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# Absorption and Activity of Some Derivatives of Griseofulvin

By L. J. FISCHER* and S. RIEGELMAN

**Some derivatives of the 4′-keto group of griseofulvin were prepared which had increased water solubility and greater absorption potential. The derivatives, which exhibited little or no *in vitro* activity against *Microsporum gypseum*, were found to be converted to griseofulvin *in vivo*. After intravenous administration to rabbits, several of the derivatives were rapidly converted to griseofulvin. Griseofulvin plasma levels resulting from oral doses of the derivatives were found to be equal to, or in most instances significantly higher than, the levels produced by equal doses of griseofulvin, but of shorter duration. Evidence for the degradation of the derivatives in the gastrointestinal tract is presented.**

PREVIOUS REPORTS have shown griseofulvin to be irregularly and incompletely absorbed from the gastrointestinal tract of man (1–3) and laboratory animals (4, 5). The incomplete absorption, which often produces low blood and tissue levels, appears to be a result of the slow rate of dissolution of the drug in the gastrointestinal fluids due to its extremely low solubility in water. Fischer and Riegelman (6) have shown that the solution-rate-limited absorption of griseofulvin in rabbits was by a pseudo zero-order process and that the drug was eliminated rather rapidly ($t^1/_2$ = 77 ± 14 min.) from the blood following intravenous injection.

Numerous attempts have been made to increase blood levels resulting from an oral dose of the drug. By increasing the specific surface area of orally administered griseofulvin threefold, many workers have reported experimental animal and human peak blood levels to be approximately twice those obtained with the same dose of regular particle size griseofulvin (7–14). However, Crounse (13) has observed that a double dose of microcrystalline griseofulvin did not significantly raise serum levels in some individuals and that these levels may still fall short of the proper inhibitory levels for the antibiotic. The administration of surfactants with an oral dose of griseofulvin generally has produced erratic results in animals (9, 12) and has not significantly increased blood levels in man (14). The oral administration of griseofulvin with lipids or after meals with high fat content has been shown to increase blood levels (9, 13, 15); however, the mechanism by which absorption is facilitated by lipids has not been clarified.

Although many attempts have been made to increase griseofulvin absorption by various methods, no previously published report has investigated the preparation and administration of derivatives of griseofulvin having increased water solubility and greater absorption potential. The derivatives were chosen so that active griseofulvin would be a possible product of their enzymatic metabolism in the body. The more water-soluble derivatives were expected to be absorbed more completely from the gastrointestinal tract, and if they were converted rapidly to griseofulvin in the body, increased blood levels of the antibiotic could be expected. In this manner the concept of drug latentiation was employed to increase plasma levels of griseofulvin following the oral administration of some derivatives of the 4′-carbonyl group of griseofulvin.

The derivatives of griseofulvin (I) selected to be prepared and tested were griseofulvin-4′-alcohol (II), griseofulvin-4′-oxime (III), griseofulvin-4′-carboxymethoxime (IV), and griseofulvin-4′-hemisuccinate (V). Griseofulvin-4′-

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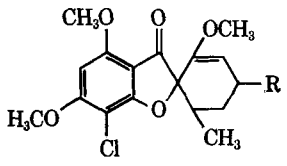
alcohol was selected for testing in this system because of a previous report suggesting *in vivo* activity in infected guinea pigs (16). Such secondary alcohols are converted to ketones or are conjugated in their normal routes of metabolism in the body (17). The hemisuccinate ester of griseofulvin-4′-alcohol, which will be called griseofulvin-4′-hemisuccinate for consistency, was prepared because its sodium salt could be administered in solution. The ester was expected to hydrolyze rapidly in the body to griseofulvin-4′-alcohol. Oximes also have been shown to be possibly converted to their corresponding ketones by enzymatic metabolism in the body (18). The carboxymethoxime derivative was chosen for testing because the molecule could be solubilized as the sodium salt and its metabolism could be expected to follow that of oximes.



R

I, =O

II, —OH

III, =N—OH

IV, =N—O—$CH_2$—COOH

V, —O—C(=O)—$CH_2$—$CH_2$—COOH

## EXPERIMENTAL

**Synthesis**—The method of preparation of the griseofulvin-4′-alcohol (II) was that of Kyburz *et al.* (16). The method of synthesis of the griseofulvin-4′-oxime (III) was that of Oxford (19). For the details of the synthesis of compounds IV and V and the evaluation of the purity of each compound, the reader is referred to the thesis (20).

**Determination of Water Solubility**—An excess of pure crystalline drug was placed in a 40-ml. glass-stoppered centrifuge tube and a 25-ml. quantity of distilled water was added. The tube was stoppered, sealed with paraffin, and heated for a brief period at 45–55° prior to being placed in a water bath at 37 ± 1° for not less than 24 hr. Agitation was accomplished by mechanically rotating the tubes end over end at 30 r.p.m.

Samples were taken with a pipet plugged with glass wool and quickly diluted to the appropriate volume with distilled water. Griseofulvin and griseofulvin-4′-alcohol were determined spectrophotofluorometrically with excitation at 315 mμ and analyzing at 450 mμ. The griseofulvin-4′-alcohol samples were also assayed using a spectrophotometric method at 293 mμ. The oxime and carboxymethoxime derivatives were determined spectrophotometrically at 294 mμ.

**Intravenous Administration and Sampling**—The derivatives which were administered intravenously were given as a solution in 75% *N,N*-dimethylacetamide (DMA) containing 20 mg. of drug/ml. The administration and sampling techniques were the same as described by Fischer and Riegelman (6) for the i.v. administration of griseofulvin to rabbits.

**Oral Administration and Sampling**—New Zealand white rabbits were used for oral administration of the drugs. A control dose of microcrystalline griseofulvin[1] was prepared as a suspension in 0.05% polysorbate 80[2] and administered using the technique previously described by these authors (6). The low concentration of polysorbate 80 was used to facilitate wetting of the powders and was not expected to influence absorption of the drug. In testing for *in vivo* activity, a dose of griseofulvin was administered to each animal, usually as the first dose of the experiment, to obtain a control level of the drug in plasma. Griseofulvin-4′-alcohol and -oxime were also administered as suspensions made by triturating the crystalline drug with the appropriate amount of polysorbate 80 and gradually adding water while mixing to make a suspension containing 20 mg. of drug/ml. No attempt was made to control the particle size of the derivatives in suspension. Griseofulvin-4′-hemisuccinate and -carboxymethoxime were administered as their sodium salts dissolved in distilled water. The interval between doses to the same animal was at least 1 week after the griseofulvin blood level from the previous dose had returned to zero.

The oral doses were administered through a stomach tube to rabbits briefly anesthetized with halothane as previously described (6). Samples were taken by making a small cut in the marginal ear vein with a scalpel blade and allowing the blood to flow into a heparinized centrifuge tube. Plasma was separated from the blood samples by centrifuging at 3500 r.p.m. The rabbits were permitted normal food (Purina commercial rabbit chow) and water *ad libitum* during the experiment unless specified otherwise.

**Microbiological Assay for Griseofulvin Activity**—Activities of the griseofulvin derivatives on *Mycrosporum gypseum* were compared to the activity of griseofulvin on the same organism using the cylinder plate assay reported by Knoll, Bowman, and Kirshbaum (21). Activities were determined in phosphate buffer at pH 8.0 and in fresh rabbit plasma. The activity of griseofulvin in the presence of some of the derivatives was also tested. All activities reported were an average of three or more separate determinations.

After a dose of griseofulvin or a griseofulvin derivative, the plasma griseofulvin activity reported was a result of six to nine determinations on a particular plasma sample as described by Knoll (21) in the assay procedure.

**Thin-Layer Fluorimetric Analysis**—Griseofulvin and griseofulvin-4′-alcohol were determined quantitatively in plasma using the TLC fluorimetric assay reported by Fischer and Riegelman (22).

Griseofulvin could be separated from griseofulvin-4′-oxime in a plasma ether extract by using

[1] Griseofulvin (lot No. 0524, specific surface area 13.–1.7 M.²/Gm.) was obtained from McNeil Laboratories, Fort Washington, Pa.

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

a solvent system of 55% chloroform, 25% ether, and 20% acetone. Griseofulvin ($R_f$ approximately 0.5), separated from the oxime derivative ($R_f$ approximately 0.3) in the aforementioned solvent system, could be determined quantitatively using the TLC fluorimetric assay. It was found that standard griseofulvin readings were the same regardless of whether the ether–acetone (3:2) or the above-mentioned solvent system was used. Griseofulvin-4′-carboxymethoxime could also be separated from its parent compound by employing the 55% chloroform, 25% ether, and 20% acetone system. In this system the carboxymethoxime derivative would not move from the original spot while griseofulvin had an approximate $R_f$ of 0.5.

Thin-layer chromatograms of plasma ether extracts were scanned with the adapted Photovolt TLC Densitometer to provide recording of the fluorescent spots over the entire length of the chromatogram. The scans were started on the original spot (labeled "S" on the TLC fluorimetric recordings) and were terminated when no more spots were observed or the fluorescent residue from plasma was detected near the end of the chromatogram. The heights of the observed peaks were proportional to the amount of fluorescent material on the spot when the previously described (22) application and development techniques were employed.

It should be noted that griseofulvin and griseofulvin-4′-alcohol concentrations determined by the TLC fluorimetric assay will be reported as mcg./ml. in plasma. Griseofulvin concentrations determined by the microbiological assay will be referred to as mcg./ml. of griseofulvin *plasma activity*. The activity, in this case, means the inhibition of growth of the test organism compared to griseofulvin, and not pharmacological activity as such.

## RESULTS AND DISCUSSION

The griseofulvin-4′-derivatives which are shown in Table I exhibited varying degrees of increased water solubility. Reduction of the 4′-carbonyl group of griseofulvin to hydroxyl increased the solubility of the molecule approximately eighteen-fold. The solubility of the 4′-alcohol derivative was determined by fluorescence and ultraviolet methods and good agreement occurred between the different assays. A ninefold increase in solubility occurred for the 4′-oxime derivative, whereas the 4′-carboxymethoxime exhibited a somewhat smaller increase. The additional molecular weight of the 4′-carboxymethoxime molecule seems to account for a solubility lower than that of the oxime derivative. Because of its instability in aqueous solution, the solubility of griseofulvin-4′-hemisuccinate was not determined.

Griseofulvin-4′-oxime was the only derivative exhibiting *in vitro* antifungal activity, as shown in Table II. The activity of this derivative appeared to be somewhat characteristic upon observation of the zones of inhibition on the test plates. The zones consistently had a characteristic yellow color on their periphery which was not observed on the inhibition zones of griseofulvin. On closer examination, the oxime derivative appeared to inhibit production of aerial mycelium, and possibly sporulation, beyond the zone of total inhibition. Generally, the oxime appeared to be $^1/_7$th as active as griseofulvin on *M. gypseum* when tested in buffer and in serum. The combinations of griseofulvin and oxime tested produced slightly higher activity than griseofulvin alone; however, the oxime and griseofulvin activities were not proportionally additive. The griseofulvin alcohol and carboxymethoxime derivatives were found not to be active at the 50 mcg./ml. level. The hemisuccinate was not active at 10 mcg./ml. in buffer and was not tested further because its logical hydrolysis product, griseofulvin-4′-alcohol, was found not to be active.

The fact that little or no *in vitro* activity was exhibited by the derivatives was not surprising in view of the previous work on the structure–activity relationships of griseofulvin analogs. Crosse *et al.* (23) have studied the activity of more than 300 analogs of griseofulvin against dermatophytic and plant pathogenic fungi. Generally, it was found that substitutions on the griseofulvin molecule altered the spectrum and diminished the activity of the antibiotic. However, some substitutions at the 2′- and 3′-positions by groups which impart lipid solubility to the molecule increased and then decreased activity as a particular series was ascended. Many of the derivatives showed increased *in vivo* activity on plant and dermatophytic fungi (23–25). From the results of previous testing of griseofulvin analogs, it appears that increased *in vitro* activity

TABLE I—SOLUBILITY OF GRISEOFULVIN AND DERIVATIVES IN WATER AT 37°

| Compd. | Method | Solubility, mcg./ml. |
|---|---|---|
| Griseofulvin (I) | Fluorescent | 14.3 |
| Griseofulvin-4′-alcohol (II) | Fluorescent | 250.0 |
| Griseofulvin-4′-alcohol | Ultraviolet | 256.0 |
| Griseofulvin-4′-oxime (III) | Ultraviolet | 132.0 |
| Griseofulvin-4′-carboxymethoxime (IV) | Ultraviolet | 75.3 |

TABLE II—*In Vitro* ANTIFUNGAL ACTIVITY OF GRISEOFULVIN DERIVATIVES, TEST ORGANISM: *M. gypseum*

| Amt./ml. Buffer | Griseofulvin Activity, mcg./ml. |
|---|---|
| Oxime, 10 mcg. | Inhibitory, but no definite zone |
| Oxime, 25 mcg. | 3.6 |
| Oxime, 50 mcg. | 6.5 |
| Oxime, 10 mcg., and griseofulvin, 5 mcg. | 6.5 |
| Oxime, 25 mcg., and griseofulvin, 5 mcg. | 6.5 |
| Alcohol, 50 mcg. | 0 |
| Carboxymethoxime, 50 mcg. | 0 |
| Hemisuccinate, 10 mcg. | 0 |
| **Amt./ml. Rabbit Serum** | |
| Oxime, 25 mcg. | 3.4 |
| Oxime, 50 mcg. | >5.0 |
| Oxime, 25 mcg., and griseofulvin, 2.0 mcg. | 2.7 |
| Alcohol, 25 mcg., and griseofulvin, 2.0 mcg. | 3.1 |

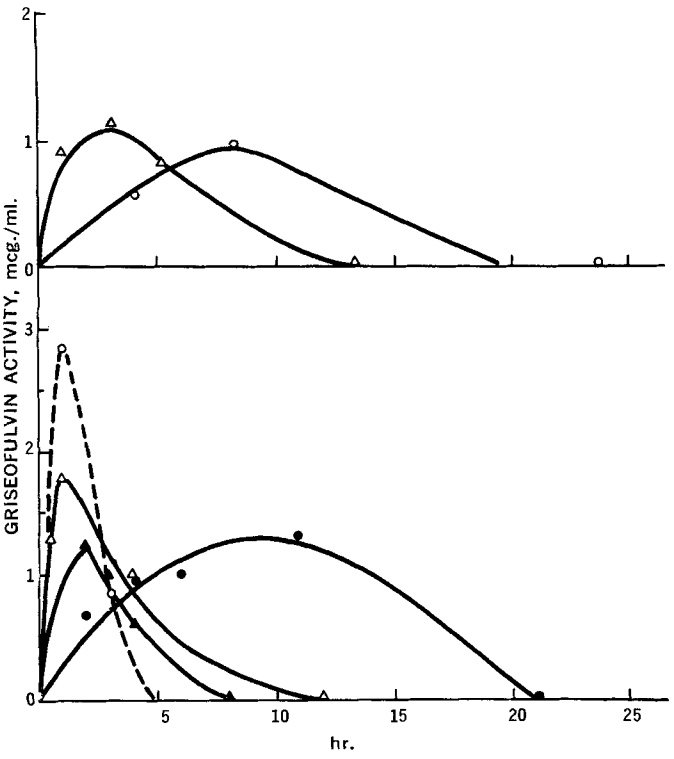



*Fig. 1—Plasma griseofulvin activity-time curves resulting from oral administration of griseofulvin and derivatives. Top, rabbit 31. Key: ○, griseofulvin, 150 mg./Kg.; △, griseofulvin oxime, 150 mg./Kg. Bottom, rabbit 30. Key: ○, griseofulvin hemisuccinate, 150 mg./Kg.; △, griseofulvin alcohol, 200 mg./Kg.; ▲, griseofulvin alcohol, 150 mg./Kg.; ●, griseofulvin, 150 mg./Kg.*

is accomplished by making particular substitutions on the molecule which increase lipid solubility; however, the more active compounds, due to their decreased water solubility, cannot be translocated efficiently *in vivo* to their site of action against the invading fungi. In such a situation the preparation of derivatives which have greater absorption potential and which will be converted to griseofulvin metabolically is a logical recourse to increase blood levels of the antibiotic.

The griseofulvin derivatives, which were prepared and tested as described, were administered to rabbits in order to evaluate their *in vivo* activity. The compounds, which showed little or no *in vitro* activity, were initially tested by assaying the plasma for antifungal activity following oral doses to test animals. As many derivatives as possible were administered to a particular animal to reduce the influence of individual animal variation on the results of the *in vivo* testing. An oral dose of griseofulvin (150 mg./Kg.) administered to rabbit 30 resulted in the typical plasma griseofulvin activity level (microbiological assay) shown in Fig. 1. A dose of griseofulvin-4′-alcohol (150 mg./Kg.) to the same animal reached a peak griseofulvin activity in 2 hr., which was approximately 8 hr. sooner than the corresponding dose of griseofulvin had reached its peak. The duration of activity, however, was much shorter when the alcohol derivative was administered. An increased dose of griseofulvin-4′-alcohol (200 mg./Kg.) produced a higher peak plasma activity of 1.8 mcg./ml. and again fell rapidly to zero at 12 hr. Griseofulvin-4′-hemisuccinate given in solution produced a rapid, higher peak plasma activity; however, the duration of activity was only 5 hr. Since these derivatives did not show griseofulvin activity *in vitro*, it appeared that conversion to griseofulvin had taken place in the body.

An oral dose of griseofulvin-4′-oxime (150 mg./Kg.) produced a more rapid but similar peak plasma griseofulvin activity compared to that produced by the same dose of griseofulvin, as represented by rabbit 31 in Fig. 1. Using the microbiological assay, it was not possible to determine definitely whether the plasma activity after a dose of the oxime derivative, which was slightly active *in vitro*, was due entirely to conversion or could be attributed in part to high blood levels of the derivative. The characteristic yellow color produced by the oxime derivative was observed on the periphery of zones of inhibition during the assay of plasma samples taken after an oral dose of oxime. Therefore, the activity appeared to be manifested in part by the oxime derivative. An oral dose of griseofulvin-4′-carboxymethoxime (200 mg./Kg.) did not exhibit plasma antifungal activity in any rabbit to which it was administered.

Following a dose of griseofulvin-4′-alcohol, the total fluorescence of the plasma was determined using the spectrophotofluorometric assay for griseofulvin (6). The fluorescence spectrum of the alcohol derivative is identical to the spectrum of griseofulvin, and the intensities were found to be additive in plasma. In three different animals given an oral dose of griseofulvin-4′-alcohol the peak plasma fluorescence time was between 4 and 8 hr., and at these times the plasma exhibited little or no antifungal activity as determined by the microbiological assay. Rabbit 30 shown in Fig. 1 (closed triangles) had peak fluorescence in the 8-hr. plasma sample which showed no griseofulvin plasma

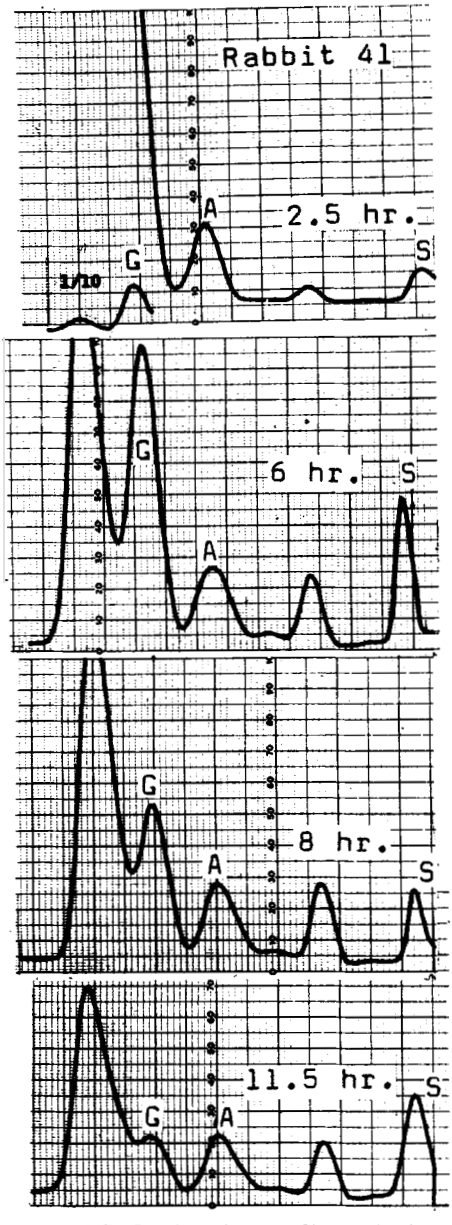



*Fig. 2—TLC fluorimetric recordings of chromatograms containing fluorescent products extracted from 1 ml. of plasma following an oral dose of 200 mg./Kg. of griseofulvin-4′-alcohol.*

activity. If conversion of the alcohol derivative to griseofulvin were taking place after absorption of the alcohol, and if only these two compounds were present in the blood, then peak plasma fluorescence should correspond to peak plasma griseofulvin activity.

In order to investigate the short duration of plasma griseofulvin activity, thin-layer chromatography was employed to separate the fluorescent constituents in the plasma following oral administration of griseofulvin-4′-alcohol. The TLC fluorimetric scans in Fig. 2 present the events which took place in the blood following an oral dose of 200 mg./Kg. of the alcohol derivative to rabbit 41. Each peak on the recording represents a fluorescent spot on the chromatogram. Fluorescent products in addition to griseofulvin (G) and griseofulvin-4′-alcohol (A) begin to make their appearance in the 2.5-hr. plasma sample. The three new fluorescent spots, one moving in front of griseofulvin and two trailing the alcohol derivative, are quite prevalent in the remaining plasma samples while griseofulvin plasma concentration falls. The appearance of other fluorescent substances in the plasma, besides the alcohol derivative and its parent compound, then, is accompanied shortly thereafter with falling griseofulvin levels. In rabbit 40, which showed high, somewhat prolonged griseofulvin levels, the appearance of the additional fluorescent substances was delayed, and they were initially observed beyond the 5-hr. sample. The discrepancy between peak plasma fluorescence and peak plasma griseofulvin activity, therefore, is due to the appearance of additional fluorescent products in the blood some time after an oral dose of griseofulvin-4′-alcohol.

Plasma samples from rabbit 39, given a 20 mg./Kg. intravenous dose of griseofulvin-4′-alcohol infused over a 15-min. period, were assayed for griseofulvin and its alcohol derivative using the TLC fluorimetric method (22). The first sample, taken 15 min. after completion of the infusion, exhibited the highest concentrations (Fig. 3). Thereafter, the plasma concentration of each substance decreased in a linear fashion when plotted against time on semilogarithmic paper. Griseofulvin, resulting from conversion of the alcohol derivative, had an apparent biological half-life of 70 min. in this animal. Griseofulvin-4′-alcohol in the plasma expectedly decreased more rapidly and had an apparent half-life of 28 min. A TLC fluorimetric scan of the extracted plasma samples showed that after an i.v. dose of the alcohol derivative only griseofulvin alcohol and griseofulvin were present in amounts detectable by the fluorescent technique. The fact that no additional fluorescent

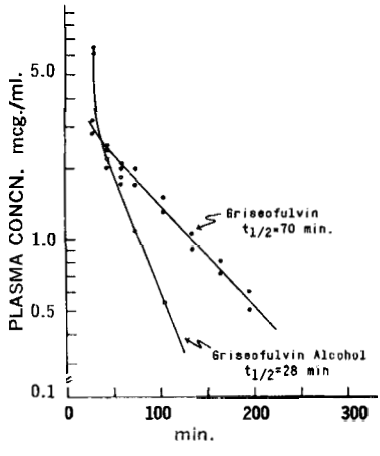



*Fig. 3—Semilogarithmic plot of griseofulvin and griseofulvin-4′-alcohol plasma concentrations following an i.v. dose of griseofulvin-4′-alcohol, 20 mg./Kg., to rabbit 39.*

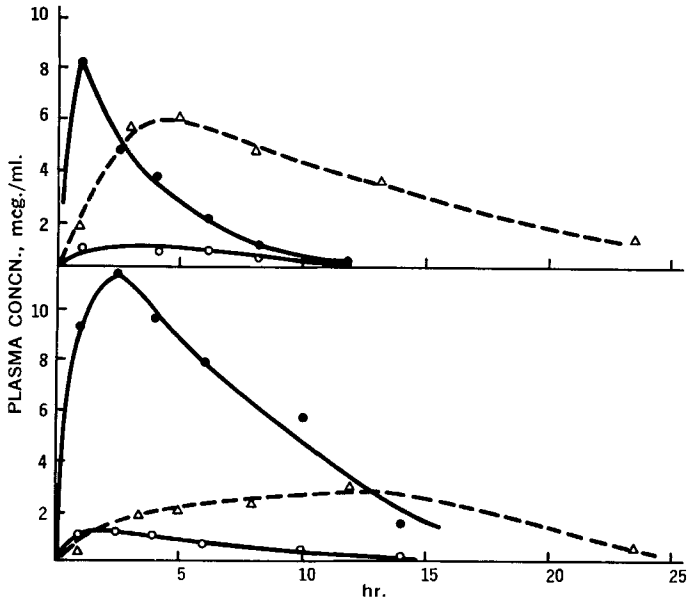



*Fig. 4—Plasma concentration-time curves of griseofulvin and griseofulvin-4′-alcohol (TLC fluorimetric assay) following an oral dose of the alcohol derivative. A plasma level curve (broken line) from a control dose of griseofulvin is also shown. Oral dose—200 mg./Kg. griseofulvin alcohol. Key: ●, griseofulvin; ○, griseofulvin alcohol. Oral dose—200 mg./Kg. griseofulvin. Key: △, griseofulvin. Top, rabbit 41; bottom, rabbit 40.*

products were found in the blood following i.v. administration of the alcohol derivative suggested that their appearance following an oral dose might be attributed to the degradation of the 4′-alcohol in the gastrointestinal tract.

An oral dose of griseofulvin-4′-alcohol (200 mg./Kg.) given to rabbit 40 (Fig. 4) produced high griseofulvin plasma levels and corresponding low griseofulvin-4′-alcohol levels as determined by the TLC fluorimetric assay. Griseofulvin reached an abnormally high plasma concentration of 11.5 mcg./ml. and had a duration of 20 hr. One week later the same animal was given an oral dose of griseofulvin (200 mg./Kg.) and exhibited a normal blood level picture as seen in the broken line curve on Fig. 4. This animal appeared to have an unusually high capacity for converting the alcohol derivative to griseofulvin. Another animal (No. 41) given a 200 mg./Kg. oral dose of the alcohol derivative exhibited the more typical blood level seen in Fig. 4. Plasma griseofulvin rose to a peak of 8.0 mcg./ml. in 1 hr. and decreased rapidly. The griseofulvin-4′-alcohol level again was consistently low. The control dose of griseofulvin produced rather high levels of griseofulvin (broken curve) showing this animal to be a good absorber of the drug.

It is particularly interesting to note that the level of griseofulvin-4′-alcohol, as represented by the data shown by rabbits 40 and 41 in Fig. 4, remains at a low, essentially constant level in the blood and at the same interval the griseofulvin level reaches a peak and rapidly falls. The alcohol derivative was shown to be approximately 18 times more soluble in water than griseofulvin and one can assume, therefore, that it should be more rapidly absorbed. The data obtained from the i.v. injection of the alcohol indicated that it has a very rapid elimination rate constant. The persistent griseofulvin-4′-alcohol blood level from an oral dose, then, must be due to a solution-rate-limited process where the low consistent blood level results from a steady state between the absorption and the rapid elimination processes. One then is left with the problem of how to explain why the griseofulvin blood levels (with a slower elimination rate) do not continue to persist at a high level as long as the griseofulvin-4′-alcohol remains in the blood and can be converted to griseofulvin.

Figure 5 shows plots of the heights of the fluorescence peaks found by a TLC fluorimetric scan, against time for one of the rabbits (No. 40) receiving an oral dose of griseofulvin-4′-alcohol. The curves give a semiquantitative measure of the appearance and elimination of all extracted and resolved fluorescent substances in the plasma follow-

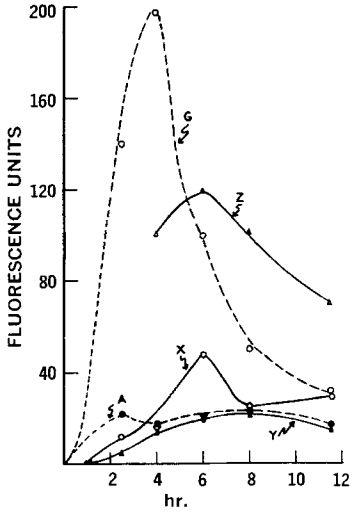



*Fig. 5—Peak intensity of fluorescent spots from TLC fluorimetric scans of plasma extract chromatograms plotted against time. The rabbit (No. 41) received a 200 mg./Kg. oral dose of griseofulvin-4′-alcohol. Key: A, griseofulvin-4′-alcohol; G, griseofulvin; X, Y, and Z, unidentified compounds.*

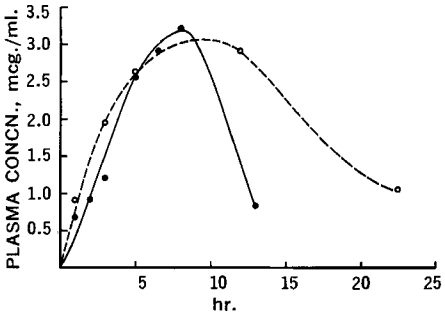



*Fig. 6—Griseofulvin plasma concentration-time curves following single 200 mg./Kg. oral doses of griseofulvin (○) and griseofulvin-4′-oxime (●). (Rabbit 45.)*

ing an oral dose of the alcohol derivative. From the curves, one observes that the unidentified spot, labeled X, which has the lowest $R_f$ value, and another unidentified spot, labeled Y, trailing behind griseofulvin alcohol, appear after a short period of time and have consistently low values as does griseofulvin-4′-alcohol. The more intense spot (labeled Z in Fig. 5) moving slightly ahead of griseofulvin also has a later peak time than griseofulvin and has somewhat higher values than the other unidentified fluorescent products. It should be pointed out that none of these secondary fluorescent products are 6-demethyl griseofulvin, a metabolite of griseofulvin which does not fluoresce under these conditions and has a different $R_f$ than any of the new compounds. Assuming that the fluorescent yield of each of the spots is not drastically different, it appears from the shapes of the curves that either substance X or Y could be converted to substance Z by a mechanism similar to the conversion of the alcohol derivative to griseofulvin. The proposed oxidative conversion of one of the assumed products (X or Y) to substance Z may compete with griseofulvin alcohol for enzymatic metabolism sites. This competition would explain the falling griseofulvin levels upon the appearance of the unidentified fluorescent substances. Even though the griseofulvin plasma level falls rapidly, it is obvious that some conversion of the alcohol is taking place as the apparent half-life of griseofulvin in the plasma after the peak level is approximately 2 to 4 hr., which is longer than the 77-min. half-life observed after an i.v. dose. It appears, then, that the short duration of griseofulvin plasma levels following an oral dose of the alcohol deriva-

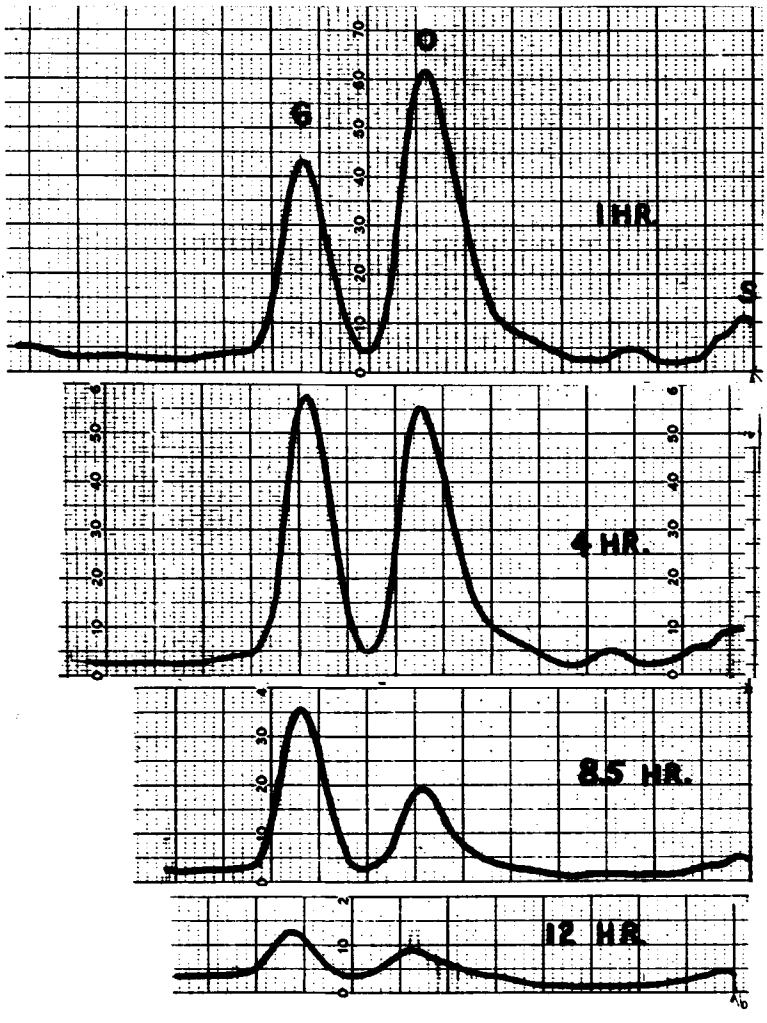



*Fig. 7—TLC fluorimetric recordings of chromatograms showing fluorescent products extracted from plasma samples (1 ml.) following oral administration of 200 mg./Kg. of griseofulvin-4′-oxime. The fluorescent spot attributed to griseofulvin is labeled G, and that of the 4′-oxime as O. (Rabbit 46.)*

tive was due to the degradation of the derivative in the gastrointestinal tract and the possible competition of the degradation products or products with griseofulvin-4′-alcohol for oxidative enzyme sites.

Due to the low fluorescence intensity of the oxime derivative, only griseofulvin plasma concentration was determined by the TLC fluorimetric method following administration of griseofulvin-4′-oxime to rabbits. The 4′-oxime derivative appeared to be converted to griseofulvin at a much slower rate than the 4′-alcohol. After intravenous administration of the 4′-oxime, low levels of griseofulvin from 0.38 to 0.6 mcg./ml. were observed in the plasma sample and during a 100-min. study reach a peak in 1 hr. Unlike the 4′-alcohol derivative, high griseofulvin plasma levels exhibiting apparent first-order elimination from the blood were not observed upon i.v. administration of the oxime. The oxime derivative was observed to be toxic when administered intravenously and the rabbit expired in 2 hr. Another animal given an identical i.v. dose of the oxime derivative exhibited a similar blood level and did not die during the experiment; however, heavy hemolysis was observed in the plasma samples of this animal.

An oral dose of griseofulvin-4′-oxime (200 mg./Kg.) to rabbit 45 produced a plasma griseofulvin curve (closed circles) similar in peak concentration, but of shorter duration than the control dose of griseofulvin (open circles) as seen in Fig. 6. As a result of oral doses of griseofulvin-4′-oxime the possible toxicity of this derivative was evidenced by the hemolysis of the early blood samples.

The slow conversion of the oxime derivatives to griseofulvin was seen in the TLC fluorimetric scans (Fig. 7) of plasma extracts after administration of a 200-mg./Kg. oral dose of griseofulvin-4′-oxime. In interpreting Fig. 7, it must be recalled that the oxime is approximately $^1/_{10}$th as fluorescent as griseofulvin. The earlier blood samples showed high levels of the oxime to be present along with lower levels of griseofulvin. From a preliminary extraction curve, there appeared to be approximately 15 to 20 mcg./ml. of the oxime derivative in the 4-hr. sample which contained 12 mcg./ml. of griseofulvin. Griseofulvin plasma concentration essentially decreased with the fall in oxime concentration and at 12 hr. only small amounts were present in the plasma. Oximes have been reported (18) to have other metabolites in addition to their corresponding ketones. It is probable, therefore, that griseofulvin is not the only metabolite of the oxime derivative. The spot trailing griseofulvin-4′-oxime on the 1- and 4-hr. TLC scans in Fig. 7 may be another metabolite or possibly a gastrointestinal degradation product of the oxime derivative. No attempts were made to characterize any metabolite other than griseofulvin.

Oral doses of griseofulvin-4′-carboxymethoxime have been shown to produce no *in vivo* griseofulvin activity, using the microbiological assay. In order to substantiate this finding, an intravenous dose (15 mg./Kg. in 75% DMA) of this derivative was given to rabbit 43. No evidence of hemolysis was observed in the blood samples taken from this animal. The first plasma sample, taken 15 min. after the 10-min. infusion of the drug, was found to contain only a trace amount of griseofulvin. A 30-min. sample exhibited no trace of the antibiotics. It was disappointing to observe that griseofulvin-4′-carboxymethoxime was not converted to griseofulvin after i.v. or oral administration. This derivative may be excreted rapidly by the kidneys, possibly by an active process, due to the similarity of the carboxymethoxime moiety to many substances actively secreted by the tubules. In addition, the $=N-O-CH_2-$ linkage in this compound may be stable to oxidative metabolism in the rabbit.

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