4	2.9	1.5	1.9
2.0	2.0	4.9	2.9	2.0	1.4
5.0	6.0	5.5	3.6	1.9	1.9
10.0	2.0	4.0	2.6	1.4	1.8
50.0	24.0	2.2	1.3	0.9	1.4

Figure 7—Urinary excretion of metolazone and metabolites following the oral and intravenous administration of 5 mg./kg. to dogs. The amount of drug to be excreted is defined as the total drug excreted in the urine minus the total amount of drug excreted at any given time period.

dose. At lower doses, administered both orally and intravenously, a single exponential rate of excretion in the urine was observed, which was equivalent to the slower phase for the intravenous dose in Fig. 7.

DISCUSSION

Drug distribution and excretion studies were conducted with single intravenous and oral doses of metolazone. Peak blood levels are related exponentially to the amount of drug administered over a wide dosage range, up to and including the 50-mg./kg. dose. Studies conducted in the rat (11) demonstrated a loss of linearity above this dose. This relationship has not been studied in dogs at higher oral doses, but there is a tendency (Fig. 4) that indicates that linearity may have been lost at the 50-mg./kg. dose level. This phenomenon may be explained in part by the low solubility of the drug and decreased absorption at higher doses, as evidenced by the inverse relationship between bioavailability and dose. In addition, Table III shows that the ratios (value at selected dose/value of lowest dose) for drug excreted over 24 hr. and also for peak blood levels reached during each 24-hr. interval are less than the ratios for the doses. The ratios for these two parameters, however, are approximately equal for any given dose level. By implication, therefore, one would expect that urinary drug excretion would follow a pattern related to the peak blood levels.

An important property of metolazone is believed to be the extent to which it is bound by erythrocytes and plasma proteins of many species, including man (12). In the present studies, the red cell/plasma ratio of metolazone plus its metabolites was always greater than unity; with chlorothiazide, this ratio was always less than unity (13). Another important parameter is the extent of protein binding in plasma, since the concentration of the unbound drug in this fraction is believed to be a reasonable estimate of both the free concentration in the extravascular fluids and the pharmacologically active concentration in vivo (14). Within the plasma fraction, approximately 80% of the metolazone is bound. Other diuretic drugs are bound to plasma proteins to a variable extent in mammals: chlorothiazide (15, 16), 40–45%; hydrochlorothiazide (16), 66%; trichlormethiazide (16), 80%; mefruside (16), 80–85%; poly-

thiazide (15, 16), 85%; and hygroton (17), 85%. The apparent volume of distribution of metolazone, calculated from the blood level data, was found to be in excess of the total body water, indicating extensive binding by not only blood but by tissue proteins as well.

Because of the limited intestinal absorption and low water solubility of this drug, it is difficult, if not impossible, to obtain high enough blood levels of the drug to saturate the binding sites on plasma proteins and to decrease the ratio of bound to free drug. In the case of the 2-mg./kg. oral dose (Figs. 4 and 5), only about 20% of the total drug found in the plasma of dogs was actually in solution as an unbound entity throughout the 2-day period of the study, regardless of the drug concentration in the whole plasma. A similar inability to saturate the plasma binding capacity was observed in the rat where doses as high as 1.25 g./kg. were administered (11, 12).

Rather than express the association of metolazone with plasma proteins as percent bound, it would be more informative to calculate the dissociation constant of the drug–protein complex. To gain further insight into this interaction, the apparent dissociation constant (k_{dp}) was calculated from the plasma levels of the dog given 2 mg./kg., using certain assumptions. Preliminary in vitro experiments from this laboratory, with the use of various canine plasma protein fractions, indicated that under in vivo conditions only a small percentage of the total drug in plasma could be bound by proteins other than albumin. When assuming the plasma albumin concentration to be approximately 5×10^{-4} M, the apparent dissociation constants (k_{dp}) were calculated using standard procedures (18, 19) from the plasma levels of the drug in the dog that received the oral 2-mg./kg. dose. Over a range of plasma concentrations of 18–699 ng./ml., measured at five different time intervals, the calculated dissociation constant was $(1.23 \pm 0.6) \times 10^{-4}$ (mean ± SD). The uniformity of the dissociation constants over this range

Table VI—Distribution of Metolazone and Its Metabolites[a] in 24-hr. Urine Samples from Dogs

Dose, mg./kg.	Route	720-22	763-409	760-2	775-643	760-375	Aqueous Solution
		Percent Distribution of Counts Appearing in Urine					
0.1	Intravenous	33.25	9.60[b]		8.07	3.20	45.88
1.0	Intravenous	41.37	4.01	1.98	1.25	1.19	50.20
5.0	Intravenous	62.29	0.39	1.04	2.56	4.18	29.54
0.1	Oral	57.73	1.0	0.25	0.87	2.35	37.80
2.0	Oral	61.00	5.07[b]		4.80	2.93	26.20
5.0	Oral	44.92	0.77	0.67	0.52	2.40	50.72

[a] Metolazone were identified by comparison of their known R_f's in two-dimensional TLC and quantitated by liquid scintillation counting. Structures are shown in Scheme I. [b] Sum of metabolites 763-409 and 760-2.

of concentration is attributed to the fact that only one binding site on plasma albumin is involved. The molar ratio of drug to albumin at the highest drug concentration was 3.8×10^{-3}.

The half-life of metolazone in plasma and whole blood of dogs receiving intravenous doses of the drug ranged from 5 to 6 hr. The enterohepatic circulation of the drug and the strong binding affinity of the red cell and plasma proteins no doubt strongly influenced the half-life. At this time it is not known how much unconverted drug and metabolites were present in the total concentration of label found in the bile, blood, or plasma. By implication, the high distribution of converted drug found in the feces of the animal given a 2-mg./kg. i.v. dose suggests that most of the biliary excretion consists of metabolic products. Bioavailability determinations suggest that above the 0.1-mg./kg. oral dose level, a large percentage of unconverted drug in the feces of these dogs was due to a lack of absorption.

The total percent of the administered dose recovered in the excretion was between 90 and 100% in four of the five orally dosed dogs. The one dog showing a lower percent of drug recovery had been given a very high dose of 50 mg./kg. No doubt this was due to some residual drug in the body. The total metabolites are excreted at a rate comparable to that of metolazone. This observation, coupled with the good recoveries with therapeutic doses, indicates that there is no residual drug remaining in the tissues and that no buildup of drug or metabolites should be expected.

It has been reported[6] that quinethazone, which is closely related to metolazone, is not metabolized in experimental animals. The extent to which thiazides and other related diuretics are metabolized in mammals has been reported to be variable. Chlorothiazide, hydrochlorothiazide, flumethiazide, and hydroflumethiazide have been reported to be rapidly excreted without undergoing metabolic transformation (16). By contrast, bendroflumethiazide, trichlormethiazide (16), and polythiazide (16, 20, 21) are partially metabolized by the dog. The metabolism of metolazone in animal species would be classified in the latter group. The metabolites of metolazone, which are products of hydroxylation and oxidation, have been found in both the urine and feces. The origin of the metabolites in the feces are presumably the results of the biliary secretion of the drug.

The extent of metabolism of metolazone was unexpectedly high for such a polar drug. In a 24-hr. period following administration of the drug, 30–60% of the drug appearing in the urine of dogs was metabolites. In the human, by contrast, the amount of metabolites found in the urine in 24 hr. was only 10–15% of the excreted drug (12). In view of the unexpectedly high degree of metabolism in dogs, the metabolites were synthesized and assayed for diuretic activity. The activities of these metabolites administered orally have been considerably less than that of metolazone and, in addition, exhibited no evidence of toxic reactions.

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