Metabolism, Plasma or Serum Levels, and Elimination of Phenformin in Guinea Pigs, Rats, and Dogs

DAVID ALKALAY ˣ, JOSEPH VOLK, WILLIAM ROTH, LAKSHMI KHEMANI, and M. FRED BARTLETT

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Abstract □ ^{14}C-Phenformin hydrochloride was used for investigating the metabolism, plasma or serum levels, and elimination of the drug following 1.5-mg/kg po or iv doses to guinea pigs, rats, and dogs. The amounts of individual metabolites and unchanged drug were assessed in urine as well as in plasma or serum. The glucuronide of 1-(*p*-hydroxyphenethyl)biguanide was a major metabolite in the blood and urine of all three species. Guinea pig serum and urine contained a sizable quantity of unchanged drug. Dog plasma and urine had significant amounts of nonconjugated 1-(*p*-hydroxyphenethyl)biguanide and of an unidentified major metabolite. In all three species following intravenous drug administration, unchanged drug contributed significantly to the radioactivity found in blood and urine. The apparent half-lives of phenformin elimination were 0.3–0.8 day for guinea pigs and rats and 1–1.5 days for dogs. Urinary excretion data indicate apparent half-lives of approximately 1.3–1.5 days for the elimination of each of the three major metabolites in dogs.

Keyphrases □ Phenformin—metabolism, plasma or serum levels, and elimination in guinea pigs, rats, and dogs, TLC-autoradiographic analysis □ Metabolism—phenformin in guinea pigs, rats, and dogs, TLC-autoradiographic analysis □ Plasma or serum levels—phenformin in guinea pigs, rats, and dogs, TLC-autoradiographic analysis □ Elimination—phenformin in guinea pigs, rats, and dogs, TLC-autoradiographic analysis □ TLC-autoradiography—analysis, phenformin in biological fluids □ Autoradiography-TLC—analysis, phenformin in biological fluids □ Antidiabetic agents—phenformin, metabolism, plasma or serum levels, and elimination in guinea pigs, rats, and dogs, TLC-autoradiographic analysis

The absorption, metabolism, and elimination of phenformin (I) in several animal species and in humans were first investigated when specific and sensitive methods for analysis of this hypoglycemic drug were not available. By relying on the use of ^{3}H-phenformin and various chromatographic techniques, Beckmann (1, 2) demonstrated that 1-(*p*-hydroxyphenethyl)biguanide (II) and its *O*-glucuronide conjugate (III) were the major drug metabolites. He reported that rats excreted II and III in urine; that mice, guinea pigs, and rabbits excreted I, II, and III; and that humans eliminated only I and II. The identities of II and III were confirmed by Murphy and Wick (3).

Recent methodology that permits the determination of trace amounts of phenformin in biological fluids (4, 5) was applied to investigate phenformin pharmacokinetics in humans (6). In the present work, TLC in conjunction with autoradiography was used for investigating the pharmacokinetics, metabolism, and disposition of the drug in guinea pigs, rats, and dogs.

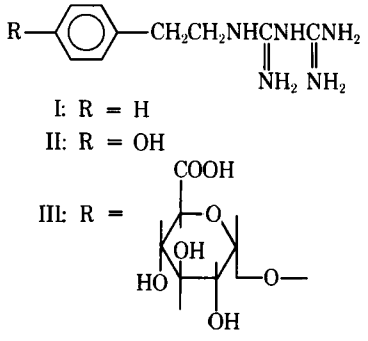

Table I—Percent of Radioactivity Excreted Renally by Three Guinea Pigs during the 24 hr following 1.5 mg of ^{14}C-Phenformin Hydrochloride/kg

Guinea Pig Number	Guinea Pig Weight, g	Drug Administration	Percent of Dose Excreted Renally as I[a]	I[b]	II[a]	III[a]	Unaccounted	Total[c]
28	725	Intravenous	34.3	34.5	3.2	14.9	7.3	59.7
31	745	Oral	17.1	17.0	5.3	8.7	4.8	35.8
34	805	Oral	15.8	14.3	4.8	17.9	6.8	45.3

[a] Determined by quantitative TLC. [b] Determined by GLC. [c] Determined by liquid scintillation counting of urine radioactivity.

EXPERIMENTAL

Chemicals—The reagents and solvents were: 28% ammonium hydroxide[1], reagent grade; 1-butanol[1], reagent grade; chloroform[1], reagent grade; ^{14}C-I and II (as hydrochlorides)[2]; 2,2-dichloro-1,1-difluoroethyl methyl ether[3]; heparin[4]; liquid scintillation counting medium[5]; methane[6], technical grade; phenformin hydrochloride[7]; and 2-propanol[1], reagent grade.

Animal Experiments—Healthy, young, male animals were fasted overnight prior to dosing. Unless otherwise specified, the drug was administered as an aqueous solution. Throughout the experiments, the animals were housed in appropriate metabolism cages. Food and excess water were supplied once daily. Feces and urine were collected and kept frozen until needed.

Guinea pigs[8], ~800 g, were given oral doses by intubation and intravenous doses in the jugular vein following sedation[3]. Blood specimens were withdrawn by heart puncture and stored overnight at 4°. After removal of the clots, the resulting serum samples were frozen until further use.

The rats[9], ~275 g, were given oral doses by intubation and intravenous doses into the tail vein. Blood samples, obtained from the tail, were centrifuged, and the resulting serums were frozen for further use. The total amount of collected blood usually was kept below 2.5 ml/animal.

Purebred beagle dogs, ~12 kg, were given the drug orally in a gelatin capsule[10] and intravenously into the cephalic vein. Blood specimens were withdrawn from the jugular vein with a heparinized syringe. They were centrifuged within 1 hr, and the separated plasma samples were kept frozen until needed.

Quantitative TLC—Prescored 250-μm silica gel plates[11] were divided into 20 parallel bands of 1-cm width each with a scriber[12]. Alternate bands were spotted with samples, in amounts not exceeding 10 μl, using disposable microsampling pipets[13].

Two solvent systems proved suitable. System A contained ammonium

[1] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.
[2] The ^{14}C-radioactivity was associated with the β-carbon atom in the phenethyl moiety of I (18 μCi/mg) and with the two carbon atoms in the biguanide moiety of II (24 μCi/mg).
[3] Metaphane, Pitman-Moore, Inc., Washington Crossing, NJ 07902.
[4] Liquaemin Sodium "10", Organon, Inc., West Orange, N.J.
[5] Scintisol Complete, Isolab, Inc., Akron, OH 44321.
[6] Matheson Gas Products, East Rutherford, NJ 07073.
[7] Ciba-Geigy Corp., Summit, NJ 07901.
[8] Hartley variety, Elm Hill Farms, Chelmsford, MA 01824.
[9] Sprague-Dawley CD variety, Charles River Breeding Laboratories, Wilmington, MA 01887.
[10] Eli Lilly and Co., Indianapolis, IN 46206.
[11] Silica gel GF Uniplates (20 × 20 cm), Analtech, Inc., Newark, DE 19711.
[12] TLC scriber, Applied Science Laboratories, State College, PA 16801.
[13] Corning Glass Works, Corning, NY 14830.

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Table II—Metabolic Profiles of Serum Samples from Three Guinea Pigs after 1.5 mg of ^{14}C-Phenformin Hydrochloride/kg

Guinea Pig	Drug Administration Route	Blood Withdrawal Time, hr	Percent of Radioactivity Attributed to				
			I[a]	I[b]	II[a]	III[a]	Unaccounted
28	Intravenous	0.27	68	84	<3[c]	17[c]	12
28	Intravenous	1	31[c]	35	<3[c]	58	8
31	Oral	1	29[c]	43	7[c]	56[c]	8
31	Oral	3	14[c]	12	≤3[c]	73[c]	10
34	Oral	2	11[c]	14	<3[c]	79	7
34	Oral	3	9[c]	11	<3[c]	79	8

[a] Determined by quantitative TLC. [b] Determined by GLC. [c] Sample radioactivity was less than three times the background level.

hydroxide (10 ml), water (10 ml), 2-propanol (45 ml), 1-butanol (25 ml), and chloroform (20 ml). System B was similar, except that the amounts of water and chloroform were 16 and 14 ml, respectively. At room temperature and in tanks[14] containing 100 ml of the chosen solvent system, the plates required approximately 4 hr for development.

Autoradiograms were obtained in cassettes[15], modified to accept TLC plates, by exposing X-ray film[16] to the radioactivity of the developed plates. The time of exposure (1 week to several months) depended on the amount of radioactivity of the spotted sample. Film development proceeded as recommended by the manufacturer.

Each TLC plate was separated into zones assigned to various metabolites on the basis of the visual appearance of the corresponding autoradiogram. The silica gel material of these zones was carefully scraped into glass vials, and the radioactivity was assessed by liquid scintillation counting. This procedure permitted determination of the relative proportion of different metabolites found on the TLC plate and, consequently, their quantification in the body fluid used for spotting the plate. Thus, quantitative metabolic profiles were obtained for selected serum or plasma samples. By correcting for the volumes of all urine fractions and by adding all individual results, cumulative renal excretion data were obtained both for unchanged drug and for various metabolites.

Enzymatic Hydrolysis of Urine Samples—Selected urine samples were subjected to enzymatic hydrolysis[17,18] in accordance with published procedures (7).

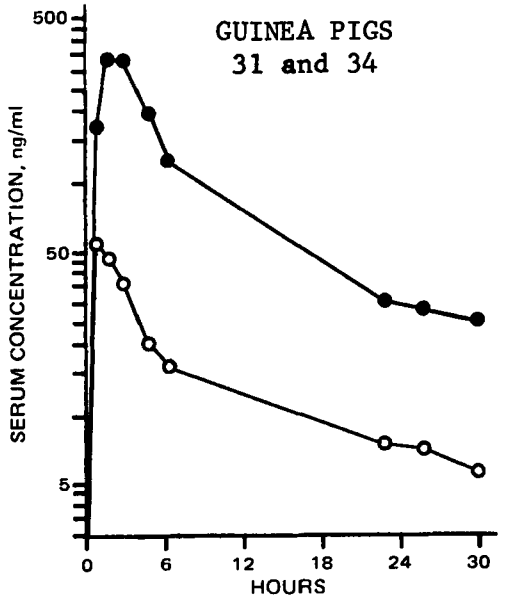

Figure 1—*Average concentration-time plots of phenformin (○) and total radioactivity (●) in serum resulting from 1.5-mg/kg po doses of ^{14}C-phenformin hydrochloride administered to two guinea pigs.*

[14] Desaga TLC tanks, Brinkmann Instruments, Westbury, NY 11590.
[15] Wafer Rigidform cassettes, Halsey X-ray Products, Brooklyn, N.Y.
[16] Blue brand type BB54, Eastman Kodak Co., Rochester, NY 14650.
[17] Glusulase, Endo Laboratories, Garden City, NY 11530.
[18] Ketodase, General Diagnostics Division, Warner-Chilcott, Morris Plains, N.J.

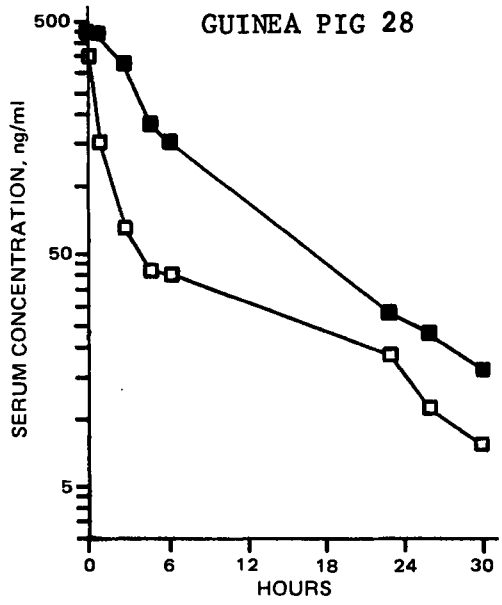

Figure 2—*Concentration-time plots of phenformin (□) and total radioactivity (■) in serum resulting from a 1.5-mg/kg iv dose of ^{14}C-phenformin hydrochloride administered to a guinea pig.*

Fecal Samples—Representative samples were obtained by pretreatment of collected fecal materials.

Rat feces were dried in an oven at 60°. The dry residues were weighed and then ground to a powder with a mortar and pestle.

Preweighed dog feces were homogenized in a blender with a threefold amount of distilled water. Several 10-ml fractions were measured with a pipet and transferred into 20-ml glass vials for lyophilization[19]. The residues were broken up to a powder.

Radioactivity Measurements—A radioscanner[20] was used for scanning developed TLC plates. Operating parameters included ×3 for high voltage, 50-mv sensitivity, 10-counts/sec range, 10-power/time constant, 10-cm/hr scanning speed, 25-ml/min methane quench gas flow, and 2 × 16-mm window slit.

The radioactivity of feces was assessed by placing powdered fecal

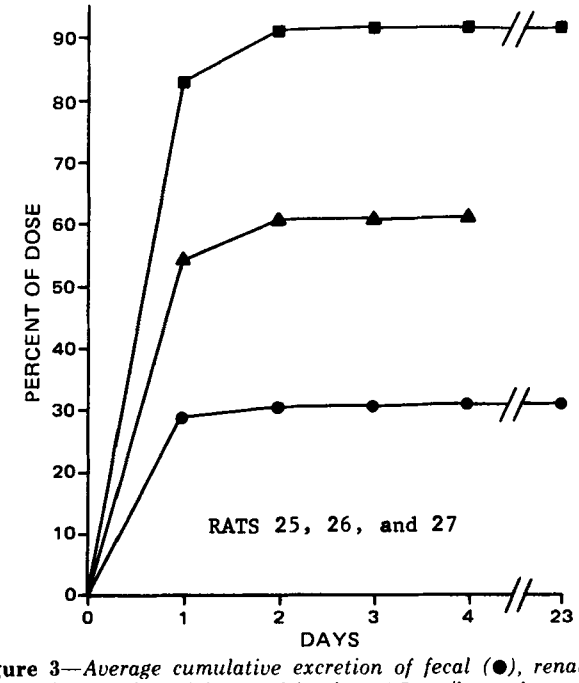

Figure 3—*Average cumulative excretion of fecal (●), renal (▲), and total (■) radioactivity resulting from 1.5-mg/kg po doses of ^{14}C-phenformin hydrochloride administered to three rats.*

[19] Model 10-145 MR-BA lyophilizer, Virtis Co., Gardiner, N.Y.
[20] Model LB 2722, Varian Instrument Division, Palo Alto, CA 94303.

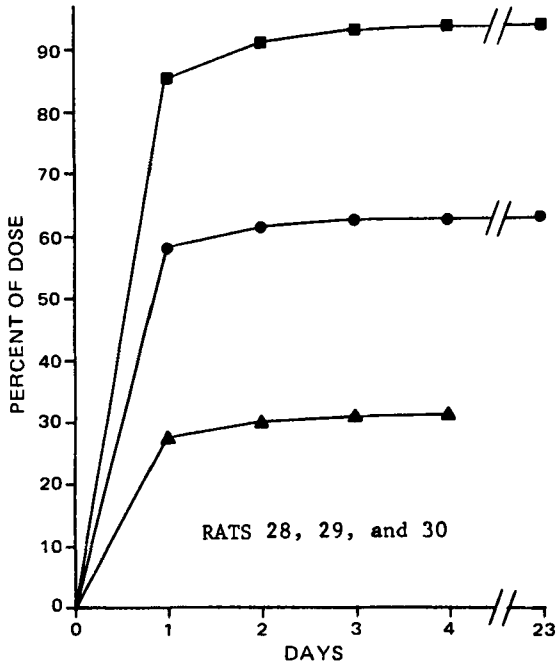

Figure 4—*Average cumulative excretion of fecal (●), renal (▲), and total (■) radioactivity resulting from 1.5-mg/kg iv doses of ^{14}C-phenformin hydrochloride administered to three rats.*

residue samples into size 0 gelatin capsules and burning them in an oxidizer[21]. The resulting $^{14}CO_2$, trapped in ethanolamine, was used for liquid scintillation counting[22]. The latter was done with 10-ml amounts of counting medium[5] for both liquid and silica gel radioactive samples.

GLC Determination of Phenformin—The specific and sensitive method employed is based on the conversion of biguanides into substituted *s*-triazines assayable by GLC and electron-capture detection or selected ion monitoring (4, 5). The gas chromatograph had ^{63}Ni-

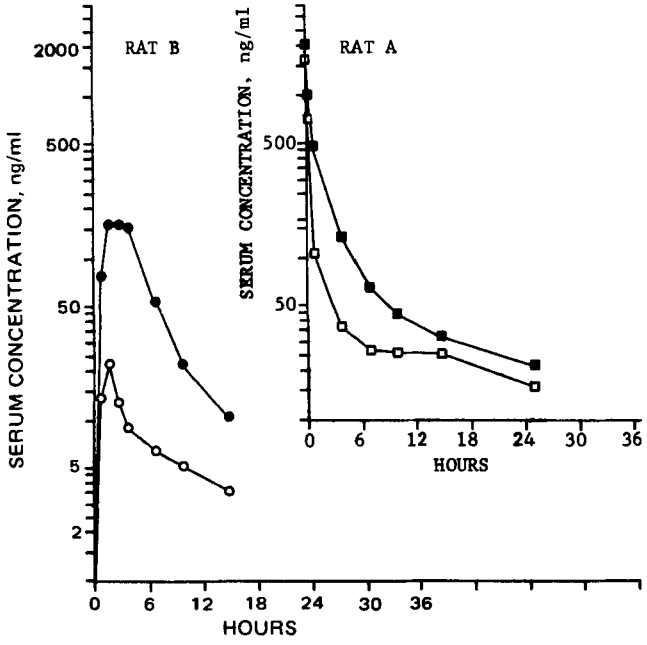

Figure 5—*Concentration–time plots of phenformin (□,○) and total radioactivity (■,●) in serum resulting from 1.5-mg/kg doses of ^{14}C-phenformin hydrochloride administered intravenously to Rat A and orally to Rat B.*

[21] Tri-Carb model 305, Packard Instrument Co., Downers Grove, IL 60515.
[22] Intertechnique model SL-40, Teledyne-Intertechnique, Westwood, NJ 07675.

Table III—Percent of Radioactivity Excreted Renally by Six Rats following 1.5 mg of ^{14}C-Phenformin Hydrochloride/kg

Rat	Drug Administration Route	Percent of Dose Excreted Renally as I[a]	I[b]	II[a]	III[a]	Unaccounted	Total[c]
25	Oral	0.3	0.4	1.0	25.1	2.4	28.7
26	Oral	0.6	0.5	1.9	24.8	2.0	29.2
27	Oral	0.6	0.2	0.7	28.5	3.1	32.9
28	Intravenous	18.6	18.3	9.1	26.6	5.0	59.3
29	Intravenous	16.7	15.5	15.1	25.9	4.4	62.0
30	Intravenous	16.9	12.6	15.2	28.0	4.7	64.8
Mean	Oral	0.5	0.4	1.2	26.1	2.5	30.3
Mean	Intravenous	17.3	15.5	13.2	26.8	4.7	62.1

[a] Determined by quantitative TLC. [b] Determined by GLC. [c] Determined by liquid scintillation counting of urine radioactivity.

electron-capture detection capabilities[23] and was equipped with a silanized glass column, 180 cm × 2 mm i.d., packed with 3% OV-17 on 80–100-mesh Chromosorb W HP[24].

RESULTS AND DISCUSSION

The sensitivity of quantitative TLC was extended significantly by autoradiography. This technique permitted the analysis of biological fluid samples with radioactivity levels undetectable by radioscanning. Its accuracy was estimated by comparison with independent GLC results. Renal phenformin data, representing the excretion of less than 6% of the administered radioactivity, and serum or plasma data, from samples whose phenformin radioactivity was less than three times the background level, were less accurate. They were associated with a relative standard deviation[25] of 26%. The accuracy of all other phenformin data was con-

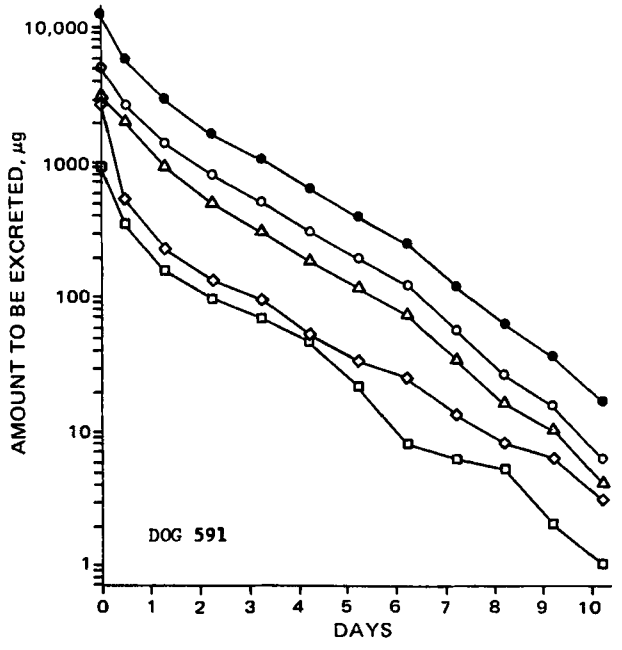

Figure 6—*"Sigma minus" plots for phenformin (□), II (○), III (△), IV (◇), and total radioactivity (●) based on renal data resulting from a 1.5-mg/kg po dose of ^{14}C-phenformin hydrochloride administered to a beagle dog.*

[23] Model 2100 gas chromatograph, Varian, Palo Alto, Calif.
[24] Ohio Valley Specialty Chemicals, Inc., Marietta, OH 45750.
[25] The relative standard deviation was calculated from:

$$S_{rel}(x) = 100\sqrt{\frac{2\Sigma(1/2\, d/m)^2}{n-1}}$$

where d is the difference between duplicates, m is the arithmetic mean of each duplicate determination, and n is the number of determinations.

Table IV—Metabolic Profiles of Serum Samples Obtained from a Rat after 1.5 mg of ^{14}C-Phenformin Hydrochloride/kg iv

Blood Withdrawal Time, min	Percent of Radioactivity Attributed to				
	I[a]	I[b]	II[a]	III[a]	Unaccounted
5	86	83	3	2	9
15	83	73	3[c]	4[c]	13
60	26[c]	23	15[c]	45[c]	14

[a] Determined by quantitative TLC. [b] Determined by GLC. [c] Sample radioactivity was less than three times the background level.

siderably better and was associated with a relative standard deviation of 7%. These values are based on analytical data abstracted from Tables I–VI.

Half-lives were estimated by least-squares fitting of the last five or more data points on the terminal log-linear parts of the concentration–time curves[26].

Guinea Pigs—A single 1.5-mg/kg dose of ^{14}C-phenformin hydrochloride was administered orally to two guinea pigs and intravenously to a third animal. Excretion data were obtained from urine collected for 24 hr (Table I). Phenformin and Metabolites II and III were assessed by quantitative TLC. The amounts of phenformin also were confirmed by GLC determinations.

The identity of phenolic Metabolite II is indicated by the similarity in TLC behavior of an authentic sample. Metabolite III exhibited properties similar to a metabolite in the rat and dog, which, as will be shown, was identified as the glucuronide conjugate of II.

Metabolic profiles were obtained for selected serum samples by use of quantitative TLC (Table II). Glucuronide III was the major radioactive component in all serum samples taken at 1 or more hr after oral or intravenous drug administration.

Serum concentration–time data for phenformin and for total radioactivity are presented graphically in Figs. 1 and 2. The apparent half-lives of elimination were comparable and averaged 11 hr[26].

Rats—Single 1.5-mg/kg doses of ^{14}C-phenformin hydrochloride were given to five rats orally and to five rats intravenously. The resulting five pairs of rats were used as follows.

The rats from one pair were placed individually in metabolism cages[27]. For 24 hr, exhaled carbon dioxide was trapped without detection of any radioactivity.

Excretion data were obtained from three pairs of rats, whose urine and feces were collected for 23 days. About 90% of the administered radioactivity was excreted within 2 days. After the 4th day, the elimination of radioactivity was negligible and amounted to less than 0.05% of the dose. Cumulative radioactivity excretion data for orally and intravenously treated rats are plotted in Figs. 3 and 4, respectively. About 31% of the radioactivity was found in the feces even after intravenous drug administration. Consequently, the biliary route of excretion appears to be significant for rats. Fecal radioactivity amounted to about 61% of the oral dose. This value agrees with data obtained by Beckmann (1), who reported that 5–12% of oral doses were eliminated by the biliary route and concluded that oral absorption was incomplete.

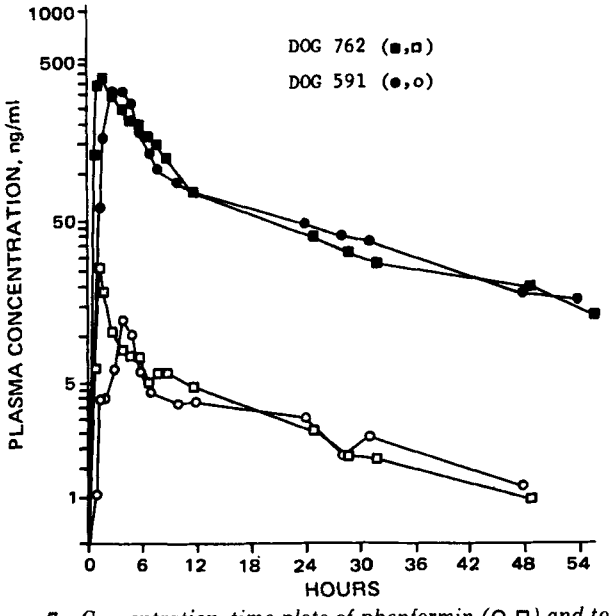

Figure 7—*Concentration–time plots of phenformin (○,□) and total radioactivity (●,■) in plasma resulting from 1.5-mg/kg po doses of ^{14}C-phenformin hydrochloride administered to two beagle dogs.*

[26] Apparent half-lives for the radioactivity elimination data presented in Figs. 1 and 5 (Rat A) were based on the last four data points.
[27] Roth.

Table V—Percent of Radioactivity Excreted Renally by Two Dogs during the Indicated Collection Periods following 1.5 mg of ^{14}C-Phenformin Hydrochloride/kg

Dog	Drug Administration Route	Collection Hours	Percent of Dose Excreted Renally as						
			I[a]	I[b]	II[a]	III[a]	IV[a]	Unaccounted	Total[c]
591	Intravenous	150	26.8	23.0	15.8	13.7	13.9	6.7	77.0
762	Intravenous	270	25.7	23.9	17.4	14.6	12.3	6.6	76.5
591	Oral	270	4.9	2.3	27.5	17.0	14.5	8.1	72.0
762	Oral	150	5.4	2.5	22.3	15.3	14.2	5.9	63.1
Mean	Intravenous		26.3	23.5	16.6	14.2	13.1	6.6	76.7
Mean	Oral		5.2	2.4	24.9	16.1	14.3	7.0	67.5

[a] Determined by quantitative TLC. [b] Determined by GLC. [c] Determined by liquid scintillation counting of urine radioactivity.

Phenformin, II, and III were found in urine and assessed by quantitative TLC. Due to rapidly decreasing radioactivity levels, only samples collected for 2 days after the oral dose and for 3 days after the intravenous dose were analyzed. Phenformin analyses were performed also by GLC (Table III). Independently of the drug administration route, III proved to be the major radioactive component in rat urine. Its identity was established by enzymatic hydrolysis.

Prior to and after hydrolysis, the samples were analyzed by GLC and by quantitative TLC. Only trace amounts of phenformin were found before and after hydrolysis. The radioactivity associated with III was essentially lost during hydrolysis and was replaced by radioactivity associated with II. Considering the experimental conditions, the choice of enzymes[17,18], and the analytical results, it may be concluded that III is a glucuronide conjugate of 1-(*p*-hydroxyphenethyl)biguanide.

A serum metabolic profile was obtained with samples from an intravenously treated rat. Quantitative TLC results (Table IV) indicate that III was the major radioactive component not only in urine but also in blood. The serum concentration–time curve for this animal and the one obtained for an orally dosed rat are plotted in Fig. 5. The intravenous data indicate half-lives for phenformin and for total radioactivity that were comparable and averaged about 17 hr. Oral dose concentrations were too low for estimation of apparent half-lives with a reasonable degree of confidence.

Dogs—Two dogs were given single oral and intravenous ^{14}C-phenformin hydrochloride doses of 1.5 mg/kg. They were treated in a crossover fashion with a 5-week washout period between drug administrations. Unlike rats, the fecal radioactivity excretion of dogs was not very significant, averaging 7% of the intravenous dose and 11% of the oral dose. Most radioactivity was excreted renally.

In analogy with both guinea pigs and rats, dog urine contained significant amounts of phenformin, II, and III. In addition, a radioactive urinary component (Metabolite IV) was also present. In Solvent System B, the R_f values of I, II, III, and IV were 0.39, 0.34, 0.10, and 0.48, respectively. The identities of I and II were confirmed by TLC and radio-

Table VI—Metabolic Profiles of Plasma Samples from Two Dogs following 1.5 mg of ^{14}C-Phenformin Hydrochloride/kg

Dog	Drug Administration Route	Blood Withdrawal Time, hr	Percent of Radioactivity Attributed to					
			I[a]	I[b]	II[a]	III[a]	IV[a]	Unaccounted
591	Oral	3	6[c]	5	28[c]	10[c]	30[c]	26
591	Oral	4	11[c]	4	24[c]	16[c]	27[c]	22
591	Oral	6	6[c]	3	15[c]	23[c]	8[c]	48
762	Intravenous	0.08	77	92	4[c]	2[c]	2[c]	15
762	Intravenous	0.25	48	51	20[c]	3[c]	17[c]	12
762	Intravenous	2	30[c]	20	8[c]	14[c]	17[c]	32

[a] Determined by quantitative TLC. [b] Determined by GLC. [c] Sample radioactivity was less than three times the background level.

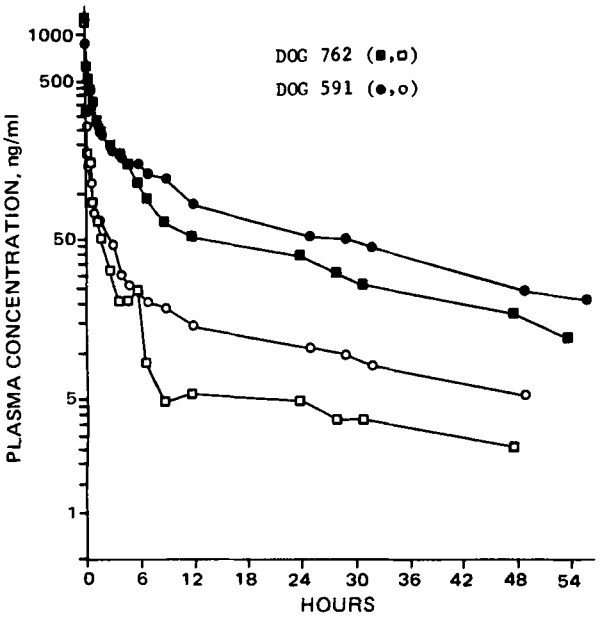

Figure 8—*Concentration-time plots of phenformin (○,□) and total radioactivity (●,■) in plasma resulting from 1.5-mg/kg iv doses of ^{14}C-phenformin hydrochloride administered to two beagle dogs.*

scanning comparisons, and the identity of III was determined by enzymatic hydrolysis experiments similar to those described for rats. Metabolite IV remains to be identified.

The amounts of renally excreted I–IV, as percentages of total administered radioactivity, are presented in Table V. The relatively high urinary radioactivity levels and the lengthy time of collection permitted the preparation of "sigma minus" plots for phenformin, for each metabolite, and for total radioactivity (as apparent drug). These plots allowed the estimation of apparent disposition half-lives for I–IV and total radioactivity. The values averaged 35–37 hr. Figure 6 illustrates the results obtained from one dog after oral drug administration. Similar data were obtained from the second dog after intravenous drug administration. The theoretical considerations and an appraisal of this method were discussed by Martin (8).

Plasma metabolic profiles were obtained after oral and intravenous drug administrations. Quantitative TLC results (Table VI) indicate the presence in plasma of significant amounts of unchanged drug and of each of the three metabolites. Plasma concentration–time curves resulting from single oral and intravenous doses were obtained for both animals (Figs. 7 and 8). The terminal log-linear parts of the curves indicate comparable elimination half-lives for phenformin and for total radioactivity. They averaged about 26 hr.

REFERENCES

(1) R. Beckmann, *Diabetologia*, **3**, 368 (1967).

(2) R. Beckmann, *Ann. N.Y. Acad. Sci.*, **148**, 820 (1968).

(3) P. Murphy and A. Wick, *J. Pharm. Sci.*, **57**, 1125 (1968).

(4) S. Matin, J. Karam, and P. Forsham, *Anal. Chem.*, **47**, 545 (1975).

(5) D. Alkalay, J. Volk, and M. Bartlett, *J. Pharm. Sci.*, **65**, 525 (1976).

(6) D. Alkalay, L. Khemani, W. Wagner, Jr., and M. Bartlett, *J. Clin. Pharmacol.*, **15**, 446 (1975).

(7) W. Conway, H. Minatoya, A. Lands, and J. Shekosky, *J. Pharm. Sci.*, **57**, 1134 (1968).

(8) B. Martin, *Br. J. Pharmacol. Chemother.*, **29**, 181 (1967).

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Fluorocarbon Aerosol Propellants XII: Correlation of Blood Levels of Trichloromonofluoromethane to Cardiovascular and Respiratory Responses in Anesthetized Dogs

YASH M. AMIN, EMMANUEL B. THOMPSON, and WIN L. CHIOU ˣ

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Abstract □ Anesthetized mongrel dogs were exposed to various concentrations of trichloromonofluoromethane. Before, during, and after the inhalation, arterial and venous blood samples were obtained for fluorocarbon analysis. After the cessation of fluorocarbon inhalation, a multiexponential decline from the blood was observed. This finding was similar to that of a previous study in which the fluorocarbon was administered intravenously to unanesthetized dogs. The half-life calculated from the terminal phase was about 280 min, and the pseudodistribution equilibrium was reached about 100 min after dosing. Study of the relationship between blood fluorocarbon levels and effects on the respiration rate and arterial blood pressure indicates that the sites of these pharmacological activities are located in the blood or central compartment rather than in the peripheral compartment. The effect on the heart rate appears to be quite instantaneous after inhalation. These results might shed some light on the fast effect of the fluorocarbon propellants, which caused sudden deaths after inhalation of a large quantity.

Keyphrases □ Trichloromonofluoromethane—blood levels correlated to cardiovascular and respiratory responses in dogs □ Cardiovascular responses—trichloromonofluoromethane, correlated to blood levels in dogs □ Respiratory responses—trichloromonofluoromethane, correlated to blood levels in dogs □ Aerosol propellants—trichloromonofluoromethane, blood levels correlated to cardiovascular and respiratory responses in dogs □ Fluorocarbon aerosol propellants—trichloromonofluoromethane, blood levels correlated to cardiovascular and respiratory responses in dogs

Because of the wide use of fluorocarbon aerosol propellants in various household, cosmetic, and pharmaceutical pressurized packages, the possible adverse effects or toxicities of these compounds have been studied extensively. These studies concerned the effects on the cardiovascular system (1–8), enzyme activities (5, 9–11), muta-

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