Absorption, Metabolism, and Excretion of 5-Methylpyrazole-3-carboxylic Acid in the Rat, Dog, and Human

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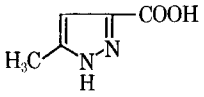
5-Methylpyrazole-3-carboxylic acid is completely absorbed and rapidly excreted in the rat, dog, and human. After 1- or 9-mg./Kg. doses to the rat, the urinary excretion half-life was 1.0 hr., with 57 per cent of the dose excreted unchanged and the remainder as an acidic conjugate. Pretreatment of rats with high doses of the drug did not significantly alter the absorption, metabolism, or excretion of the drug. After oral and intravenous administration of about 7 mg./Kg. to the dog, all of the drug was excreted unchanged with a half-life of 1.8 hr. (average of blood and urine data). After oral and intravenous doses of 37.5 mg. to six human volunteers, the average half-life for the excretion of radioactivity was 1.9 hr. At this dose the human converts an average of 31 per cent of the drug to the same metabolite produced by the rat. In the human, the following averages were not statistically different after oral and intravenous administration: total urinary excretion, urinary excretion half-life, total fecal excretion, half-life of disappearance from plasma, apparent volume of distribution, area under the plasma curve, and percentage of drug metabolized. The human data have been summarized by general equations, which were derived for a simple kinetic model. The average first-order rate constants ($hr.^{-1}$) for the six humans were as follows (average of oral and intravenous data): over-all loss, 0.32; excretion of drug, 0.22; formation of metabolite, 0.10; excretion of metabolite, 1.5.

IN PREVIOUS studies (1) 5-methylpyrazole-3-carboxylic acid (I) was isolated as a metabolite



I

from the urine of rats treated with 3,5-dimethylpyrazole and was shown to be 116 times more potent than tolbutamide as a hypoglycemic agent in the intact, fasted, glucose-primed rat (2).

Studies in the rat with ^{14}C-labeled 3,5-dimethylpyrazole (3) showed the oral dose to be completely absorbed and rapidly excreted as four metabolites: 5 - methylpyrazole - 3 - carboxylic acid (13%), conjugated 5-methylpyrazole-3-carboxylic acid (14%), conjugated 4-hydroxy-3,5-dimethylpyrazole (71%), and an unidentified metabolite (1%).

All of the hypoglycemic activity of 3,5-dimethylpyrazole in the rat appeared to be due to I, its active metabolite. Unlike 3,5-dimethylpyrazole, I stimulates glucose utilization by adipose tissue *in vitro* and depresses blood sugar of eviscerated rats (2). It was not known whether the human would metabolize 3,5-dimethylpyrazole to the active carboxy metabolite and, if so, how much would be produced. It was therefore decided to undertake the development of the active metabolite as an antidiabetic agent. As part of this research effort, ^{14}C-labeled I (also referred to as the drug) was prepared, and its absorption, metabolism, and excretion compared in the rat, dog, and human.

EXPERIMENTAL

Materials—^{14}C-labeled I[1] was prepared from ethyl acetopyruvate-4-^{14}C and hydrazine (4). Its chemical and radiochemical purity was established by melting point, elemental analysis, infrared spectroscopy, and paper and thin-layer chromatography.

Rat Studies—Charles River CD male rats, weighing 140–200 Gm., were used. They were fasted overnight prior to the experiment (water was allowed *ad libitum*), and were housed individually in stainless steel metabolism cages. Rats *1–3* were dosed orally with 1.315 mg. (9 mg./Kg.) of labeled I (specific activity: 1.33 μc./mg.), dissolved in 0.5 ml. of 0.25% sterile methylcellulose (MC) vehicle. Sequential urine samples were collected from each rat at 2, 4, 6, 8, 15, 24, 48, and 72 hr. Since biological studies suggested that tachyphylaxis to the drug develops in rats (unpublished results) and since a change in the metabolism of the drug could explain this phenomenon, rats *10–15* were treated orally with 25 mg./Kg. of unlabeled I dissolved in 0.5 ml. of 0.25% MC vehicle twice daily for 3 days and 3 times on the fourth day. The rats were fasted on the fourth night and dosed orally with 0.184 mg. (1.0 mg./Kg.) of labeled drug (specific activity: 0.57 μc./mg.)

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[1] This compound was prepared by Dr. R. C. Thomas, The Upjohn Co.

in 4.6 ml. of physiological saline on the morning of the fifth day. The controls (rats *4–9*) were treated exactly like rats *10–15* except that they received only vehicle on days 1 through 4. Rats *4–15* were hydrated by oral administration of 25 ml./Kg. of physiological saline on the afternoon of the fourth day. Sequential urine samples were collected from rats *4–15* at 0.5, 1, 2, 3, 4, 6, 7, 8, and 24 hr. after oral administration of the ^{14}C-labeled drug.

Dog Studies—The ^{14}C-labeled I was administered both orally and intravenously in crossover fashion to two fasted beagles (one male, one female). One week was allowed between the oral and intravenous doses. In each case the dose was approximately 7.5 mg. (10 μc.). The oral dose was given as a powder in a soft gelatin capsule and the intravenous dose was dissolved in 5 ml. of water. Several blood and sequential urine samples were collected for 72 hr. The urine was collected by catheter.

Human Studies—Oral and intravenous doses of ^{14}C-labeled I were administered to six adult male volunteers in crossover fashion. Three of the subjects received the drug intravenously and the other three received it orally on the first week and then, after a 2-week rest period, the drug was administered again with the routes of administration crossed over. The ages and weights of the volunteers are given in Table V. The intravenous dose was 10 ml. of a 3.75 mg./ml. sterile aqueous solution of the labeled drug (specific activity: 1.33 μc./mg.). The oral dose was 37.5 mg. of the drug (1.33 μc./mg.) mixed with lactose in a mortar (average particle size about 70 μ; all particles less than 107 μ), and packed in capsules. The volunteers were fasted overnight and for 6 hr. after drug administration. To facilitate urine collection, each subject received 8 fl. oz. of water 1 hr. prior to drug administration and at 0, 1.5, 4, 6.5, and 10 hr. Plasma and sequential urine samples were collected for 3 days as indicated in Tables II and III. Fecal samples were collected at 24, 48, and 72 hr.

General Counting Techniques—The samples were counted in duplicate using Packard Tri-Carb liquid scintillation spectrometers, models 314EX-2A and 314X; corrections for counting efficiency were made using ^{14}C-toluene as the internal standard. Urine was counted directly by dissolving 0.1 to 0.5 ml. in 15 ml. of diotol counting solution, which was prepared by the procedure of Herberg (5). Plasma and homogenized fecal samples (0.5 to 0.9 Gm.) were placed in sample sacs made from dialysis tubing, and dried overnight. The dried samples were burned by the Schöniger technique, and the evolved $^{14}CO_2$ trapped in a 1 *M* solution of 2-phenylethylamine in methanol. For counting, an aliquot of the latter was mixed with an equal volume of a solution consisting of 4.6 Gm. PPO and 8 mg. POPOP per liter of toluene.

Paper Chromatography—The following paper chromatographic systems were employed, using Whatman No. 2 paper: BAW system, *n*-butanol–acetic acid–water (2:1:1; R_f values: I, 0.76; metabolite, 0.72); BPW system, *n*-butanol–piperidine–water (81:2:17; R_f values: I, 0.37; metabolite, 0.32). The radioactive zones were measured with a 4π paper-strip scanner, Vanguard model 880. The percentage of the total radioactivity represented by each peak was determined by cutting out the peaks and weighing them.

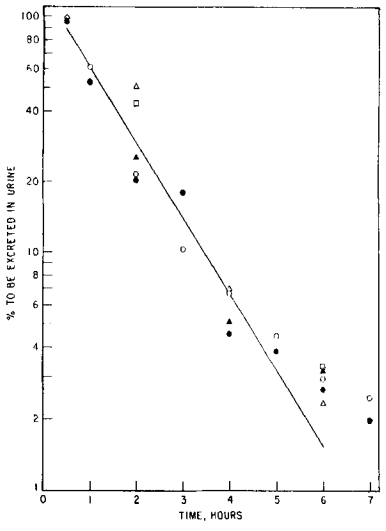


Fig. 1—Rate of urinary excretion of equivalents of 5-methylpyrazole-3-carboxylic acid-^{14}C in the rat. Key: △, rat 1; ▲, rat 2; □, rat 3; ○, rats 4–9; ●, rats 10–15. Average $t_{1/2}$ = 1.0 hr.

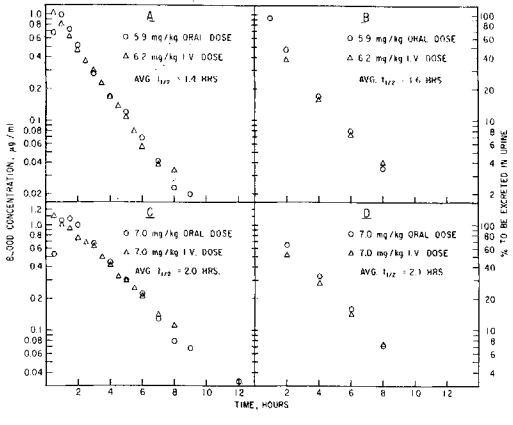


Fig. 2—Rate of excretion of 5-methylpyrazole-3-carboxylic acid-^{14}C in the dog. Key: A, blood levels in the female; B, urinary excretion by the female; C, blood levels in the male; D, urinary excretion by the male.

RESULTS

Rat Studies—After oral administration of the ^{14}C-labeled drug to three rats at a dose of 9 mg./Kg., 88.4% of the radioactivity was found in the urine and 0.2% in the feces. By paper chromatography, an average of 57% of the urinary radioactivity was unchanged drug while the remainder was a single metabolite. The excretion of radioactivity in the urine by the rat obeyed first-order kinetics with a half-life of about 1.0 hr. (Fig. 1).

The six drug-pretreated rats excreted an average of 98.8% of the dose in the urine with 54.0% unchanged. The six nonpretreated rats given the 1-mg./Kg. dose excreted an average of 100.3% of the dose in the urine with 59.7% as unchanged drug. For these two groups there was no significant difference in average percentage of radio-

TABLE I—BLOOD HALF-LIFE, URINARY EXCRETION HALF-LIFE, AND APPARENT VOLUME OF DISTRIBUTION OF 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID-^{14}C IN THE DOG

Dog	Sex	Route	Half-Life, hr. Blood	Half-Life, hr. Urine	Apparent V_d, L.	Apparent V_d, % Body Wt.
1	M	i.v.	2.12 ± 0.09[a]	2.15	4.21	39.5
1	M	Oral	2.06	1.95	4.08	38.7
		Mean	2.09	2.03	4.15	39.1
2	F	i.v.	1.43 ± 0.08[a]	1.65	5.24	43.1
2	F	Oral	1.42	1.45	5.20	40.5
		Mean	1.43	1.55	5.22	41.8
		Over-all mean	1.76	1.79	4.69	40.5

[a] Straight line least squares fit (95% confidence limits given).

TABLE II—AVERAGE PLASMA CONCENTRATIONS OF DRUG EQUIVALENTS AFTER ADMINISTRATION OF A 37.5-mg. DOSE OF 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID-^{14}C TO THE HUMAN

Time, hr.	mcg./ml. ± S.E.M. i.v. Route	Oral Route
0.00	0.00 ± 0.00	0.00 ± 0.00
0.05	2.78 ± 0.08	...
0.3	2.08 ± 0.03	...
0.5	1.83 ± 0.05	0.78 ± 0.24
0.8	1.59 ± 0.05	...
1.0	...	1.42 ± 0.29
2.0	1.23 ± 0.04	1.24 ± 0.04
3.0	...	0.89 ± 0.04
4.0	0.58 ± 0.04	0.55 ± 0.02
5.0	0.32 ± 0.03	0.38 ± 0.01
7.0	0.13 ± 0.02	0.16 ± 0.01
9.0	...	0.073 ± 0.005
10.0	0.041 ± 0.006	...
12.0	...	0.024 ± 0.003
24.0	0.00 ± 0.00	0.00 ± 0.00

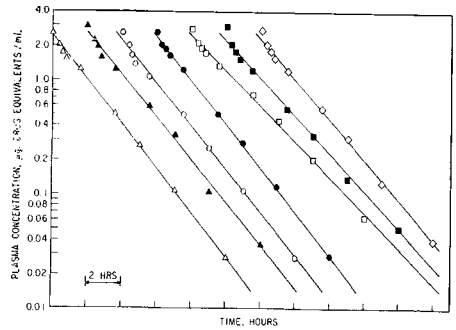


Fig. 3—Plasma levels of equivalents of 5-methylpyrazole-3-carboxylic acid-^{14}C in humans after a 37.5-mg. intravenous dose (log scale). Each curve is displaced 2 hr. from the one preceding it, and the last point on each curve is at 12.0 hr. Key: △, subject No. 1; ▲, No. 2; ○, No. 3; ●, No. 4; □, No. 5; ■, No. 6; ◇, average of No. 1–6.

activity represented by unchanged drug in each of the urine collection intervals. For example, in the first 30 min. 63.7 ± 3.0% (±S.D.) of the radioactivity excreted by the nonpretreated rats was unchanged drug, whereas 61.8 ± 1.5% of the total excreted during this interval by the pretreated rats was unchanged. Therefore, pretreatment with drug for 4 days did not alter the excretion pattern of labeled drug given on the fifth day. Both the rate of excretion (Fig. 1) and the total amount of labeled drug excreted were essentially identical in both the pretreated and nonpretreated groups.

Dog Studies—*Intravenous Dose*—After intravenous administration of approximately 7 mg./Kg. to two dogs, 104.6% of the dose was recovered in the urine and 0.3% in the feces. By paper chromatography, all of the urinary radioactivity was unchanged drug. The whole blood concentrations are plotted in Fig. 2. In the case of one dog, both the whole blood and plasma levels of radioactivity were measured. The plasma levels, which paralleled the blood levels, averaged 28% higher. The elimination of drug from the blood of both dogs obeyed first-order kinetics with half-lives of 1.40 and 2.15 hr. for the female and male dog, respectively (Fig. 2, A and C). No radioactivity was detected in the blood of either dog at 24 hr. The urinary excretion of radioactivity into the urine also obeyed first-order kinetics with half-lives of 1.65 and 2.15 hr. for the female and male dog, respectively (Fig. 2, B and D).

Oral Dose—After oral administration of approximately 7 mg./Kg. to two dogs, an average of 100.2% of the dose was recovered in the urine, and 0.1% in the feces. By paper chromatography all the urinary radioactivity was unchanged drug.

In both dogs the peak levels of radioactivity in the blood were attained within 1 hr. after the oral dose (Fig. 2). The elimination of radioactivity from the blood obeyed first-order kinetics with half-lives of 1.25 and 1.75 hr. for the female and male dog, respectively (Fig. 2, A and C). After the oral dose, urinary excretion of the radioactivity obeyed first-order kinetics with half-lives of 1.45 and 1.95 hr. in the female and male dog, respectively (Fig. 2, B and D).

The half-lives calculated from the blood and urine levels after oral and intravenous doses in the dog are summarized in Table I.

Human Studies—*Intravenous Dose*—The average concentrations of drug equivalents in plasma and their standard errors at several time intervals after the 37.5-mg. intravenous dose are summarized in Table II, and the data for the individual subjects are plotted in Fig. 3. The log plasma concentration time curves show two segments, the first representing rapid equilibration with body fluids (average half-life ~7 min.); the second segment is rate-determining, having an average half-

TABLE III—CUMULATIVE URINARY EXCRETION OF DRUG EQUIVALENTS AFTER ADMINISTRATION OF A 37.5-mg. DOSE OF 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID-^{14}C TO HUMANS

Time, hr.	mg. ± S.E.M. i.v. Route	Oral Route
0	0.00 ± 0.00	0.00 ± 0.00
1	8.19 ± 1.66	1.92 ± 0.66
2	14.88 ± 1.41	...
3	...	12.20 ± 1.64
5	27.13 ± 1.06	27.31 ± 1.01
9	31.18 ± 0.50	31.54 ± 0.78
11	32.26 ± 0.56	...
12	...	34.07 ± 0.49
24	33.18 ± 0.84	34.76 ± 0.43

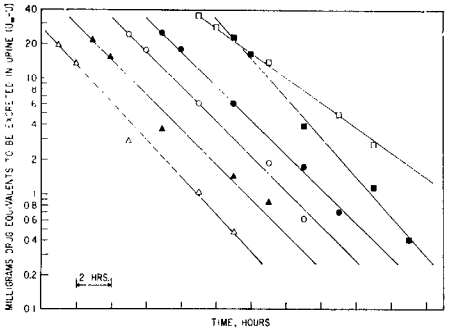


Fig. 4—Urinary excretion of equivalents of 5-methylpyrazole-3-carboxylic acid-^{14}C by humans after a 37.5-mg. intravenous dose (log scale). Each curve is displaced 2 hr. and the first point on each curve is at 1.0 hr. Subject identification is the same as that in Fig. 3.

TABLE IV—PERCENTAGE AS METABOLITE IN THE HUMAN URINE COLLECTIONS[a]

Urine Collection Interval, hr.	% as Metabolite[b] ± S.E.M. i.v. Route	Oral Route
0–1	16.7 ± 1.7	15.0 ± 1.7
1–2	30.0 ± 3.0	...
1–3	...	30.3 ± 3.0
2–5	32.6 ± 2.6	...
3–5	...	36.2 ± 4.9
5–9	35.3 ± 2.8	40.9 ± 1.9
9–11	38.7 ± 5.1	...
9–12	...	39.1 ± 4.7
11–24	33.5 ± 2.8	...
12–24	...	35.9 ± 3.7
0–24	38.6 ± 2.8	33.7 ± 3.2

[a] Average of six subjects. [b] The remainder was excreted as intact drug.

life (±S.E.M.) of 1.66 ± 0.05 hr. in the six subjects.

The urinary excretion of drug equivalents following the intravenous dose is presented in Table III. The urine data for the individual subjects are plotted in Fig. 4. The average half-life (±S.E.M.) for the excretion of drug equivalents in urine after the intravenous dose was 1.91 ± 0.15 hr.

An average (±S.E.M.) of 71.4 ± 2.8% of the urinary radioactivity was found to be unchanged drug (Table IV); the remainder (28.6 ± 2.8) was present as a metabolite, which corresponded by

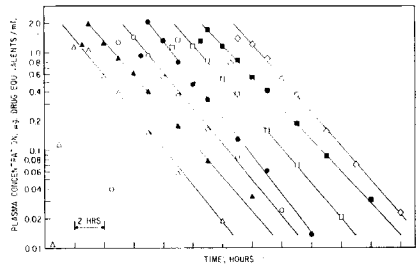


Fig. 5—Plasma levels of equivalents of 5-methylpyrazole-3-carboxylic acid-^{14}C in humans after a 37.5-mg. oral dose (log scale). The displacement of the curves from the origin and the subject identification are the same as in Fig. 3.

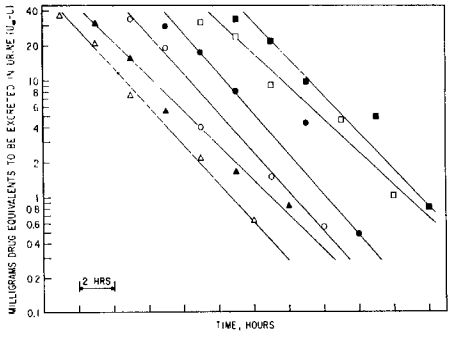


Fig. 6—Urinary excretion of equivalents of 5-methylpyrazole-3-carboxylic acid-^{14}C by humans after a 37.5-mg. oral dose. The displacement of the curves from the origin is the same as Fig. 4. Subject identification is the same as that in Fig. 3.

paper chromatography to the same metabolite excreted by the rat.

An average cumulative total (±S.E.M.) of 0.003 ± 0.002, 0.009 ± 0.002, and 0.009 ± 0.002 equivalents of the intravenous 37.5-mg. dose was found in the feces at 24, 48, and 72 hr., respectively.

Oral Dose—The average concentrations of drug equivalents and their standard errors in plasma at several time intervals after the 37.5-mg. oral dose are summarized in Table II and the data for the individual subjects are plotted in Fig. 5. The average plasma half-life (±S.E.M.) for the six subjects was 1.71 ± 0.05 hr. The average plasma levels beyond about 2 hr. were actually higher after the oral dose than after the intravenous dose.

The urinary excretion of drug equivalents following the oral dose is presented in Table III, and the data for the individual subjects are plotted in Fig. 6. The average (±S.E.M.) half-life for the excretion of drug equivalents into the urine after the oral dose was 2.22 ± 0.20 hr.

Following the oral dose, an average (±S.E.M.) of 66.3 ± 3.2% of the radioactivity recovered in the urine was unchanged drug, and the remainder was excreted as the metabolite (Table IV).

An average (±S.E.M.) cumulative total of 0.016 ± 0.014, 0.052 ± 0.038, and 0.058 ± 0.038 equivalents of the oral 37.5-mg. dose was found in the feces at 24, 48, and 72 hr., respectively.

TABLE V—CALCULATED CONSTANTS FROM HUMAN PLASMA DATA: AREAS UNDER PLASMA CURVES, PLASMA RATE CONSTANTS, AND APPARENT VOLUMES OF DISTRIBUTION

Subject[c]	i.v. Route: Plasma Area, mcg. hr./ml.	i.v. Route: Plasma K,[a] hr.$^{-1}$	i.v. Route: Vd,[b] L.	Oral Route: Plasma Area, mcg. hr./ml.	Oral Route: Plasma K,[a] hr.$^{-1}$	Oral Route: Vd,[b] L.
1 (29, 65)[d]	5.43	0.435	13.1	3.86	0.431	21.9
2 (30, 73)	5.92	0.424	12.6	6.02	0.379	14.7
3 (50, 72)	5.22	0.432	14.9	5.02	0.402	17.5
4 (27, 65)	5.62	0.434	13.1	5.55	0.448	14.1
5 (43, 85)	7.03	0.356	14.7	5.20	0.406	16.3
6 (26, 68)	6.17	0.389	12.6	5.92	0.366	16.5
Mean	5.90	0.412	13.5	5.26	0.405	16.8
S.E.M.	±0.31	±0.013	±0.4	±0.32	±0.013	±1.1

[a] $t_{1/2}$ (hr.) = 0.693/K. [b] $Vd = \frac{U_\infty}{(\text{plasma area})\ (\text{plasma } K)}$ (see Table VI for U_∞ values). [c] Subjects *1*, *2*, and *3* received the intravenous dose on week 1 and the oral dose on week 4. Subjects *4*, *5*, and *6* received the oral dose on week 1 and the intravenous dose on week 4. [d] Age and weight (Kg.), respectively.

TABLE VI—CALCULATED CONSTANTS[a] FROM HUMAN URINARY EXCRETION DATA: INFINITE URINARY EXCRETION (U_∞), URINARY RATE CONSTANTS, AND LAG TIMES (t_0)

Subject	i.v. Route: U_∞, mg.	i.v. Route: Urine K,[b] hr.$^{-1}$	i.v. Route: t_0, hr.	Oral Route: U_∞, mg.	Oral Route: Urine K,[b] hr.$^{-1}$	Oral Route: t_0, hr.
1	31.05	0.433	0.003	36.52	0.338	1.034
2	31.46	0.410	0.085	33.63	0.381	0.786
3	33.57	0.330	0.042	35.27	0.389	1.046
4	31.96	0.347	0.328	35.05	0.264	0.546
5	36.87	0.243	0.944	34.36	0.239	0.866
6	34.32	0.406	0.074	35.81	0.261	0.823
Mean	33.21	0.362	0.243	35.10	0.312	0.852
S.E.M.	±1.51	±0.029	±0.047	±0.49	±0.027	±0.075

[a] Using these fitting constants, the urinary excretion data for each subject may be fitted to the equation: $U = U_\infty[1 - e^{-K(t-t_0)}]$, where U = the milligrams of drug equivalents excreted at time, t, in hours. [b] $t_{1/2}$ (hr.) = 0.693/K.

DISCUSSION

Absorption—The drug is very efficiently absorbed in the rat, dog, and human as shown by the essentially quantitative recovery of the dose in the urine after oral administration. In the dog the drug was absorbed so rapidly that the blood levels after oral administration were essentially identical to those after intravenous administration (Fig. 2). The superimposition of the oral and intravenous blood level curves does not mean, however, that oral absorption was complete in the 20 sec. that was required for intravenous administration, but rather that the oral absorption rate and excretion rate counterbalance each other, *i.e.*, since the intravenous dose gets in faster, a larger fraction is being excreted sooner.

The drug was absorbed very rapidly in the human. The absorption rate could not be estimated even though the first blood sample was taken 0.5 hr. post-drug administration. Nearly quantitative absorption of the oral dose in the human is shown by the fact that the total urinary excretion (Table III) and the areas under the plasma curves (Table V) were not significantly different after oral and intravenous administration. Based on the drug equivalents excreted in the urine (Table VI), the average per cent absorbed was 92.7% of the oral dose. This is a minimum estimate since this method of calculation gives only 89.0% absorbed estimated from the intravenous data.[2]

[2] The fact that the urinary excretion is lower after the intravenous dose than after the oral dose may indicate nonquantitative intravenous administration.

The per cent absorbed is 88.1 and 105.7% calculated from oral/intravenous ratios of the areas under the plasma curves and urinary output, respectively. Since the 72-hr. fecal excretion averaged only about 0.2% of the oral dose, the actual absorption may be as high as 99.8% of the dose.

Distribution—The apparent volumes of distribution (V_d) of the drug in the male and female dogs, after both routes of administration, are summarized in Table I. The apparent volumes are based on the plasma concentrations. Expressed as per cent of body weight, the V_d was very similar in the two dogs and was essentially identical after oral and intravenous administration. The average V_d (40.5% of body weight) is about twice the volume of extracellular water, but is less than total body water.

In the human, the early portion of the plasma curves after the intravenous dose reveals a rapid disappearance phase, having an average half-life (estimated from residuals) of about 7 min. in the six subjects, which undoubtedly reflects distribution and equilibration of the drug in body fluids. The apparent volumes of distribution of the drug in the six subjects, after both routes of administration, are summarized in Table V. The apparent volumes of distribution are independent of route of administration. The average V_d in the six subjects was found to be 15.2 L. (21.5% of their average body weight). This volume corresponds (perhaps fortuitously) to extracellular fluid volume as determined by insulin and sucrose (6).

Metabolism—The dog excreted all of the drug

TABLE VII—CALCULATED CONSTANTS[a] FROM URINARY EXCRETION DATA: INFINITE URINARY EXCRETION (U_∞), RATE CONSTANTS (K), AND LAG TIMES (t_0) FOR THE DRUG AND ITS METABOLITES

		Drug			Metabolite		
		Urine			Urine		
Subject	Route	U_∞, mg.	K, hr.$^{-1}$	t_0, hr.	U_∞,[b] mg.	K, hr.$^{-1}$	t_0, hr.
1	i.v.	23.93	0.451	−0.156	7.12	0.390	0.546
2	i.v.	23.62	0.407	−0.076	7.84	0.417	0.503
3	i.v.	21.51	0.343	−0.188	12.08	0.313	0.455
4	i.v.	20.33	0.347	0.132	11.64	0.346	0.641
5	i.v.	24.63	0.272	0.925	11.56	0.226	1.049
6	i.v.	27.50	0.417	0.007	6.84	0.361	0.348
Mean		23.59	0.373	0.107	9.51	0.342	0.590
S.E.M.		1.04	0.027	0.170	1.02	0.027	0.100
1	Oral	24.57	0.356	1.020	12.15	0.294	1.064
2	Oral	20.67	0.393	0.705	12.90	0.367	0.924
3	Oral	20.74	0.416	1.033	14.79	0.349	1.075
4	Oral	20.70	0.314	0.425	12.72	0.244	0.817
5	Oral	22.88	0.257	0.801	11.52	0.208	1.037
6	Oral	28.84	0.275	0.799	7.02	0.214	0.971
Mean		23.07	0.335	0.797	11.85	0.279	0.981
S.E.M.		1.32	0.026	0.091	1.06	0.028	0.040

[a] Using these fitting constants, the urinary excretion of drug and metabolite for each subject may be fitted to the equation: $U = U_\infty[1 - e^{-K(t-t_0)}]$, where U = mg. of drug equivalents excreted at time t. [b] Expressed as drug equivalents.

unchanged; two paper chromatographic systems revealed only a single zone corresponding to intact drug. The 15 rats excreted an average of 57% of the drug unchanged and the remainder as an acidic metabolite. The percentage of drug metabolized was essentially identical at the 1-mg./Kg. and 9-mg./Kg. dose levels. This metabolite can be converted to the free drug with acid but not with sulfatase or glucuronidase and is therefore believed to be a glycine conjugate. The R_f values of this metabolite correspond to one of the metabolites of 3,5-dimethylpyrazole in two paper chromatographic systems (2).

In the human, an average (±S.E.M.) of 71.4 ± 2.8% of the intravenous dose and 66.3 ± 3.2% of the oral dose was excreted as unchanged drug. The remainder of the dose in the human was excreted as a metabolite, which corresponded by paper chromatography to the one excreted by the rat. The distribution of the drug and its metabolite in each sequential urine sample from the rat and human was also determined, in order to calculate the individual rates of excretion of the drug and its metabolite (*vide infra*).

Effect of Pretreatment with Drug on Its Absorption and Metabolism in the Rat—The data strongly suggest that tachyphylaxis to the drug in rats is not due to altered metabolism. Absorption from the gastrointestinal tract was also not altered with repeated administration. Paper chromatography indicated that the metabolite was identical in both the pretreated and nonpretreated groups and the amounts of metabolite excreted were essentially the same in the two groups. Since no alteration in metabolism occurs, tachyphylaxis cannot be explained on the basis of drug metabolism.

Excretion—The average half-life for the excretion of radioactivity by the rats was 1.0 hr. Excretion by the dog was slightly slower; the half-lives obtained from blood level and urine data were essentially identical, averaging about 1.8 hr. in the two dogs.

The over-all (plasma and urine) half-life for the excretion of drug equivalents in the six subjects was essentially identical to that found in the dog, averaging 1.9 ± 0.1 hr. The plasma levels and rates of elimination of drug equivalents from the plasma were remarkably uniform in the humans. The half-lives for the appearance of radioactivity in the urine of the humans were obtained by fitting the data by the method of least squares to the first-order equation (Eq. 1):

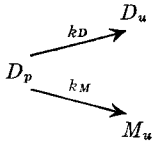
$$U = U_\infty[1 - e^{-K(t - t_0)}] \qquad \text{(Eq. 1)}$$

where U is the milligrams of drug equivalents excreted to time t, U_∞ is the amount excreted at infinite time, K is the observed rate constant for excretion ($t_{1/2} = 0.693/K$), and t_0 is a "lag time" which corrects for the delays caused by absorption, metabolism, and distribution.

Kinetics of Metabolism and Excretion in the Human—Since the over-all rate constant for the loss of radioactivity in the human was known to be a composite of the rates of excretion of the unchanged drug and the formation of its metabolite, it seemed desirable to assay for each of these compounds in all of the sequential urine collections. Hopefully, the individual rate constants could then be estimated. The percentages of radioactivity as metabolite in each of the sequential human urine samples, determined by quantitative paper chromatography, are presented in Table IV. After the first hour, the average ratio of drug to metabolite remained constant within the error of the assay. From the distribution of radioactivity in each sample, the cumulative excretion and the 24-hr. distribution[3] of the drug and its metabolite were calculated. The cumulative excretion data and Eq. 1 were used to estimate the over-all rate constant for the appearance in the urine of the drug and its metabolite. The constants of Eq. 1 which were obtained by this procedure are summarized in Table VII.

[3] The 24-hr. distribution obtained by this procedure was in excellent agreement with that found by paper chromatography of a pooled sample consisting of one-tenth of every sequential urine sample.

Model I—After both oral and intravenous administration, the average rate constant for the appearance of drug in the urine is not significantly different from the corresponding average constant for the appearance of metabolite (Fig. 7), *i.e.*, the ratio of drug to metabolite remains relatively constant with time. This suggests the following simple model:



where D_p is the amount of drug in the body and D_u and M_u are the amounts of drug and metabolite excreted in the urine. This model describes the data only in the mathematical sense; it ignores at least one of the rate constants involved in the transfer of drug in the plasma to metabolite in the urine. The over-all rate constant (K) for the appearance of drug or metabolite in the urine is equal to the sum of k_D and k_M. This model requires that the experimentally determined ratio of D_u to M_u be constant with time and equal to the ratio of k_D to k_M. Also, according to this model, the over-all rate constant (K) for the appearance of drug in the urine must be equal to the rate constant for the appearance of metabolite in the urine as well as to the rate constant for the disappearance of drug from the plasma. The model is described by the following equations (7):

$$D_p = D_0 e^{-Kt} \quad \text{(Eq. 2)}$$

$$D_u = (k_D D_0/K)(1 - e^{-Kt}) \quad \text{(Eq. 3)}$$

$$M_u = (k_M D_0/K)(1 - e^{-Kt}) \quad \text{(Eq. 4)}$$

$$K = k_D + k_M \quad \text{(Eq. 5)}$$

where D_0 is a constant equal to the amount of drug absorbed, and t is the time. The elimination of drug from the plasma data obeys Eq. 2 and the excretion of drug into the urine after the intravenous dose obeys Eq. 3, but the appearance of both drug and metabolite after the oral dose are described best by Eqs. 6 and 7, which include the correction term, t_0:

$$D_u = (k_D D_0/K)[1 - e^{-K(t - t_0)}] \quad \text{(Eq. 6)}$$

$$M_u = (k_M D_0/K)[1 - e^{-K(t - t_0)}] \quad \text{(Eq. 7)}$$

Fitting the average human urine data to Eqs.

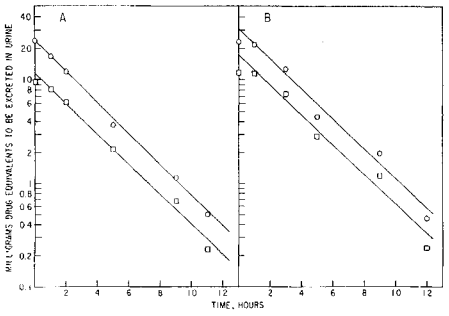


Fig. 7—Urinary excretion of 5-methylpyrazole-3-carboxylic acid-^{14}C and its metabolite (log scale) as determined by paper chromatography. Average of 6 subjects. Key: A, *37.5-mg. i.v. dose;* B, *37.5-mg. oral dose;* O, *drug;* □, *metabolite.*

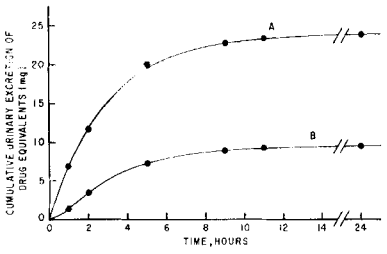


Fig. 8—Average cumulative excretion of 5-methylpyrazole-3-carboxylic acid-^{14}C and its metabolite in the 6 humans after the intravenous dose. Curves are calculated from Eqs. 3 and 13; K = *0.34,* k_D = *0.24,* k_m = *0.10,* k'_m = *1.5; points are experimental values. Key:* A, *drug;* B, *metabolite.*

TABLE VIII—COMPARISON OF OBSERVED AND CALCULATED[a] CUMULATIVE URINARY EXCRETION OF 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID AND ITS METABOLITE FOLLOWING 37.5-mg. ORAL AND INTRAVENOUS DOSES

		Av. Excreted by the 6 Subjects, mg.					
		Drug			Metabolite		
Time, hr.	Route	Obs.	Calcd.	Deviation	Obs.	Calcd.	Deviation
1	i.v.	6.88	6.41	0.47	1.31	1.30	0.01
2	i.v.	11.57	11.33	0.24	3.31	3.65	0.34
5	i.v.	19.83	19.11	0.72	7.29	7.38	0.09
9	i.v.	22.42	22.38	0.04	8.76	8.94	0.18
11	i.v.	23.05	22.71	0.34	9.21	9.21	0.00
24	i.v.	23.55	23.51	0.04	9.45	9.49	0.04
				Mean 0.31			Mean 0.11
1	Oral	1.63	1.26	0.37	0.29	0.06	0.23
3	Oral	10.55	11.20	0.65	4.29	5.41	1.12
5	Oral	18.53	16.60	1.93	8.78	8.32	0.46
9	Oral	20.98	21.12	0.14	10.42	10.76	0.34
12	Oral	22.48	22.26	0.22	11.37	11.37	0.00
24	Oral	22.94	23.00	0.06	11.61	11.77	0.10
				Mean 0.56			Mean 0.38

[a] From Eqs. 8–11.

TABLE IX—ESTIMATES OF PARAMETERS OF MODEL *II* FOR METABOLISM AND EXCRETION OF 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID AFTER INTRAVENOUS ADMINISTRATION

Parameter	Subject 1	2	3	4	5	6	Av.
k_m (hr.$^{-1}$)	0.111	0.108	0.139	0.130	0.051	0.081	0.103
k'_m (hr.$^{-1}$)	1.14	1.76	1.41	1.31	0.618	2.21	1.32
k_D (hr.$^{-1}$)	0.377	0.325	0.251	0.222	0.084	0.332	0.256
V_d (L.)	13.4	13.5	16.6	15.5	38.9	14.3	15.2

6 and 7 (the oral and intravenous doses were treated separately), gave the following constants for excretion of the drug and metabolite:

	Intravenous Dose	Oral Dose
D_0 (mg.)	32.95	34.76
K (hr.$^{-1}$)	0.339	0.305
k_D (hr.$^{-1}$)	0.242	0.202
k_M (hr.$^{-1}$)	0.097	0.103

The cumulative excretion of drug and metabolite after the 37.5-mg. intravenous and oral doses is described, therefore, by Eqs. 8–11.

i.v. dose

$$D_u = 23.52\,[1 - e^{-0.34(t\,-\,0.06)}] \quad \text{for } t \geq 0.06 \text{ hr.} \quad \text{(Eq. 8)}$$

$$M_u = 9.49\,[1 - e^{-0.34(t-0.56)}] \quad \text{for } t \geq 0.56 \text{ hr.} \quad \text{(Eq. 9)}$$

oral dose

$$D_u = 23.02\,[1 - e^{-0.31(t-0.83)}] \quad \text{for } t \geq 0.83 \text{ hr.} \quad \text{(Eq. 10)}$$

$$M_u = 11.78\,[1 - e^{-0.31(t-0.98)}] \quad \text{for } t \geq 0.98 \text{ hr.} \quad \text{(Eq. 11)}$$

The experimental data are compared with the values calculated from Eqs. 8–11 in Table VIII. The deviation of the average cumulative urinary excretion of the six humans from that calculated from the above equations averaged less than 1.3% of the dose.

Model II—Although model *I* yields equations which give a satisfactory mathematical description of the data, it has an obvious defect. The appearance of the metabolite in the urine must be effected by *at least* two rate processes, first the formation of the metabolite with a rate constant k_m, and second, its excretion with a rate constant k'_m, *viz.*:

$$\begin{array}{l} \overset{k_D}{\longrightarrow} D_u \\ D_p \xrightarrow{k_m} M_p \xrightarrow{k'_m} M_u \end{array}$$

Since first-order kinetics are nearly obeyed (Figs. 3–6), one of these processes must be much faster than the other.

Since rapid metabolism followed by slow excretion of the metabolite would not be consistent with the observed ratio of drug to metabolite, the rate-determining step is undoubtedly the formation of the metabolite (k_m). Therefore, the rate constant k_M in model *I* must be essentially equal to k_m. Since the rate constant for excretion of metabolite is larger than the rate constant for formation, the plasma levels of metabolite are undoubtedly quite low compared to plasma levels of drug, *i.e.*, the plasma half-life obtained by measurement of total radioactivity (Table III) reflects primarily intact drug.

The magnitude of k'_m, the rate constant for the excretion of the metabolite, was determined by both analog and digital computer techniques. Using the above constants for D_0, K, and k_D, and setting k_m equal to k_M, the analog computer[4] gave an excellent fit for both the intravenous and oral data when k'_m was set at approximately 1.5 hr.$^{-1}$, *i.e.*, when the half-life for the excretion of the metabolite is about 28 min. An analog computer plot of the intravenous data is presented in Fig. 8.

The digital computer was also used as a tool for the fitting of the data to model *II*. A digital computer program,[5] which simultaneously fits both the urine and plasma data, was used to estimate the rate constants. Equations 3, 12, and 13, which describe model *II* for the intravenous dose, were incorporated into the computer program.

$$C_D + C_M = D_0[(K - k'_m)V_d]^{-1}[(k_D - k'_m)e^{-Kt} + k_m e^{-k'_m t}] \quad \text{(Eq. 12)}$$

$$M_u = k_m D_0 K^{-1}[1 + k'_m(K - k'_m)^{-1} e^{-Kt} - K(K - k'_m)^{-1} e^{-k'_m t}] \quad \text{(Eq. 13)}$$

where C_D and C_M represent the concentrations of drug and metabolite in the plasma, and the other terms have already been defined. These equations satisfactorily describe not only the average data, but also the data of the individual subjects. The parameters obtained are presented in Table IX. The average values do not differ significantly from those obtained by the use of the analog computer. For the best fit of the oral data, at least one more rate constant (representing gastrointestinal absorption) would have to be introduced into model *II*.

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[4] The assistance of Dr. R. H. Baker, Jr., in the fitting of the data by the analog computer is gratefully acknowledged.
[5] This program was written by Dr. Carl L. Metzler, The Upjohn Co.