Absorption and Distribution of Griseofulvin in Rabbits

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Griseofulvin was administered intravenously and orally to rabbits to evaluate the absorption and distribution characteristics of the drug from blood level data. Following an i.v. dose, the apparent half-life of the drug in the blood showed wide animal to animal variation and appeared to be independent of the dose administered. The area per i.v. dose under the concentration-time plot was not constant and therefore could not be used to evaluate the absorption of an oral dose of the drug. Blood levels after an oral dose were also subject to individual animal variation. Rabbits which were not permitted food following an oral dose of griseofulvin exhibited a characteristic blood level response consisting of a prolonged plateau in the plasma concentration-time curve comparable to that observed during constant intravenous infusion of a drug.

THE DISAPPEARANCE of griseofulvin from the blood, following intravenous administration to rabbits, occurs by a first-order process after an initial distribution phase has transpired. The work of Bedford *et al.* (1) suggests that the apparent half-life of the drug in the blood varies with the dose administered. This dependence of half-life on the amount of drug in the blood is unusual if the elimination were following a simple model containing first-order rate constants.

Many workers have shown that the oral administration of griseofulvin leads to irregular and incomplete absorption from the gastrointestinal tract of man and laboratory animals (1–4). This may be explained on the basis that the extremely low water solubility of the drug probably makes the dissolution step the rate-limiting step in the absorption process. Oral administration of griseofulvin having a high specific surface area will generally provide increased serum levels (4–7); however, in some individuals this may still fall short of adequate levels to produce a therapeutic response (2). Serum levels of griseofulvin are limited by the highly variable capacity of the individual to absorb griseofulvin, and increased doses of griseofulvin may not significantly raise circulating drug levels (2).

The purpose of this study was to investigate the absorption and elimination of griseofulvin from the blood of rabbits after administering the drug intravenously and orally under various conditions. It was initially hoped that the intravenous study would allow the utilization of the area under the blood curve from the known dose as a means of estimating the absorption characteristics of the drug when administered orally. However, this was not possible due to an unexplained variation of area even at constant dosage. The data obtained after oral administration lead to several interesting observations on the relations among the physical properties of the drug, the absorption state of the animal's gastrointestinal tract, and the amount and duration of absorption.

EXPERIMENTAL

Intravenous Administration and Sampling.—The marginal ear vein of New Zealand white rabbits weighing from 2.5 to 3.5 Kg. was catheterized for a distance of approximately 15 cm. with Intramedic PE 50 tubing. In this manner the catheter entered a larger vein and permitted adequate dilution of the injected fluid so that hemolysis was avoided. Heparin, 1 ml. (1000 u./ml.), was injected through the catheter to prevent clotting. A 2-ml. blank blood sample was taken prior to injection of the test drug. Because of its insolubility in water, griseofulvin (McNeil Laboratories, lot No. 0524)[1] was dissolved in redistilled *N,N*-dimethylacetamide (DMA) to make a solution containing 40 mg./ml. Immediately before infusion into the animal, this solution was diluted with an equal part of 50% DMA in water to make an infusion solution of 20 mg./ml. in 75% DMA. The drug was infused into the animal using a Harvard instrument model

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[1] The authors acknowledge the kindness of McNeil Laboratories for furnishing samples of pure griseofulvin.

600–900 infusion pump fitted with a 5-ml. glass syringe. The entire dose was infused into the animal at a constant rate over a 10-min. period unless specified otherwise. The animals were kept immobilized in a metal rabbit box and were not permitted food or water during the sampling period.

Blood samples could be taken from the marginal ear vein by withdrawing the catheter a few cm. until blood could be drawn freely. The first 0.5 ml. of each blood sample was discarded, and each 2.0-ml. sample was taken with a fresh disposable syringe. Samples taken from the catheter were transferred to a 15-ml. centrifuge tube containing 0.05 ml. of a 1000 u./ml. heparin solution. After the sample was taken, heparinized saline was injected to clear the catheter. Occasionally, due to clotting or veno-collapse, it was not possible to obtain all samples from the catheter. Samples were then taken by nicking the marginal ear vein with a scapel blade and allowing the blood to flow into a heparinized centrifuge tube. Plasma was obtained by centrifuging the fresh heparinized blood at 3500 r.p.m. and taking the straw-colored plasma for analysis. The success of the infusion procedure could be noted from the lack of hemolysis frequently found in injecting water miscible organic solvents.

Oral Administration and Sampling.—New Zealand white rabbits as previously described were used for oral administration of the drug. Griseofulvin was administered orally as a suspension of the microcrystalline powder (McNeil Laboratories, lot No. 0524, specific surface area 1.3–1.7 M.²/Gm.), 20 mg./ml. in water containing 0.05% polysorbate 80[2] as a wetting agent. The suspension was made by mixing griseofulvin powder with the appropriate amount of polysorbate 80 and gradually adding water while stirring to make a suspension containing 20 mg. drug/ml.

The rabbits were briefly anesthetized with halothane, and a soft rubber stomach tube was inserted. The drug in suspension was administered through the stomach tube and the tube rinsed with 25 ml. of water. After the tube was removed, the rabbits recovered fully from the anesthesia within 5 min. Samples were taken by nicking the marginal ear vein with a scalpel blade and allowing 2 to 3 ml. of blood to flow into a centrifuge tube containing 0.05 ml. of heparin (1000 u./ml.). Plasma was obtained as previously described. Between samples the rabbits were kept in their customary cages. Food (Purina commercial rabbit chow) and water were permitted unless the rabbits were fasted during the experiment, in which case only water was permitted.

Spectrophotofluorometric Assay of Griseofulvin.—The assay of Bedford *et al.* (8) was modified slightly in the determination of plasma griseofulvin concentration. A 0.5-ml. plasma sample was placed in a 15-ml. glass-stoppered tube, and 1.0 ml. of distilled water was added to the sample. Seven milliliters of freshly distilled ether was added to the tube, and the plasma was extracted by shaking on a mechanical shaker for 10 min. Upon separation, a 5.0-ml. aliquot of the ether layer was placed in a 10-ml. weighing bottle, and the solvent was evaporated to dryness on a steam bath. Five milliliters of distilled water was added to the residue, and the stoppered container was allowed to stand with occasional shaking for 0.5 hr. before reading.

The ether used in the assay was freshly distilled through a 35-cm. column of $^3/_{16}$ in. glass helices before use. Approximately 6 hr. after distilling, the ether contained enough fluorescent impurities to make it unfit for use. Special distilled water prepared in a Barnstead still used for the production of pyrogen-free water was used in all cases because this water was found to give a consistently low fluorescent reading, and could be stored in polyethylene containers without contamination. All glassware was washed by boiling in 10% nitric acid for 5 min., then rinsing successively with tap water, distilled water, and special distilled water.

Fluorescence was determined using the Aminco Bowman spectrophotofluorometer by activating at a wavelength of 315 mμ (uncorrected) and analyzing at 450 mμ (uncorrected). Slits were placed in the following order: $^1/_8$, $^1/_{16}$, $^1/_8$, $^1/_8$, $^1/_{16}$, $^1/_8$, and $^3/_{16}$, respectively.

Standard solutions of griseofulvin (0.05, 0.08, 0.10, and 0.20 mcg./ml.) in water were prepared by diluting a stock solution of 200 mcg./ml. dissolved in freshly distilled ethanol. Because the slope of the standard curve varies with temperature, the standard solutions were read immediately before reading a series of unknown solutions.

An extraction curve was prepared by adding known amounts of griseofulvin (0.5 to 5.0 mcg./ml.) to fresh rabbit serum and performing the above-mentioned analysis. Duplicate determinations were taken on each sample. The amount of griseofulvin extracted was found to be 91.6%, standard error 6.9, as a result of six assays.

RESULTS AND DISCUSSION

The data obtained following an intravenous dose of griseofulvin result in a curve which may be fitted

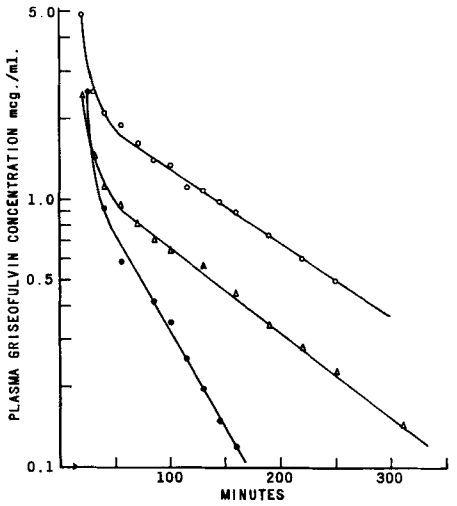

Fig. 1.—Semilogarithmic plot of blood data from three rabbits administered an intravenous dose of 10 mg./Kg. of griseofulvin. (Arrow on abscissa indicates infusion time.) Key: ○, rabbit *F*, apparent half-life 107 min.; △, rabbit *B*, apparent half-life 94 min.; ●, rabbit *E*, apparent half-life 42 min.

[2] Marketed as Tween 80 by Atlas Chemical Industries, Wilmington, Del.

TABLE I.—RESULTS OF INTRAVENOUS ADMINISTRATION OF GRISEOFULVIN TO RABBITS

i.v. Dose, mg./Kg.	Rabbit	Apparent Half-Life, min. (.693/b)	Apparent Half-Life, min. (.693/a)	Area under i.v. Blood Level Curve, mcg. min./ml.
5	*16*	74	...	204
	16A	67	7.0	226
	5	60	...	452
	25	50	...	398
	3	57	...	472
				350 ± 134[b]
10	*19A*	48	5.0	304
	19[a]	58	...	454
	17	112	...	388
	18[a]	51	...	292
	A	90	4.8	440
	B	94	5.5	179
	C	92	5.5	187
	D	102	9.0	340
	E	42	...	103
	F	107	5.3	378
				306 ± 81[b]
15	*4*	90	...	679
	16C	80	...	507
		77.1 ± 13.7[c]		Mean 593

[a] Forty-minute infusion time. [b] Mean ±95% confidence level. [c] Mean ±95% confidence level for first dose to each animal.

to the biexponential function $C_p = Ae^{-at} + Be^{-bt}$. An initial distribution phase occurred which had a duration of between 50 and 75 min. The nonlinear portion of the curves in Fig. 1 represents the distribution phase and is followed by a linear metabolic and excretion phase, from which the apparent rate constant, *b*, of the drug in the blood was calculated. When the linear portion of the curve was extrapolated to zero time, the value of the coefficient, *B*, was obtained. The difference between the observed values in the distribution phase and the extrapolated values resulted in a linear semilogarithmic plot from which the rate constant, *a*, and the coefficient, *A*, could be obtained.

Identical 10 mg./Kg. i.v. doses of griseofulvin given to three different animals produced the curves seen in Fig. 1. All the curves show good linearity in the metabolic phase, and the animal to animal variation of half-life is exhibited. Generally, it was found that the apparent half-life of griseofulvin in the blood following i.v. doses to different, previously untreated, animals was subject to wide variation. The range of half-lives from the 14 animals appearing in Table I is 42 to 112 min.

Figure 2 shows the half-life of the distribution period of animals *F* and *B* to be 5.3 and 5.5 min., respectively. The seven animals having an adequate number of points in the distribution phase exhibited distribution half-lives of 4.8 to 9.0 min.

To determine the effect of dose on the half-life of the drug, it was necessary to repeat the injection by administering different doses to the same animal. Technically, this was difficult to accomplish because the venocatheter could not be kept open for long periods of time. This was accomplished on one animal, however, and the results are represented in Fig. 3. Rabbit *16*, previously untreated, was given an i.v. dose of 5 mg./Kg. of griseofulvin in 75% DMA by infusing the drug over a 15-min. period. The half-life obtained from the first 5 mg./Kg. dose was 74 min., and a repeat dose of 5 mg./Kg. 3 days later exhibited a half-life of 67 min. Two weeks later, a dose of 15 mg./Kg. given to the same rabbit exhibited a half-life of 80 min. Thus, a threefold increase in i.v. dose to this particular rabbit did not significantly effect the half-life of the drug in the blood.

The dependence of half-life on dose suggested by Bedford *et al.* (1) was exhibited partially perhaps as a result of individual variation in the animals reported to be tested. Another factor producing the de-

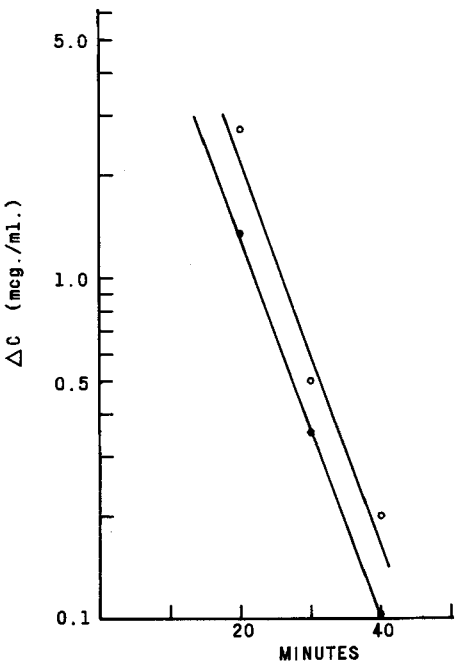

Fig. 2.—Semilogarithmic plot showing method of obtaining half-lives of the distribution phase of animals *F* and *B*. Key: O, rabbit *F*, distribution phase apparent half-life, 5.3 min.; ●, rabbit *B*, distribution phase apparent half-life, 5.5 min.

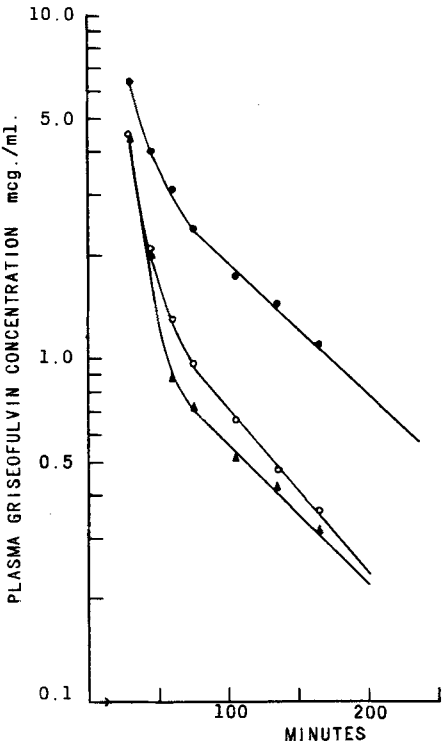

Fig. 3.—Semilogarithmic plot of blood data from an animal which was administered different doses of griseofulvin intravenously. Key: ▲, rabbit *16*, i.v. dose, 5 mg./Kg., apparent half-life, 74 min.; ○, rabbit *16A*, i.v. dose, 5 mg./Kg., apparent half-life, 67 min.; ●, rabbit *16C*, i.v. dose, 15 mg./Kg., apparent half-life, 80 min.

pendence of half-life on the dose administered would be the precipitation of some of the insoluble drug at the site of injection. By use of a venocather inserted through the marginal ear vein into a larger vessel and by using a longer infusion time, the possibility of precipitation of the drug in the blood has been significantly reduced. No hemolysis of early blood samples was observed, as had been reported by Bedford (1). The data in Table I suggest there may be some trend to longer half-lives upon increasing doses, but more animals would be needed for statistical evaluation.

Experimental factors, such as the total infusion time and the amount of DMA administered which could affect the distribution and metabolism of the drug following an i.v. dose were investigated and found to have no effect on the half-life. Rabbit *19* was given a dose of 10 mg./Kg. using a prolonged infusion time of 40 min. and exhibited a half-life of 58 min. A repeat dose using the normal 10-min. infusion time exhibited a somewhat shorter half-life of 48 min. Precipitation of the drug in the blood during the shorter infusion time should result in an increase of apparent half-life and not the slight decrease observed.

The area under the concentration-time curve also shows wide variation, as seen in Table I. This area is usually accepted as being directly proportional to the amount of drug in the body. The mean ±95% confidence intervals of the concentration-plots of animals administered 5 and 10 mg./Kg. i.v. doses were 350 ± 134 and 306 ± 81 (mcg. min./ml.), respectively. This clearly indicates that doubling the dose did not produce the expected results. The area under the i.v. curve of rabbit *16C* administered 15 mg./Kg. is approximately 2.5 times that obtained from the first 5 mg./Kg. There appeared to be at least two groups of animals present in the testing, and one group appeared to exhibit twice the area under the plasma level curve than the other. On examining the semilogarithmic plots, the most significant feature which separated these two groups was the plasma concentration at the end of the distribution phase. The animals having a large area under the plasma concentration-time curve had roughly twice the plasma drug concentration at the termination of the distribution phase.

Examination of the data reported by Bedford *et al.* (1) on the distribution of griseofulvin following single oral and intravenous doses to rats suggests that the injection solvent, 75% *N,N*-dimethylformamide (DMF), may have had some influence on the distribution of the drug. These workers reported persisting high concentrations of the drug in lung and fat tissue following intravenous doses in 75% DMF. The high levels in these particular tissues were not observed following oral doses of the drug. The method and site of injection are not defined in Bedford's report which precludes further analysis. Also, these results in rats may not be applied indiscriminately to other animals. It should be noted that in our work rabbit *16C* received three times more DMA than rabbit *16* without exhibiting a significant difference in half-life. If a change in distribution was occurring as a result of the injection solvent, it was not reflected in the apparent half-life of the drug in the blood of this animal.

As was noted in the beginning of this article, the blood curves can be fitted to a biexponential function. This is usually accepted (9) as meaning that the drug may be conceived as being distributed

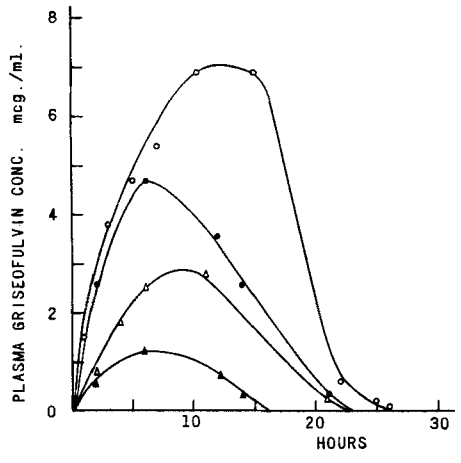

Fig. 4.—Blood level curves following oral doses of 150 mg./Kg. of microcrystalline griseofulvin to rabbits. (Rabbits *12* and *12A* are identical doses to the same animal.) Key: ○, rabbit *12A*; ●, rabbit *12*; △, rabbit *30G*; ▲, rabbit *14*.

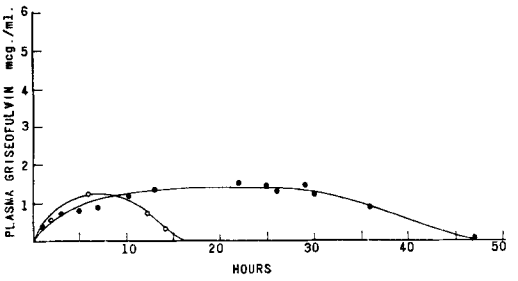

Fig. 5.—Blood level curves exhibiting the effect of food intake on the duration of absorption of griseofulvin following oral doses of 150 mg./Kg. to rabbit *14*. Key: O, normal food and water permitted during the experiment; ●, only water permitted after administration of the drug.

between a central compartment and one additional compartment. While superficially this appears to hold, it is clear that the volume of distribution is not constant even at the same dose level. Thus, the area method in this instance is not a valid method of assessing the per cent absorbed from an orally administered dose.

Oral doses of suspension of a microcrystalline griseofulvin (150 mg./Kg.) were given to rabbits in an effort to evaluate their absorption characteristics. The variability of absorption by different animals is seen on the plasma concentration-time plots in Fig. 4. All of these animals had had free access to food and water before and during the experiment. Rabbit *R12*, an unusually good absorber of the drug, exhibited plasma levels from identical doses of the drug seen on the top two curves. Data obtained from the two different animals, *30G* and *14*, are also shown in Fig. 4. They show maximum blood levels in 6 to 10 hr. and dropped to zero between 15 and 25 hr. Generally oral doses of microcrystalline griseofulvin produced peak plasma concentration between 1.0 and 3.0 mcg./ml.; however, some animals did not exhibit blood levels above 0.3 mcg./ml. at this dosage level.

A group of animals were fasted following an oral dose of griseofulvin in an effort to reduce the effect of food intake on absorption. Rabbit *14* was given a 150 mg./Kg. oral dose, and only water was permitted during the sampling period. The plasma level data (closed circles) are shown in Fig. 5, with the plateau level starting at approximately 10 hr. and extending for some 20 hr. This same animal was previously given a dose of 150 mg./Kg. of griseofulvin suspension orally, and food and water were permitted during the experiment. The plasma levels obtained from this dose are shown in Fig. 5 by the curve with open circles. Thus, griseofulvin was detectable in the plasma for an additional 30 hr. by simply withholding food from the animal. This sustained peak plasma level continuing for a long duration is similar to the blood picture produced by constant infusion of a drug into the blood (10).

Griseofulvin is a poorly water-soluble, relatively lipoid-soluble compound containing no ionizing groups. It is probable that griseofulvin is rate-limited in its absorption by the solution rate step, and that this process appears to be zero order due to the large amount of drug administered. Such a phenomenon would explain the zero-order plateau seen in the blood data obtained from animals which were not permitted food following an oral dose of the drug. It is unique, however, that the steady-state condition persisted in this group of animals for 20 hr. or longer. Several factors may contribute to this persisting blood level. The nonionic character of the drug eliminates the influence of pH changes in the gut on its absorption. Several workers have shown that food contents and fat in particular (2) influence absorption; however, the rabbit diet is particularly low in fat (approximately 2%). The ingestion of food will induce peristalsis and potentially move the griseofulvin through the gastrointestinal tract until the colon is reached, where absorption may be expected to be minimal. The duration of the blood level following oral administration of griseofulvin may be controlled almost entirely by the length of time the drug is present in the portion of the gastrointestinal tract where absorption is taking place. Further studies are being carried out to investigate this effect in detail.

SUMMARY

1. Wide individual variation in apparent half-life of the drug in the blood is exhibited following intravenous administration of griseofulvin to rabbits. It appears that this first-order rate constant for elimination of the drug from the blood during the metabolic and excretion phase is independent of the i.v. dose administered.

2. The areas under the concentration-time curves from intravenous injections of griseofulvin vary significantly from animal to animal and are not proportional to the dose administered.

3. The blood levels obtained from oral doses of griseofulvin vary considerably with the individual animal. Food intake appears to reduce the comparative time during which the drug is absorbed.

4. Animals fasted following oral administration of the drug appear to show a constant zero-order absorption process for as long as 20 hr.

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