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Disposition Kinetics of Griseofulvin in Dogs

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Abstract □ Griseofulvin and its main metabolite, 6-demethylgriseofulvin (6-DMG), have been administered i.v. to dogs. The plasma data of griseofulvin were found to fit biexponential equations while the urinary excretion rate data of 6-DMG after its i.v. dose (and after griseofulvin administration) were found to fit triexponential equations. When increasing doses of griseofulvin were administered intravenously, some dogs followed dose-independent disposition kinetics, while others showed definite evidence of dose-dependent disposition kinetics. The distribution rate constants and the volume of distribution were found changed with dose in dogs showing dose-dependent disposition kinetics. However, the urinary recovery of the main metabolite, 6-DMG, remained almost constant. It is, therefore, postulated that the dose-dependent disposition kinetics of griseofulvin might be attributed to changes in tissue distribution rather than changes in the intrinsic metabolic activity.

Keyphrases □ Griseofulvin—disposition kinetics, dogs □ 6-Demethylgriseofulvin—disposition kinetics, dogs □ Kinetics—dose-dependence, -independence, griseofulvin □ Metabolite excretion—griseofulvin

Griseofulvin (gris), a water-insoluble antibiotic, is widely used in both man and animals for treatment of superficial fungal diseases. Although it has been used in dogs for almost a decade, its metabolic fate in dogs has been only recently reported from this laboratory (1). The purpose of this investigation is to engage in more extensive pharmacokinetic study of this drug in dogs. It is interesting to note that some dogs follow dose-dependent disposition kinetics and some do not. The implication and the possible cause of dose-dependent disposition will be discussed. The absorption characteristics after oral administration of different dosage forms to dogs will be discussed in a future communication.

EXPERIMENTAL

Materials—Gris USP grade,[1] polyethylene glycol (PEG) 400 and 6000,[2] and bacterial beta-glucuronidase, Type II,[3] were used. 6-Demethylgriseofulvin (6-DMG) was isolated from the urine of dogs after administration of large doses of gris.

Formulation of Intravenous Dosage Forms—Pure PEG 400 or 35% PEG 6000 aqueous solution was used as a vehicle for the parenteral administrations of gris. The concentration of the drug ranged from 2–10 mg./ml. The parenteral 6-DMG dosage form was prepared in the aqueous alkaline solution with a strength of about 8 mg./ml. Fifty milligrams of 6-DMG was administered to dogs.

Animal Procedures—Male mongrel, unanesthetized, conditioned dogs weighing between 19–22 kg. were used throughout the study. They were fasted for 16–18 hr. prior to experiments. Food was withdrawn during study, while water was available *ad libitum*. Solutions of gris or 6-DMG were administered i.v. in 1 or 2 min. For intravenous studies of gris, 3–5 ml. of blood samples were usually taken at 2, 4, 6, 8, 10, 15, 25, 35, 50, 70, 90, and 120 min. after administration, while urine samples were usually collected at 20, 40, 60, 80, 100, 120, 150, 180, 210, 240, 300, 360, 420, and 480 min. from the indwelling urethral catheter. The dogs were then returned to metabolic cages. Total urine samples at 24 and 48 hr. were collected. At least 20 ml. of saline was used each time to flush the bladder after initial withdrawing of urine samples. Both the washing and the urine sample were mixed and the total volume recorded. Only a portion was retained in a plastic container for the assay. The plasma was collected after centrifugation of the heparinized blood specimen. Both plasma and urine samples were stored at 5° until assayed.

Assay of Plasma and Urine Samples—The plasma concentration

[1] McNeil Laboratories Inc., Fort Washington, Pa.
[2] Union Carbide Chemicals Co., New York, N. Y.
[3] Sigma Chemical Co., St. Louis, Mo.

Table I—Pharmacokinetic Data from Griseofulvin i.v. Injections in Dogs

Exptl.	Weight of Dogs, kg.	Dose, mg.	$t_{0.5}^{\alpha}$, min.	$t_{0.5}^{\beta}$, min.	A, mcg./ml.	B, mcg./ml.	V_p, ml.	$V_{dist.}$, ml.	Area[a], min. mcg./ml.
A-0	19	60	3.3	46	3.8	2.0	10,400	22,900	144
C-3	21	50	3.2	35	3.2	1.6	10,430	22,800	96
E-1	20	100	3.0	45	5.7	3.75	10,570	21,300	134
G-7	22	50	2.3	35	1.6	2.5	12,200	18,700	132
H-9	21	50	4.4	52	1.6	2.5	12,200	18,350	198
K-14	22	50	3.2	48	2.05	1.95	12,500	22,500	145
Av.			3.2	43.5	2.53	2.10	11,400	21,100	125± 16[b]

[a] Area: all corrected for 50 mg. griseofulvin. [b] Av. ±*SE*.

of gris was determined spectrophotofluorometrically by the method of Rowland, *et al.* (2). The urinary concentrations of 6-DMG and its glucuronide were assayed spectrophotometrically according to the method of Rowland and Riegelman (3).

RESULTS AND DISCUSSION

Gris Disposition Kinetics Following Single i.v. Dose—As reported in man (2), rabbits (4) and dogs (1), the plasma level decay curves of gris in six dogs studied after a single intravenous injection could also be described by a biexponential Equation $Ae^{-\alpha t} + Be^{-\beta t}$, which corresponds to a two-compartmental open model. The pharmacokinetic parameters based on a two-compartmental model (5, 6) are shown in Table I.

The fast disposition phase had a half-life of about 3 min., while the slow disposition phase had a half-life ranging from 35–52 min. It should be noted that the half-life of the slower phase found in man ranged from 9.3–21 hr. (2), while that of dogs (1), rabbits (4) and rats (7) was all very short and in the same range. The volume of distribution of gris, based on the same body weight, is also different. A dog weighing about 20 kg. has a value of about 21,000 ml., while a man weighing about 70 kg. has a value of about 100,000 ml. (2).

Although the weight and the volume of distribution of dogs studied were quite similar, the area under the plasma concentration time curve varied considerably. An almost twofold difference was found in Dogs C and H.

Dose-Independent Kinetics of Gris—In order to be able to utilize the plasma concentration time curve to evaluate the oral absorption characteristics of gris from different dosage forms, one of the essential requirements is that the area under the plasma concentration time curve must be proportional to the dose, *i.e.*, dose-independent kinetics must be met. To test this, different doses were injected intravenously into the same dog. The half-life and the area were then compared. In two dogs, 25 mg. of gris was first administered, and then 100 mg. was given when the gris concentration from the first dose became negligible. It was found that the half-life remained the same and the area was proportional to the dose as shown in Fig. 1. It should be noted that the dose-independent kinetics was also found in man (2).

Dose-Dependent Kinetics of Gris—Dose-dependent kinetics of gris was found in three dogs during the authors' study. The pharmacokinetic data of nine i.v. experiments in Dog B are summarized in Table II and the typical plasma curves are shown in Fig. 2. In this dog, 30- and 60-mg. doses showed different elimination half-lives. It was suspected that this might be due to the day-to-day variation. Therefore different doses were given in the same day, one in the morning and a higher dose in the afternoon. On one day, a 30-mg. dose (B-3) produced a half-life of 38 min. and a 60-mg. dose (B-4) gave 56 min. In another experiment, 32 min. were found for the 15-mg. dose (B-7) and 86 min. for the 120-mg. dose (B-8). It

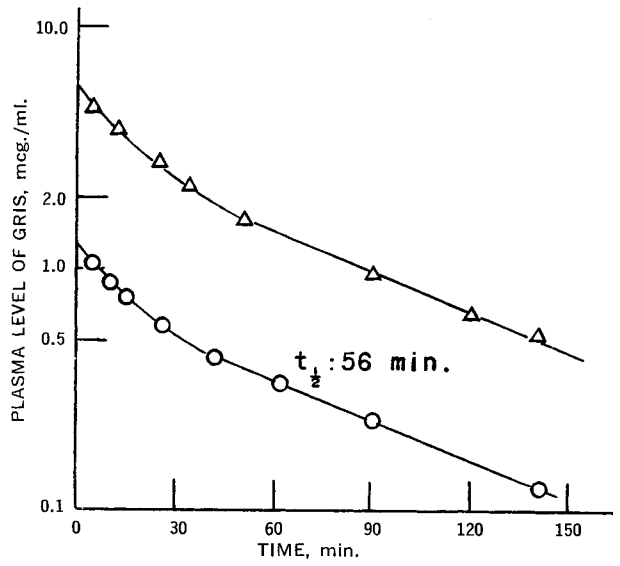

Figure 1—*Data showing dose-independent kinetics after i.v. administration of griseofulvin to Dog H. Key: ○, 25-mg. dose; △, 100-mg. dose.*

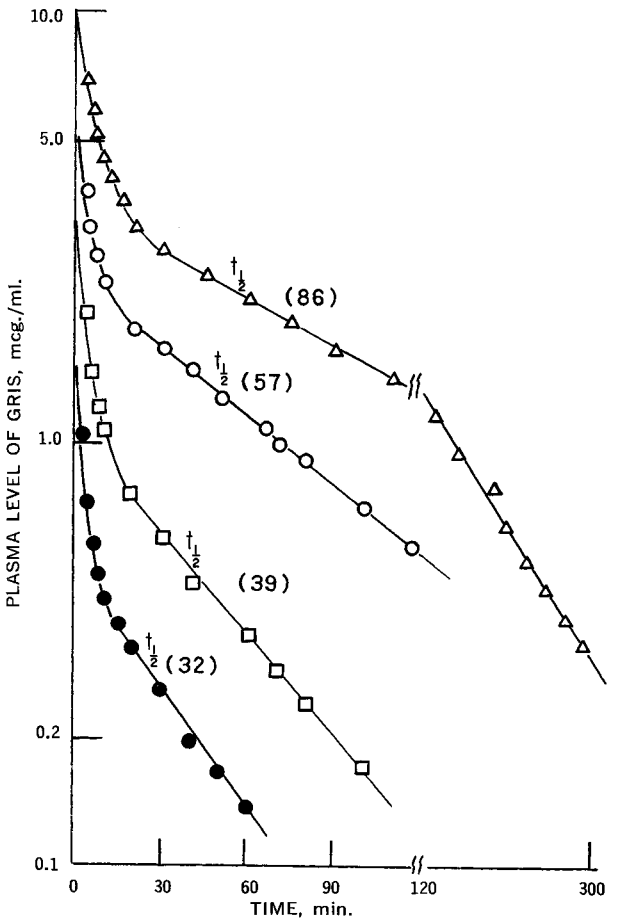

Figure 2—*Data showing dose-dependent kinetics after i.v. administration of griseofulvin to Dog B. Key: ●, 15-mg. dose (B-7); □, 30-mg. dose (B-5); ○, 60-mg. dose (B-6); △, 120-mg. dose (B-8).*

Table II—Pharmacokinetic Data for Dog B after Different i.v. Doses of Griseofulvin

Expt.	Date of Expt., 1967	Dose, mg.	A, mcg./ml.	B, mcg./ml.	C_p^0, mcg./ml.	$t_{0.5}^{\alpha}$ min.	$t_{0.5}^{\beta}$ min.	V_p, ml.	$V_{dist.}$, ml.	Clearance, ml./min.	Area, min. mcg./ml.
B-1	9–26	60	3.10	2.25	5.35	3.8	49	11,200	21,900	338	176.0
B-2	9–28	30	1.20	1.13	2.23	3.4	40	12,900	22,900	420	71.4
B-3	10–2	30	2.2	1.45	3.65	1.8	38	8,200	18,100	352	85.2
B-4	10–2	60	3.5	2.26	5.76	3.0	56	10,400	22,000	306	197.2
B-5	10–17	30	2.7	1.03	3.73	2.3	39	8,050	22,400	440	68.2
B-6	10–18	60	2.7	2.37	5.07	2.5	57	11,800	23,000	288	203.7
B-7	10–27	15	1.00	0.50	1.50	2.3	32	10,000	23,200	244	26.5
B-8	10–27	120	6.00	3.70	9.70	3.8	86	12,400	28,600	565	492.0
B-9[a]	11–10	30	2.05	0.93	2.98	3.5	59	10,150	25,800	355	89.1

[a] After 120-mg. dose.

should be noted in Fig. 2 that the curves representing the slower disposition phase are linear out to the limit of the assay of gris in plasma. The half-life obtained from 30- and 60-mg. doses was quite reproducible from isolated experiments. In the last experiment, a 30-mg. dose was given at 7 hr. after the intravenous injection of 120 mg. The half-life increased to 59 min. (B-9) which is much longer than previously observed. As noted in Table II the clearance decreased and the area under the plasma curve increased with the increase of dose. It is interesting to note that there was a linear relationship between the slow disposition half-life and the dose administered, with the exception of Experiment B-9. In these nine

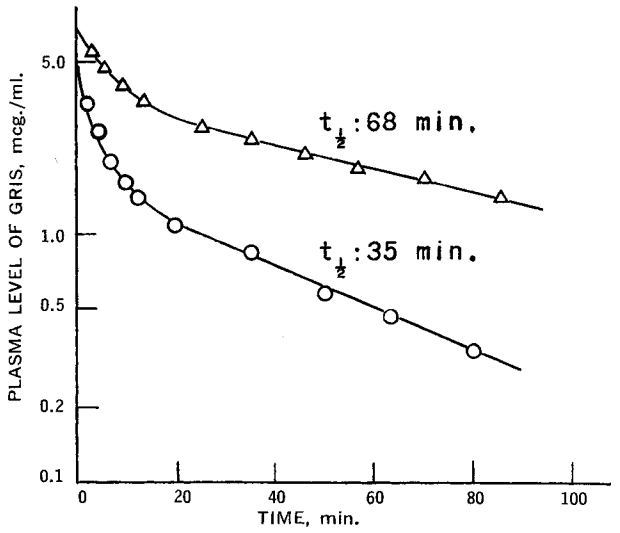

Figure 3—*Data showing dose-dependent kinetics after i.v. administration of griseofulvin to Dog C. Key: ○, 50-mg. dose; △, 100-mg. dose.*

experiments, the volume of the central compartment and the total volume of the distribution showed a 25–40% variation, respectively.

The pharmacokinetic data for Dogs C and K are summarized in Table III. In Dog C a dose of 50 mg. resulted in a half-life of 35 min. while one week later a dose of 100 mg. gave rise to a half-life of 68 min. This is shown in Fig. 3. In addition, the volume of the central compartment was also found to increase by 40%. Another interesting example of dose-dependent kinetics was found in Dog K. A single dose of 50 mg. produced a half-life of 49 min. (Fig. 4, K-14). Several weeks later, three intravenous injections, 25-, 100-, and 25-mg. doses, were given successively in the same day (Fig. 4, K-16, 17, 18). The initial 25-mg. dose resulted in the same half-life as that obtained from the previous 50-mg. dose. However, the half-lives for the later disposition phase from the next 100- and 25-mg. doses all increased twofold and the half-life for the fast disposition phase from 100-mg. dose all increased from 3.2–20 min. Furthermore, the volume of the central compartment also increased about 60%. Since the 25- and 50-mg. doses resulted in the same half-life and the proportional area under the plasma concentration time curve, it seems that there exists a threshold for the dog to become dose-dependent in the disposition of the drug. This phenomenon was also found for heparin in dogs (8).

The phenomenon of dose-dependent kinetics of gris found in dogs may have significant clinical importance. The gris is usually administered to dogs orally for the treatment of certain types of superficial fungal diseases. If a small dose is given at relatively short intervals, then the metabolic rate of gris will be quite fast and the resulting blood level will be lower. However, if a large dose is administered at longer intervals, the metabolic rate may decrease considerably and the blood level may be much higher and prolonged, provided the fraction of the drug absorbed is relatively independent of dose (which can be questioned). Another implication of dose-dependent kinetics is in the measuring of the absorption rate or total availability from the blood level data. Since the pharmacokinetic parameters are a function of dose, it is difficult to measure the absorption rate or availability from such data. However, it will be shown below that urinary data offer an alternative method of availability assessment.

Urinary Excretion of Metabolites After i.v. Injection of Gris—The urinary metabolites in dogs after intravenous administration of gris have been previously investigated in this laboratory (1). Intact gris was found excreted in a negligible amount. Only one metabolite of gris was found, 6-DMG, which was converted to a small extent to 6-DMG glucuronide. However, the excretion rates of metabolites were not studied previously.

The excretion rates of the metabolite, 6-DMG, after intravenous doses of 50 or 100 mg. of gris to four dogs are shown in Fig. 5. The excretion rate reached a maximum after the first 20-min. sampling interval. This is obviously due to the build-up of the body concentration of the metabolite. Beyond this peak, the excretion rate can be separated into fast and slow phases. The slow phase (half-life between 2–5 hr.) usually began after the gris level in the blood had decreased to a negligible value. Since the half-life for gris elimination is less than 1 hr., the slow elimination of 6-DMG is undoubtedly due to its rate of tissue distribution. This was confirmed by the intravenous administration of 6-DMG to the same dogs which will be discussed later. The excretion rate of 6-DMG was quite fast, generally, increasing with the increase of the metabolic rate of gris.

Table III—Pharmacokinetic Data of Griseofulvin in Two Dogs Showing Dose-Dependent Disposition Kinetics

Exptl.	Dose, mg.	$t_{0.5}^{\alpha}$, min.	$t_{0.5}^{\beta}$, min.	A, mcg./ml.	B, mcg./ml.	Area, min. mcg./ml.	Clearance K_{el} ml./min.$^{-1}$	K_{12}, min.$^{-1}$	K_{21}, min.$^{-1}$	V_p, ml.	V_t, ml.
C-3	50	3.2	35	3.20	1.60	96	0.0500	0.100	0.085	10,430	12,100
C-4	100	4.8	68	3.52	3.48	360	0.0192	0.0579	0.0771	14,300	10,700
K-14	50	3.2	48	2.05	1.95	145	0.0275	0.0905	0.1134	12,500	9,700
K-17	100	20.0	99	2.70	2.40	416	0.0123	0.0091	0.0203	19,700	8,500

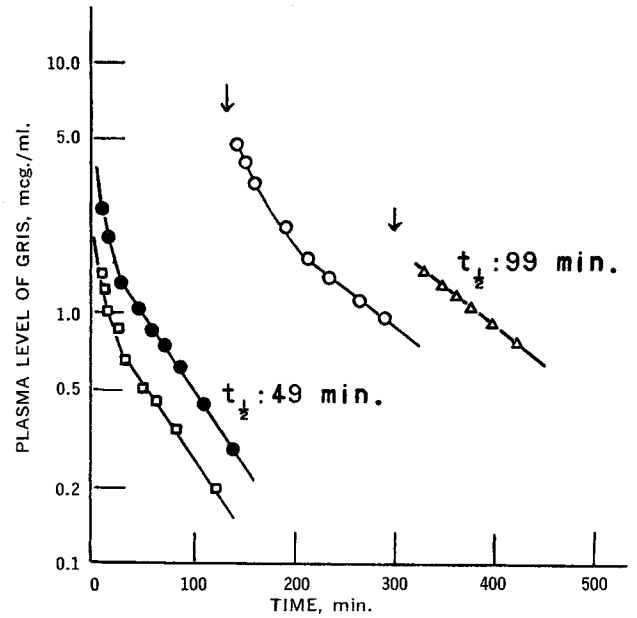

Figure 4—*Data showing dose-dependent kinetics after i.v. administration of griseofulvin to Dog K. Key: □, 25 mg. (K-16); ●, 50 mg. (K-14); ○, 100 mg. (K-17); △, 25 mg. (K-18). Arrows indicate the time of administration.*

It is interesting to note that the percentage of total recovery of 6-DMG in 48 hr. from eight studies in five dogs remained almost constant with an average of 56.4%, although their elimination half-lives differed significantly as shown in Table IV. It should be emphasized that even Dog C which was found to show dose-dependent kinetics of gris (see last section) also produced the same metabolite recovery from different doses (C-3, 4). It seems possible, therefore, that one can use the total recovery of 6-DMG to evaluate the total availability of gris after oral administration even if the dogs show dose-dependent kinetics.

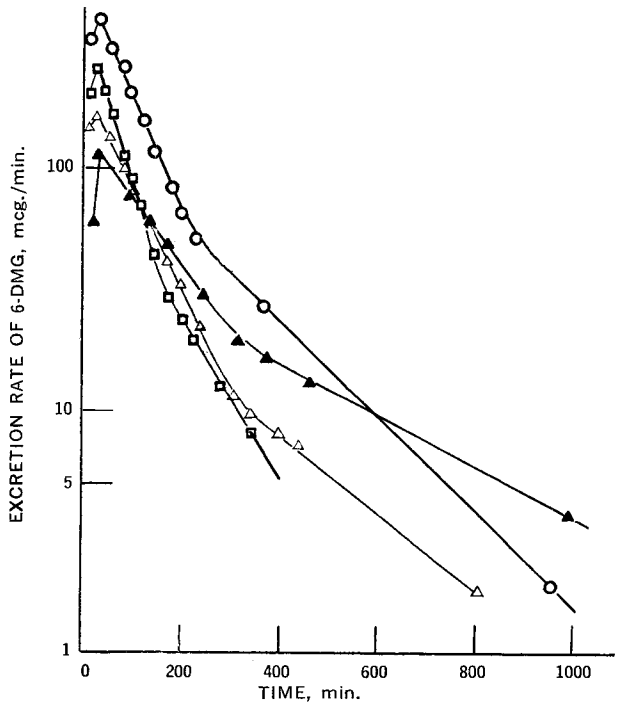

Figure 5—*Excretion rate of 6-demethylgriseofulvin after i.v. administration of griseofulvin to dogs. Key: □, C-3 (50-mg. dose); △, G-7 (50-mg. dose); ▲, E-1 (100-mg. dose); ○, H-9 (50-mg. dose). Data plotted at midpoint of collection period.*

The recovery of the total 6-DMG glucuronide was usually quite small, ranging from 3–6% of the dose from the author's study. They were measured by the difference in the optical density before and after the incubation of the sample with β-glucuronidase. Since the absorbance of the blank samples usually increased slightly after the incubation and the amount of the glucuronide formation was quite small, the error in the calculation of the glucuronide would be relatively big. Therefore, the rate of the excretion of the glucuronide was not studied intensively.

Urinary Excretion of 6-DMG After i.v. Injection of 6-DMG—The pattern of the distribution, metabolism, and excretion of 6-DMG after its intravenous injection was quite similar in the three dogs studied. The excretion rate of 6-DMG could all be represented by a triexponential equation

$$Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

except for the first 2.5 min. (Table V and Fig. 6).

If one assumes that the excretion rate of 6-DMG in the urine is proportional to the concentration of 6-DMG in the blood, then the decay curve of the excretion rate against time should reflect the blood level decay of 6-DMG. Therefore, the blood level decay of 6-DMG after its i.v. injection might be equally described by a triexponential equation with the same slope. This is equivalent to describing the distribution, metabolism, and excretion of 6-DMG by a three-compartmental open model. From the urinary data, the rate constants of elimination and the distribution between compartments and the relative size of the compartments might therefore be estimated (9). The use of urinary data to obtain pharmacokinetic parameters of drugs is particularly valuable when it is difficult to assay drugs in the blood. Since compounds like 6-DMG will be concentrated in the urine, it is easier to assay them in the urine than in the blood. Although the literature has not been scanned completely, this may be the first instance where urinary data of a drug or its metabolite was found to fit a triexponential equation.

The fast portion in the feathered curves has the half-life of 5.5–7 min., the medium portion 28–31 min., and the slowest part 197–285 min. The determination of the half-life in the fast portion was made possible by the frequent sampling of urine from the indwelling urethral catheter at 5-min. intervals for the first 30 min.

The total percentage of 6-DMG recovery in 48 hr. in three dogs from the intravenous administration of 6-DMG is shown in Table IV. The average recovery is 76.3%. Since the recovery is not complete and the excretion of the glucuronide is negligible, there must be another route of metabolism and/or excretion of the 6-DMG. The percent of recovery of 6-DMG after its i.v. injection is higher than that after gris injection, possibly indicating another pathway of elimination of gris besides the transformation of gris to 6-DMG. Although the biliary excretion of the glucuronides of 6-DMG and 4-DMG has been shown to take place in rats and rabbits (10), the biliary excretion of gris and 6-DMG in dogs was found negligible in this laboratory (1). Hence, there must exist other pathways for the elimination of gris or 6-DMG.

Possible Cause of Dose-Dependent Kinetics—Dose-dependent kinetics such as discussed above have also been reported on heparin (8), probenecid (1), phenylbutazone, biscoumacetate, diphenylhydantoin (12), and bishydroxycoumarin (13). Dayton *et al.* (11, 12) attributed such unusual phenomenon to the self-inhibition of the metabolism of a drug or the formation of a strong enzyme-drug complex. Wagner (14) has recently reviewed some of these aspects. He pointed out that a decrease in the distribution rate constant or an increase of the peripheral volume in the two-compartment open

Table IV—6-Demethylgriseofulvin Recovery after Griseofulvin i.v. Administration in Dogs

Experiment	Dose, mg.	Half-Life, min.	Recovery, %
C-3	50	35	58
C-4	100	68	57.7
E-1	100	43	55.4
G-7	50	35	51.0
G-16,17,18	150	44	57
H-7	50	—	58.6
H-9	50	52	54.2
K-14	50	49	59.2
Av. ± *SEM*			56.4 ± 2.7

Table V—Total Recovery and Excretion Rate Equations for 6-Demethylgriseofulvin after i.v. Injection of 50 mg. of 6-DMG

Experiment	Recovery, %	Excretion rate, mcg./min.[a]				
G-9	74.4	$900\ e^{-0.09t}$	+	$680\ e^{-0.0231t}$	+	$12\ e^{-0.00328t}$
H-11	74.2	$2150\ e^{-0.138t}$	+	$530\ e^{-0.0224t}$	+	$9.5\ e^{-0.00243t}$
K-3	80.2	$1400\ e^{-0.115t}$	+	$820\ e^{-0.0247t}$	+	$16\ e^{-0.00352t}$
Av.	76.3					

[a] See Fig. 6 for experimental data for dog H-11.

model system with increase in dose could result in a longer elimination half-life, even though the rate constant for the elimination was independent of dose. The data shown in Tables II and III clearly support the latter contention. It should be noted that the change of the volume in the central rather than the peripheral compartment was found in Dogs C and K. Further evidence to substantiate the argument that the enzyme activity may be unchanged in dogs showing dose-dependent kinetics was found in Dog C. The percent urinary recovery of 6-DMG remained constant, as is shown in Table IV, in spite of the change of the half-life and other pharmacokinetic parameters. One possible explanation of these observations is that the protein binding characteristics of gris may vary in these animals. This, in turn, would cause minimal change in the metabolism.

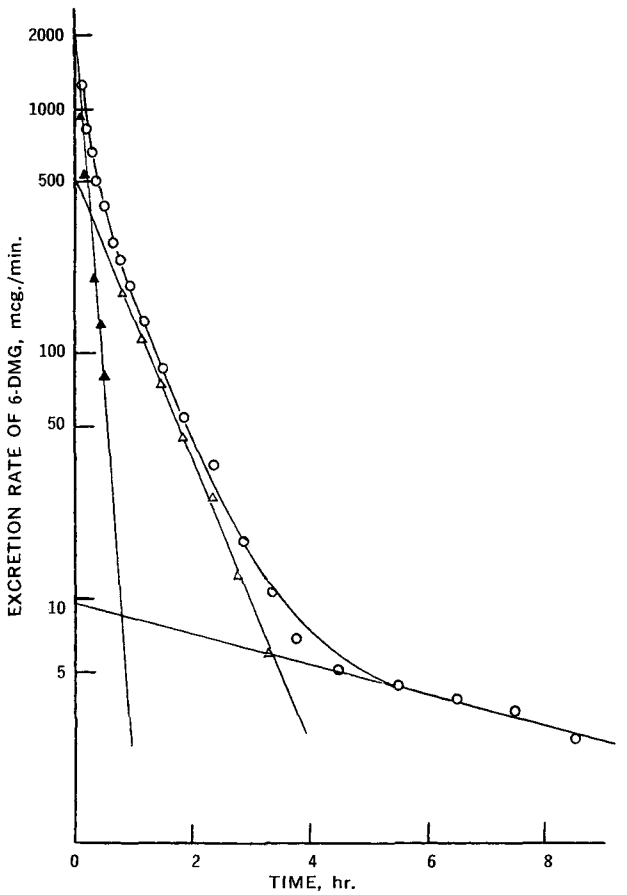

Figure 6—*The tri-excretion rate of 6-DMG after i.v. administration of 6-DMG (50 mg.) to a dog (H-11; the excretion rate at 2.5 min. is not included). Data plotted at midpoint of collection periods.*

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