Absorption Kinetics of Griseofulvin in Man

By M. ROWLAND, S. RIEGELMAN, and W. L. EPSTEIN

The distribution and elimination kinetics of griseofulvin have been examined in man. The drug was administered intravenously in polyethylene glycol 300 *via* a saline drip. The plasma-concentration time curve was satisfactorily described by a bi-exponential equation, the half-life for the first exponent was found to be 0.70–1.7 hr. and the second 9.5–21.0 hr. It was also shown that the area under the plasma-concentration time curve was proportional to the dose administered. Griseofulvin was then given orally and from the plasma concentration, the kinetics of the absorption of this drug were calculated. Absorption was found to occur up to 30 hr. after administration and 27–72.5 percent of the dose was absorbed.

SINCE ITS introduction in 1958 (1) griseofulvin still remains the only effective oral antifungal agent for the treatment of dermatophytic infections and much interest has been shown in the absorption of this drug. It was the evidence of Sharp and Tomich (2), that the oral toxicity in rats and mice was much lower than that following intraperitoneal administration, which first led to the suggestion that griseofulvin may be incompletely absorbed. This was confirmed by Davis *et al.* (3) who recovered 30–64% of an oral dose in the feces of rats. Numerous workers have since shown inefficient absorption of griseofulvin in man and have increased the degree of absorption by reduction of particle size (4–9), concomitant administration of fat (10, 11), and the preparation of a supersaturated solution of the drug in polyethylene glycol (PEG) 400 (12). However, only a change in the degree of absorption has been demonstrated and in no case has the actual amount of drug absorbed been assessed in man. The present paper reports a study of the kinetics of distribution, metabolism, and excretion following intravenous administration of griseofulvin in man. A standard dose of micronized griseofulvin was then given orally and both the percentage of drug absorbed and the kinetics of absorption were determined.

EXPERIMENTAL

Intravenous Study—A parenteral product of griseofulvin in PEG 300 (30 mg./ml.) was prepared as follows. Griseofulvin (recrystallized from benzene) was suspended in PEG 300 (Union Carbide), the suspension flushed with N_2, and autoclaved for 30 min. at 121°. Autoclaving resulted in solution of the griseofulvin in the PEG 300. The concentration of griseofulvin was determined by accurately weighing about 1 Gm. of the preparation and diluting to 100 ml. with spectroscopic grade ethanol. A 100-fold dilution of the ethanolic solution was prepared in distilled water (approximately 3 mcg. griseofulvin/ml.) and the absorbance measured at 292 mμ. The concentration of griseofulvin was calculated by reference to a standard curve of griseofulvin in 1% v/v ethanol in water (no absorbance at 292 mμ was observed with PEG 300 after autoclaving and assaying in the above manner).

Five healthy Caucasian male volunteers, 35 to 48 years of age, were used in the study. Each subject received griseofulvin intravenously, usually at two dose levels on occasions at least 1 week apart. The dose of griseofulvin 90–180 mg. (3–6 ml. PEG 300) was administered over a 3–6 min. period with a Harvard infusion pump (model 600-900) and diluted into a saline drip just before entering the vein. The drip was run in at 15 ml./min. so as to ensure that no precipitation of griseofulvin occurred in the vein. In one subject the drug was infused at a known constant rate for 2 hr., with the saline drip adjusted accordingly. The infusion pump was fitted with a 10-ml. glass syringe which had previously been calibrated so that the volume of PEG preparation administered was accurately known. Blood samples (5 ml.) were usually drawn at 0, 0.5, 1, 2, 3, 4, 5, 7.5, 10, 24, 28, 32, 36, and 48 hr. after drug administration. The sample was collected in a heparinized test tube, centrifuged, and the plasma stored at 4° until required for analysis.

Oral Study—The same volunteers as in the intravenous study were used. Each subject ingested one 0.5-Gm. micronized griseofulvin tablet (McNeil, Grifulvin, batch No. EL 9066) on an empty stomach with approximately 200 ml. water and no food was taken for at least 3 hr. Blood samples (5 ml.) were drawn at 0, 1, 2, 3, 4, 5, 6, 8, 11, 24, 28, 32, 36, and 48 and occasionally up to 72 hr. after drug administration. At least 1 month later the study was repeated in four subjects. The blood samples were collected and stored as in the intravenous study. All subjects had the same diet and were instructed to avoid drugs and food which might interfere with griseofulvin assay (especially salicylates).

Spectrophotofluorometric Assay of Griseofulvin—The plasma concentration of griseofulvin was determined by the method of Fischer and Riegelman (13) with the following modification. Plasma (0.5–1.0 ml.) was pipeted into a centrifuge tube containing 1.5 ml. distilled water, and extracted with 7 ml. distilled diethyl ether. A 5-ml. aliquot of the ether

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extract was pipeted into a weighing bottle, evaporated to dryness, and redissolved in 5 ml. of triple distilled water. After reading the fluorescence of the sample, activation wavelength 315 mμ (uncorrected) and fluorescent wavelength 450 mμ (uncorrected), 0.2 ml. of 6 *M* sulfuric acid was added to 2 ml. of the sample and the fluorescence again noted. The change in fluorescence before and after the addition of acid was used to calculate the concentration of griseofulvin. A standard curve was obtained by reading the fluorescence of known aqueous solutions of griseofulvin (0.01, 0.02, 0.04, 0.1, 0.2 mcg./ml.) before and after the addition of acid.

In the intravenous studies, it was found necessary to be able to determine levels as low as 0.1 mcg. griseofulvin/ml. plasma. With the previous assay, this was not possible as blank values equivalent to 0.2–0.3 mcg. griseofulvin/ml. plasma were frequently obtained. However, griseofulvin does not fluoresce in strongly acidic aqueous media (pH less than 1.0), whereas very little change occurred with blank plasma samples. This modification in the assay procedure enabled the blank reading to be reduced to less than 0.03 mcg./ml.

Calculation of the Absorption of Griseofulvin—The absorption of drug at various times was calculated by the method of Loo and Riegelman (14).

$$\text{Amount absorbed } A_t = VpCp + k_{13}Vp\int_0^t Cpdt + VpC_T \quad \text{(Eq. 1)}$$

$$\text{and percent of absorbed drug } \left(\frac{A_t}{A_\infty} \times 100\right) = \frac{\left[C_p + k_{13}\int_0^t Cpdt + C_T\right]}{k_{13}\int_0^\infty Cpdt} \times 100 \quad \text{(Eq. 2)}$$

where A_t is the cumulative amount of drug absorbed at time t, and Cp, C_T are the corresponding plasma concentration and tissue concentration (calculated and expressed relative to the plasma) at time t, while $\int_0^t Cpdt$ and $\int_0^\infty Cpdt$ are the areas under the concentration-time curve up to time t and infinity, respectively; k_{13} is the rate constant for metabolism and excretion and Vp is the volume of the central compartment. The equation is essentially one of mass balance in that the amount of drug absorbed at any time is the summation of that in the central compartment ($VpCp$), that eliminated ($k_{13}Vp\int_0^t Cpdt$) and that in the tissue compartment (VpC_T). As with the Wagner-Nelson method (15) the value of A_t/Vp is calculated until an asymptote (A_∞/Vp) is reached indicating that absorption has ceased. The total amount of drug absorbed was calculated by the following equation:

$$\text{percent administered dose absorbed} = \frac{\text{dose}_{\text{i.v.}}}{\text{dose}_{\text{oral}}} \times \frac{\left(\int_0^\infty Cpdt\right)_{\text{oral}}}{\left(\int_0^\infty Cpdt\right)_{\text{i.v.}}} \times 100 \quad \text{(Eq. 3)}$$

where $\left(\int_0^\infty Cpdt\right)_{\text{oral}}$ and $\left(\int_0^\infty Cpdt\right)_{\text{i.v.}}$ are the total areas under the plasma concentration-time curve which result from the oral and intravenous dose, respectively.

RESULTS AND DISCUSSION

Intravenous Study—Before the absorption kinetics of any drug can be adequately described, the pharmacokinetics following an intravenous dose of that drug must first be defined. In the case of griseofulvin, this presents a problem in that it is a neutral insoluble molecule (aqueous solubility 10 mcg./ml.) and one would require 10–20 L. of saline in order to give a 100–200 mg. dose. This difficulty may be overcome by administering griseofulvin in PEG 300 whereby a concentration of 30 mg./ml. is achieved. Although several of the low molecular weight polyethylene glycols were found to be suitable solvents for griseofulvin, PEG 300 was chosen owing to its low toxicity in man and its acceptance in several parenteral pharmaceutical products. As much as 80 ml. of this solvent has been given intravenously, over a 4-month period, without any reported ill effects (16). However, there is the problem of precipitation of griseofulvin in the vein if the PEG preparation were injected directly, and for this reason the product was given *via* a rapidly flowing intravenous saline drip.

In all the intravenous studies, it was found that the griseofulvin plasma level curve could be described by a bi-exponential equation (*e.g.*, Fig. 1). The initial, or distribution, phase had a half-life of approximately 1 hr. while the second or elimination phase had a half-life varying from 9.5 to 21.0 hr. (Table I). This bi-exponential curve can be interpreted in terms of a two-compartmental open system (17), as depicted in Fig. 2. From the bi-exponential equation, the distribution rate constants k_{12}, k_{21}, and the metabolism and excretion rate constant, k_{13}, were evaluated together with the sizes of the central (Vp) and peripheral (V_T) compartments (Table II). Since the urinary excretion of griseofulvin is negligi-

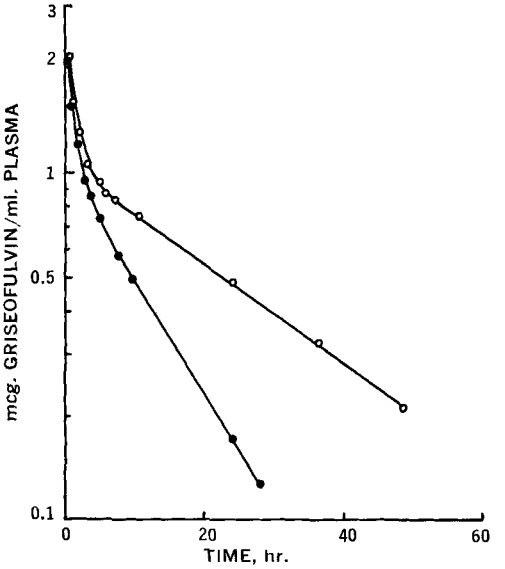

Fig. 1—Griseofulvin plasma levels following intravenous administration in man. Key: ○ subject A, 122 mg.; ●, subject C, 142 mg.

TABLE I—DATA OBTAINED FOLLOWING A SINGLE INTRAVENOUS INJECTION OF GRISEOFULVIN TO MAN

Subject[a]	Dose, mg.	Plasma Concn. Time Curve $Cp = Ae^{-\alpha t} + Be^{-\beta t}$	$0.693/\alpha$, hr.	$0.693/\beta$, hr.	Area, mcg. hr. ml.$^{-1}$	Area/ Dose
A	58	$0.75e^{-0.87t} + 0.56e^{-0.041t}$	0.80	17.0	14.7	0.254
	112	$1.60e^{-0.93t} + 1.06e^{-0.034t}$	0.75	21.0	33.7	0.276
B	142	$1.20e^{-0.60t} + 1.1e^{-0.045t}$	1.15	15.5	26.8	0.190
C	128	$1.35e^{-0.63t} + 0.93e^{-0.075t}$	1.10	9.3	14.5	0.113
	142	$1.55e^{-0.74t} + 1.0e^{-0.073t}$	0.95	9.5	15.8	0.111
D	99	$1.04e^{-0.70t} + 0.87e^{-0.039t}$	1.00	17.5	23.8	0.240
E	90	$0.90e^{-0.41t} + 0.62e^{-0.063t}$	1.70	11.0	12.1	0.134
	180	$1.7e^{-0.44t} + 1.1e^{-0.060t}$	1.55	11.5	22.1	0.123

[a] Subject weights: 73, 96, 73, 78, and 71 Kg., respectively.

TABLE II—VALUES OF CONSTANTS USED TO DEFINE THE PLASMA GRISEOFULVIN LEVELS FOLLOWING A SINGLE INTRAVENOUS INJECTION[a]

Subject	Dose, mg.	k_{12}, hr.$^{-1}$	k_{21}, hr.$^{-1}$	k_{13}, hr.$^{-1}$	Vp, ml.[b]	Vd_{ss}, ml.[c]
A	58	0.43	0.40	0.089	45,000	93,000
	112	0.49	0.39	0.080	46,000	102,000
B	142	0.25	0.31	0.085	62,000	112,000
C	128	0.27	0.30	0.154	56,000	107,000
	142	0.31	0.33	0.162	55,000	108,000
D	99	0.32	0.33	0.080	52,000	102,000
E	90	0.14	0.20	0.130	60,000	103,000
	180	0.17	0.21	0.130	64,000	116,000

[a] Calculations based on a 2-compartmental open system. [b] Vp = dose/A + B (see Table I). [c] $Vd_{ss} = Vp + V_T = Vp + k_{12}/k_{21}\ Vp$ (*Reference 21*).

ble, being less than 0.5% of the administered dose (18–20; personal observation), it may be ignored for the purpose of this model. Consequently the rate constant k_{13}, is almost exclusively associated with metabolism and it was found to have a twofold (0.08–0.16 hr.$^{-1}$) variation in this study (Table II).

In practice, there is probably at least a three-compartmental model, which would then give rise to a tri-exponential blood concentration-time curve. The first compartment into which griseofulvin distributes, depicted by a dotted circle in Fig. 2, can be considered to be the blood, liver, kidney, and other vascular rich tissues. This compartment is not seen, however, because the drug is infused over 3–6 min. so that griseofulvin can distribute into other tissues, such as muscle, skin and vascular rich fat tissue; consequently, the value of about 50,000 ml. for the central compartment (Vp) is much larger than one would have anticipated. However, as will be shown, absorption of griseofulvin is relatively slow compared to this initially expected, although not observed, distribution. Therefore, only a small error will occur in the calculation of the absorption of this drug by conceiving the body to be a two- instead of three-compartmental model. In contrast, since the observed apparent distribution phase is approximately 5 hr. following the i.v. dose (Fig. 1) any absorption rate calculations for griseofulvin must take this distribution phase into account, and therefore the model cannot be reduced further without introducing large errors.

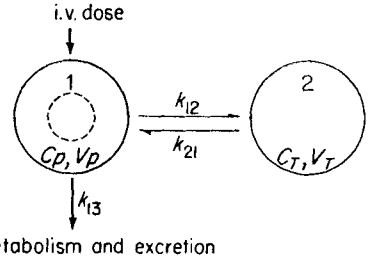

Fig. 2—Two-compartmental open model describing the distribution and elimination of griseofulvin in man. Key: 1, central compartment; 2, peripheral tissue compartment.

Regarding the second, or peripheral compartment, this can be considered to be the vascular poor fat tissue and is of significance owing to the high lipid solubility of griseofulvin. The size of this compartment is the same as that of the central compartment, so that the volume of distribution Vd_{ss} ($= Vp + V_T$; *Reference* 21) is approximately 100 L. indicating a marked extravascular concentration of this drug. It should be emphasized here, however, that the above model does not necessarily have any physiological meaning, but rather is the minimum model required to fit the data.

Another prior requirement to any quantitative absorption measurements from plasma level data is that the area under the plasma-concentration time curve must be proportional to the dose administered. This relationship was shown to hold with griseofulvin following intravenous doses of 90–180 mg. (Table I). In addition, the values of the rate constant k_{12}, k_{21}, and k_{13}, are essentially unchanged between studies showing that these parameters do not vary with time (Table II). These findings are contrary to that noted in rabbits when no relationship between an i.v. dose and the corresponding area could be established (13). The latter result may be explained by the very large interanimal

variation which these workers observed and the difficulty in using any rabbit repeatedly. In one study where they did manage to use an animal on three occasions, proportionality between dose and area appeared reasonably linear.

Oral Study—In the reported intravenous studies it was shown that, under the condition of administration, the data may be fitted by a two-compartmental open system model. Prior to proceeding to analyze data obtained after administering the dose orally, it was deemed essential to prove that the method of analyzing the resultant blood data will result in a true assessment of the absorption rate of griseofulvin. Therefore, griseofulvin was infused at a known zero-order rate (12.8 mg./min.) for 2 hr. From the values of the constants, k_{13}, k_{12}, and k_{21}, previously determined after a single rapid intravenous injection, the amount entering the body with time was calculated according to the method of Loo and Riegelman (14). The calculated absorption rate data are shown in Fig. 3 while in the insert are the plasma levels resulting from the single intravenous and zero-order infusion to that subject. The excellent fit between the known, 12.8 mg./min., and estimated, 12.4 mg./min. rate of infusion (absorption) of the drug shows that both the proposed model and the method of calculation for the absorption of this drug are acceptable.

Typical griseofulvin plasma levels following the oral dose of 0.5 Gm. micronized drug are shown in Figs. 4 and 5, together with their corresponding intravenous data. Without the intravenous study a long biological half-life might be calculated for this drug whereas in this study the levels are shown to be primarily the result of prolonged griseofulvin absorption. In fact, the dosage form exhibits the properties of a prolonged release product in that a plasma level is rapidly achieved and then maintained for 10–20 hr. Subsequent analysis of the area under the concentration time curves, following the oral and intravenous dose, shows that only 27–72% of the drug was absorbed (Table III). This not only demonstrates the variable and incomplete absorption of this drug but also illustrates one of the difficulties in trying to assess factors which might increase the extent of absorption. Obviously, the area under the plasma concentration-time curve for the person absorbing 72% drug can only increase by a further 39% whereas a 270% increase can occur with the person absorbing only 27%. Hence, the suggestion that the former was a poor responder to such factors could be misleading if, at the same time, the actual amount of drug absorbed was not determined. A further complication is that the absorption from

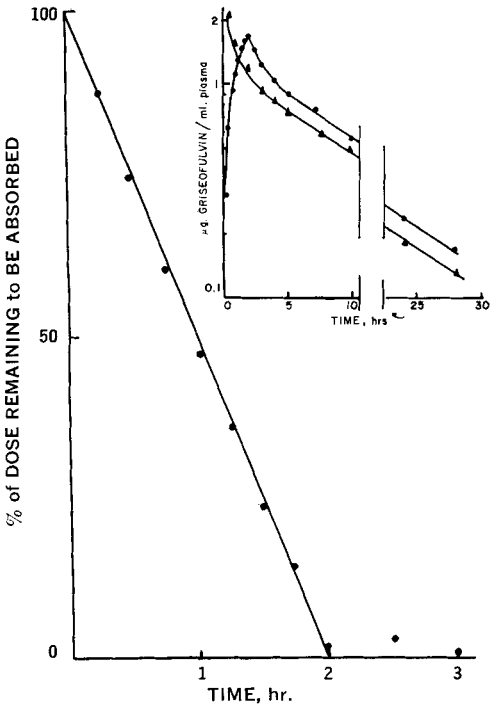

Fig. 3—Plot of the amount of griseofulvin remaining to be infused (absorbed) following a 2-hr. zero-order infusion (12.8 mg./min.) of drug in Subject C. Data calculated from plasma levels shown in insert. Key: ●, zero-order infusion; ▲, single intravenous injection (142 mg.).

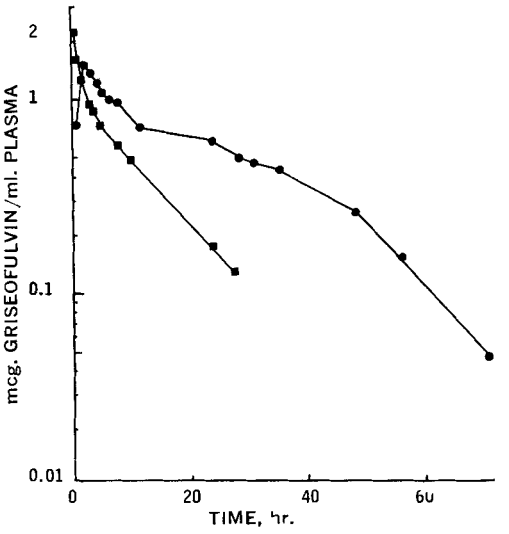

Fig. 4—Griseofulvin plasma levels following an oral dose of 0.5 Gm. micronized drug (●) and an intravenous injection, 142 mg. (■). Subject C.

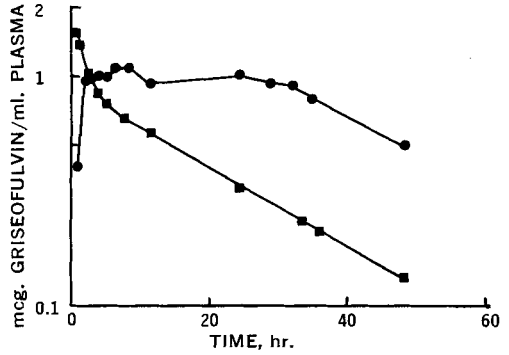

Fig. 5—Griseofulvin plasma levels following an oral dose of 0.5 Gm. micronized drug (●) and an intravenous injection, 99 mg. (■). Subject D.

TABLE III—AMOUNT OF GRISEOFULVIN ABSORBED AND THE CORRESPONDING AREA UNDER THE CONCENTRATION-TIME CURVE[a]

Subject	Date	Area, $\int_0^\infty Cpdt$	% Administered Dose Absorbed
A	12 May	52.7	38.5
B	12 May	58.5	62.4
	25 July	37.5	39.7
C	12 May	34.4	62.0
	25 July	32.4	58.0
D	12 May	58.4	48.5
	25 July	32.4	27.0
E	28 June	33.4	52.3
	25 July	46.0	72.5

[a] Following an oral dose of 0.5 Gm. micronized griseofulvin.

repeated doses of the same batch of griseofulvin is not reproducible resulting in large differences in some subjects (Table III).

Several workers have used the rationale that the area under the concentration time curve is a measure of "available griseofulvin" (4–6). This is a satisfactory response parameter only if a suitable crossover experiment is designed. However, Duncan *et al.* (6) noted a threefold variation in this parameter within a group of subjects on a standardized preparation of griseofulvin and inferred that this was due to absorption differences within this group. Obviously caution must be taken before relating area to absorption alone. In Table III both Subjects C and D gave an area value of 32.4 mcg. hr./ml. Whereas this indicates 58% drug absorption with the former, it only corresponds to 27% in the latter subject. The reason for this is clear from the following relationship:

$$\text{Area}\left(\int_0^\infty Cpdt\right) = \frac{\text{dose absorbed}}{k_{13}Vp} \quad \text{(Eq. 4)}$$

Therefore, a low area can be attributable to either poor absorption, fast metabolism (high k_{13}), or a high Vp value. With Subjects C and D, the volume Vp was essentially the same and the difference in amount absorbed for the same area is primarily due to the metabolic rate constant (k_{13}) being twice as large in Subject C as in Subject D (Table II). Consequently, within a given group on a single dose study differences in absorption can only be ascertained by reference to the intravenous dose.

Additional information was obtained by calculating the amount of griseofulvin absorbed at various times following drug ingestion (Fig. 6). From such data, it is seen that of the total drug absorbed approximately 50% occurs in the first 3–10 hr. Thereafter, absorption continues but much more slowly for a further 20–30 hr. suggesting that absorption probably takes place along the majority of the gastrointestinal tract.

Analysis for the absorption rate from the cumulative absorption plots demonstrates even more strikingly the rapid rate of absorption throughout the first 2–3 hr. following ingestion of griseofulvin (Fig. 7). After this period the absorption rate drops sharply even though only 7–28% of the drug has been absorbed. One possible explanation is that dissolution of the drug from griseofulvin particles may rate limit the absorption process in the upper part of the gut,

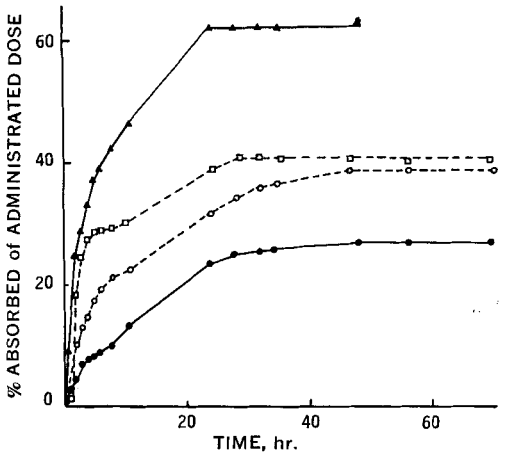

Fig. 6—Cumulative amount of griseofulvin absorbed following oral administration of 0.5 Gm. micronized griseofulvin. Key: ○, Subject A; □, Subject B; ▲, Subject C; ●, Subject D.

while further along intestinal absorption becomes the rate-limiting step. The finding by Davis *et al.* (3), that plasma griseofulvin levels fell while substantial amounts of griseofulvin was still present in the alimentary tract of rats, favors this hypothesis. Bedford *et al.* (18), suggested a simlar rationale for the absorption of this drug in cats. Alternately, the present results may have a physical rather than a biological explanation. Thus, it might be assumed that a certain fraction of the dose occurred as microfine readily dispersed particles which rapidly dissolved and was easily absorbed while a larger fraction was less readily available due to a much smaller surface for dissolution. Further studies are under way to distinguish between these two proposed explanations.

The present results confirm those of previous workers in showing that griseofulvin absorption is incomplete in man and was found to be 27–72% of the administered dose in this study. Absorption of

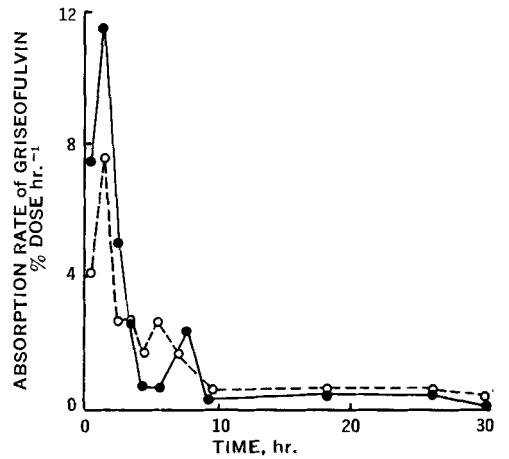

Fig. 7—Rate of absorption of griseofulvin after oral administration of 0.5 Gm. micronized drug to man. Key: ●, Subject C; ○, Subject D.

this drug was slow and whether it is due to physical or physiological factors has yet to be ascertained. Polyethylene glycol 300 has proved to be a useful solvent for griseofulvin and this and other low molecular weight polyethylene glycols might enable the pharmacokinetics of other insoluble neutral drugs to be evaluated in man.

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Keyphrases

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Distribution, excretion—griseofulvin
Plasma—griseofulvin analysis
Fluorometry—analysis

Analgesic and Anti-Inflammatory Evaluation of Thymotic Acid and Certain Homologs

By FRED J. MAROZZI and MARVIN H. MALONE

Ortho-thymotic acid and nine homologs were submitted for general, analgesic, and antistress evaluations in the rat. While all appeared to be nonspecific central nervous system depressants, only 2-methyl-5-*tert*-butylsalicylic acid appeared to have significant analgesic ability in the rat tail flick test. 2-Hydroxy-4-methyl-5-isopropylbenzoic acid, 2-hydroxy-4-isopropyl-6-methylbenzoic acid, and 2-hydroxy-3-methyl-6-isopropylbenzoic aicd were superior to sodium salicylate and inferior to morphine sulfate in providing significant protection from stress induced by unilateral hind leg tourniquets. The latter two compounds were active in suppressing the acute inflammatory process produced by pedal injection of carrageenin, but were ineffective against chronic inflammation induced by the *Mycobacterium butyricum* adjuvant.

O'BRIEN AND Thoms (1) investigated the antipyretic and analgesic activity of the sodium salts of *o*- and *p*-thymotic acid in 1958. Von Kaulla (2) demonstrated in 1965 that *o*-thymotic acid possessed significant fibrinolytic activity. More recently, Rader and Wulf (3) investigated the fibrinolytic activity of nine homologs of *o*-thymotic acid on the human and cat fibrinolytic systems and were able to show that several compounds possessed significant fibrinolytic activity beyond that of *o*-thymotic acid. The present investigation is concerned with the analgesic and general evaluation of certain of these homologs along with *o*-thymotic acid, itself.

EXPERIMENTAL

The compounds shown in Table I were obtained in limited amounts from the Division of Medicinal Chemistry of the University of Connecticut. This group was selected so as to reveal possible structure–activity relationships. Table I also summarizes the fibrinolytic activity of the various homologs as determined by Rader and Wulf (3, 4).

Qualitative Screening—The hippocratic method of Malone and Robichaud (5) was used as a prelim-

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