Griseofulvin–Phenobarbital Interaction: A Formulation-Dependent Phenomenon

F. JAMALI * and J. E. AXELSON ˣ

Received December 27, 1976, from the *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.* Accepted for publication July 13, 1977. *Present address: Faculty of Pharmacy, University of Tehran, Tehran 14, Iran.

Abstract □ The reported interaction of griseofulvin with phenobarbital was studied in the rat following oral administration of different dosage forms. A single oral dose of 15 mg of phenobarbital/kg 24 hr prior to the oral administration of a suspension of 100 mg of griseofulvin/kg in 0.5% polysorbate 80 significantly reduced plasma griseofulvin levels. An increase in the concentration of polysorbate 80 to 2% reduced the extent of the interaction from 50 to 32%. Phenobarbital did not influence plasma griseofulvin levels when griseofulvin was given in either 70% polyethylene glycol 300 (suspensions of 20 or 100 mg/kg) or 100% polyethylene glycol 600 (solution of 50 mg/kg). It is concluded that the observed interaction is formulation dependent and is a result of diminished dissolution and, consequently, reduced absorption of griseofulvin.

Keyphrases □ Griseofulvin—interaction with phenobarbital, effect of different dosage forms in rats □ Phenobarbital—interaction with griseofulvin, effect of different dosage forms in rats □ Dosage forms, various—griseofulvin, effect on interaction with phenobarbital in rats □ Interactions—griseofulvin with phenobarbital, effect of different dosage forms in rats □ Antifungal agents—griseofulvin, interaction with phenobarbital, effect of different dosage forms in rats □ Anticonvulsant–sedatives—phenobarbital, interaction with griseofulvin, effect of different dosage forms in rats

Griseofulvin is an orally administered antifungal agent whose erratic and incomplete absorption has been shown to be dissolution rate limited (1). Low aqueous solubility (15 mg/liter at 37°) and extensive metabolism make griseofulvin a potential candidate for drug interactions when agents capable of altering absorption and/or metabolism are coadministered. Phenobarbital induces the metabolism of several compounds (2–4). The systemic availability of some drugs is affected by agents capable of influencing GI motility (5–8). Existing evidence suggests that griseofulvin availability is reduced by an interaction with phenobarbital (9–12).

It was suggested that phenobarbital increases griseofulvin metabolism in humans (9) and rats (12) by enzyme induction. Riegelman *et al.* (11) observed a similar interaction when griseofulvin tablets were administered to humans. However, following intravenous administration of the drug in the presence and absence of phenobarbital, there was no perceptible effect on the plasma griseofulvin concentration *versus* time curves in test *versus* control animals. Riegelman *et al.* (11) suggested that the observed griseofulvin–phenobarbital interaction was a result of reduced absorption mediated by a complex mechanism involving enhanced bile flow and GI motility.

This proposal was based on the recognized ability of phenobarbital to enhance bile flow (13). Alternatively, if griseofulvin elimination was rate limited by its access to the liver *per se*, then enzyme induction would have no effect on the slope of the postdistribution phase of the plasma drug concentration–time curve. However, enzyme induction might significantly increase the fraction of dose metabolized on the first pass through the liver after oral administration. In the presence of such a complex mechanism, the metabolic alterations induced by phenobarbital would not be expected to alter the terminal elimination half-life of griseofulvin.

In view of the varied mechanisms postulated, it was of interest to examine the griseofulvin–phenobarbital interaction more closely. The present investigation determined and compared the oral absorption of griseofulvin from various polyethylene glycol solutions and polysorbate 80 suspension dosage forms in control and test animals receiving phenobarbital. The main interests were to evaluate the formulation dependency of the griseofulvin–phenobarbital interaction and to delineate the mechanisms involved.

EXPERIMENTAL

Animals and Treatments—Adult male Wistar rats, with an average weight of 250 g, were fasted for 24 hr prior to and during the experiments. Water was available *ad libitum*. All animals were maintained in metabolism cages in a controlled environment for at least 3 weeks prior to the experiments.

Micronized griseofulvin[1] (specific surface area of 1.32 m^2/g) was used in the preparation of five different dosage forms: (*a*) 100 mg/kg in 0.5% polysorbate 80, (*b*) 100 mg/kg in 2% polysorbate 80, (*c*) 20 mg/kg in 70% polyethylene glycol 300, (*d*) 100 mg/kg in 70% polyethylene glycol 300, and (*e*) 50 mg/kg in 100% polyethylene glycol 600. The first four preparations were suspensions, and the fifth was a solution. The dosage forms were administered *via* gastric intubations at a constant volume of 1 ml. Suspensions in 70% polyethylene glycol 300 were stirred at room temperature for 24 hr prior to administration to achieve equilibrium solubility.

Control rats received griseofulvin only. Test rats were treated orally with a single dose of a freshly prepared aqueous solution of 15 mg of phenobarbital sodium[2]/kg 24 hr prior to the griseofulvin administration.

Plasma Level Studies—Serial blood samples (100–250 μl) were taken from the tail artery of ether-anesthetized rats in heparinized caraway tubes at appropriate time intervals (Figs. 1–5).

All blood samples were immediately centrifuged for 15 min in a clinical centrifuge[3], and the plasma was assayed for intact drug.

Equilibrium Solubility of Griseofulvin in Presence of Polyethylene Glycol 300 and Polysorbate 80—Equilibrium solubility of griseofulvin in solutions containing different concentrations (percent weight per weight) of polyethylene glycol 300 (Fig. 6) and polysorbate 80 was measured. An excess amount of griseofulvin (25 mg/ml) was added to the aqueous solutions containing 0, 20, 50, 60, 70, and 100% polyethylene glycol 300 or 0.5, 2, and 5% polysorbate 80 in 25-ml flasks at room temperature and 37°. The contents of the flask were stirred using magnetic stirrers.

[1] Supplied by Dr. Milo Gibaldi, State University of New York at Buffalo, Amherst, N.Y.

[2] Lot 33150, supplied by British Drug Houses (Canada) Ltd., Toronto, Canada.

[3] IEC, Needham Heights, Mass.

Table I—Area under Plasma Concentration–Time Curves, *AUC*, Peak Plasma Concentrations, C_{max}, and Their Time of Occurrence, T_{max}, of Griseofulvin following Oral Administration of Single Doses of 100 mg/kg in 0.5% Polysorbate 80 to Control and Phenobarbital-Pretreated Rats

	Control Rats			Phenobarbital-Pretreated Rats		
	AUC, μg hr/ml	C_{max}, μg/ml	T_{max}, hr	*AUC*, μg hr/ml	C_{max}, μg/ml	T_{max}, hr
	37.5	7.35	6	26.9	4.82	5
	53.0	7.68	5	25.8	4.10	6
	37.3	6.92	6	19.3	3.64	6
	37.8	7.02	6	19.6	3.30	5
	40.8	6.02	6	19.2	2.72	5
	52.9	6.05	6	22.2	3.20	6
Mean	43.2	6.84	5.8	22.1	3.63	5.5
SE	3.1	0.28	0.2	1.4	0.30	0.2
CV, %	17.8	9.9	7.0	15.5	20.4	10.0
Statistical difference[a]				S	S	NS

[a] Determined by the Student *t* test (two tailed); $\alpha = 0.05$; S = significant; and NS = not significant.

After 24 and 48 hr, 1 ml of the contents was transferred into 15-ml centrifuge tubes and centrifuged for 20 min in a clinical centrifuge[3]. Aliquots of 20 μl of each supernate were transferred into 10-ml volumetric flasks and assayed for griseofulvin.

Assay Procedure—The electron-capture GLC method of Shah *et al.* (14) was employed with some modification to determine griseofulvin levels in plasma and various dosage forms (15). The following conditions were found to be satisfactory: glass column, 1.83 m long × 2 mm i.d. packed with 3% OV-25 on 80–100-mesh Chromosorb W; column oven temperature, 285°; injection port temperature, 250°; detector temperature, 350°; and carrier gas (95% argon–5% methane) flow, 40 ml/min. The injection port configuration permitted on-column injection. The peak height ratio (griseofulvin to diazepam) method was used to construct a standard curve and to quantitate griseofulvin.

This assay was specific for griseofulvin. The major metabolite of griseofulvin in the rat, 4-desmethylgriseofulvin, had a retention time of 7.5 min, nearly 2.5 min prior to griseofulvin. Under no circumstances was 4-desmethylgriseofulvin observed in the plasma samples analyzed.

Treatment of Data—Mean plasma griseofulvin levels and the deviation from the mean were calculated for each experiment. Plasma levels were plotted as a function of time, and the areas under the curves (*AUC*) were measured using a planimeter. The Student *t* test was used to measure statistically the significance of the differences at $\alpha = 0.05$[4].

RESULTS AND DISCUSSION

Griseofulvin Suspensions in Polysorbate 80—Mean plasma griseofulvin levels following oral administration of a single dose containing 100 mg of griseofulvin/kg in 0.5% polysorbate 80 to control rats and those pretreated orally with single doses of phenobarbital sodium

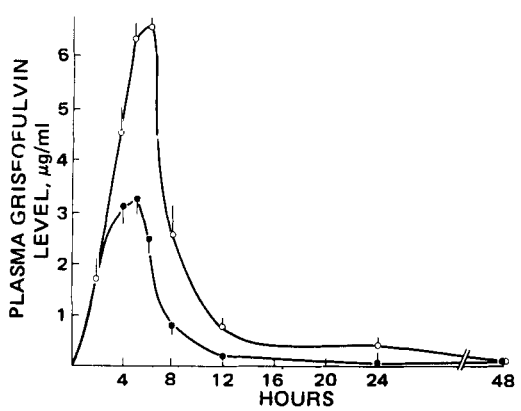


Figure 1—*Mean plasma griseofulvin levels* versus *time following oral administration of single doses of 100 mg/kg in 0.5% polysorbate 80 to control* (○, n = 6) *and phenobarbital-pretreated* (●, n = 6) *rats. Error bars represent the standard error of the mean.*

[4] Unless otherwise stated.

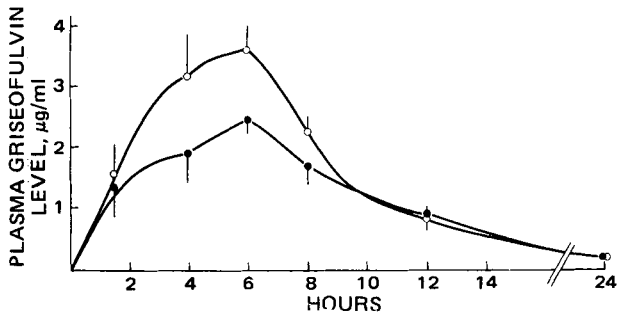


Figure 2—*Mean plasma griseofulvin levels* versus *time following oral administration of single doses of 100 mg/kg in 2% polysorbate 80 to control* (○, n = 4) *and phenobarbital-pretreated* (●, n = 5) *rats. Error bars represent the standard error of the mean.*

are shown in Fig. 1. Phenobarbital pretreatment reduced both the peak plasma griseofulvin levels and the area under the plasma griseofulvin concentration–time curve (*AUC*). The plasma levels were significantly different at all times except at 2 and 12 hr (Fig. 1).

The observed mean *AUC* in the test group was approximately 50% lower than that of the controls (Table I). The mean maximum plasma level, C_{max}, also was reduced by 47% in the test group (Table I). Phenobarbital treatment did not shorten the time of attainment of C_{max}, T_{max} (Table I). Since T_{max} is an indication of the absorption rate and since griseofulvin absorption has been shown to be dependent upon gastric emptying (5, 6), it is suggested that phenobarbital does not significantly influence gastric emptying in the rat.

The presence of surfactants has been shown to influence effectively the dissolution and, hence, the absorption of griseofulvin (16, 17). Thus, an increase in the surfactant concentration in the griseofulvin suspension dosage form was anticipated to optimize the dissolution process and indirectly to diminish the effect of phenobarbital. Therefore, a suspension containing 2% polysorbate 80 was prepared and administered to the rat in the presence and absence of phenobarbital.

Figure 2 depicts the plasma levels *versus* time of griseofulvin following administration of a single dose of a suspension of griseofulvin in 2% polysorbate 80 in the presence and absence of phenobarbital. Although phenobarbital pretreatment reduced the plasma griseofulvin levels, its effect was significant only at C_{max} ($\alpha = 0.1$) (Table II). The treatment also failed to influence significantly the *AUC* and T_{max} values (Table II). These observations indicate that a fourfold increase in the concentration of polysorbate 80 significantly reduced the extent of the interaction. Phenobarbital pretreatment, followed by the administration of a suspension of griseofulvin in 0.5% polysorbate 80, reduced the mean C_{max} by 50%. Under identical conditions when griseofulvin was formulated in 2% polysorbate 80, the barbiturate lowered the mean C_{max} by only 31%. Therefore, it is reasonable to suggest that a decreased dissolution rate may be mainly responsible for the griseofulvin–phenobarbital interaction. Since an increase in the surfactant concentration is expected to improve dissolution, the phenobarbital influence would be expected to diminish, as observed.

Comparison of control *AUC* values in Tables I and II suggests that

Table II—Area under Plasma Concentration–Time Curves, *AUC*, Peak Plasma Concentrations, C_{max}, and Their Time of Occurrence, T_{max}, of Griseofulvin following Oral Administration of Single Doses of 100 mg/kg in 2% Polysorbate 80 to Control and Phenobarbital-Pretreated Rats

	Control Rats			Phenobarbital-Pretreated Rats		
	AUC, μg hr/ml	C_{max}, μg/ml	T_{max}, hr	*AUC*, μg hr/ml	C_{max}, μg/ml	T_{max}, hr
	19.6	2.72	6	17.1	2.62	6
	35.2	4.60	6	15.7	2.42	6
	27.4	3.50	4	32.6	3.45	6
	37.6	4.22	4	22.1	2.32	4
				21.8	3.15	4
Mean	30.0	3.76	5.0	21.9	2.79	5.5
SE	4.0	0.41	0.5	1.6	0.22	0.3
CV, %	27.3	22.0	23.1	16.8	17.5	15.7
Statistical difference[a]				NS	$p <$ 0.1	NS

[a] Determined by the Student *t* test (two tailed); NS = not significant.

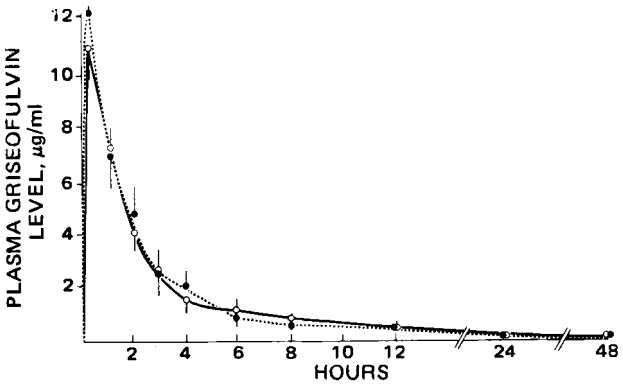


Figure 3—*Mean plasma griseofulvin levels* versus *time following oral administration of single doses of 50 mg/kg in 100% polyethylene glycol 600 to control* (○, n = 8) *and phenobarbital-pretreated* (●, n = 6) *rats. Error bars represent the standard error of the mean.*

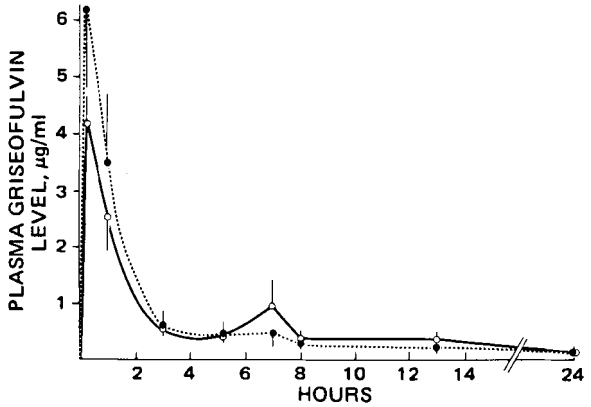


Figure 4—*Mean plasma griseofulvin levels* versus *time following oral administration of single doses of 20 mg/kg in 70% polyethylene glycol to control* (○, n = 4) *and phenobarbital-pretreated* (●, n = 4) *rats. Error bars represent the standard error of the mean.*

formation of a complex phase after an increase in the polysorbate 80 concentration decreased the relative bioavailability of griseofulvin approximately 31%. A complex opaque phase could be seen upon examination of the polysorbate 80 preparations *in vitro*. The solubility experiment revealed that the griseofulvin concentration in the clear phase of 0.5, 2, or 5% polysorbate 80 formulations was the same as in water (approximately 14 mg/liter). This result may indicate that griseofulvin is tightly bound to the complex phase and that drug availability may be limited by its release rate from this complex phase. Therefore, a shorter residence time in the region of the absorptive site should decrease the availability of the preparations with higher polysorbate 80 concentrations still further. Thus, the griseofulvin–phenobarbital interaction is unlikely to result from an increase in motility.

Addition of 2% polysorbate 80 to the griseofulvin suspension perhaps reduced relative drug bioavailability to such an extent that phenobarbital treatment could not reduce it further. Phenobarbital treatment and an increased concentration of polysorbate 80 may influence griseofulvin absorption by the same mechanism. Since the addition of extra surfactant to the preparation did not eliminate completely the effect of phenobarbital on the plasma griseofulvin levels, other mechanisms could also be involved in the interaction; preparations containing polyethylene glycol provided more information.

Griseofulvin in Polyethylene Glycol—The mean plasma levels following a single oral dose of 50 mg of griseofulvin/kg in polyethylene glycol 600 (solution) in control and phenobarbital-pretreated animals are plotted as a function of time in Fig. 3.

Phenobarbital predosing failed to affect the plasma griseofulvin levels when the drug was administered in solution (Fig. 3 and Table III). This observation clearly suggests that the griseofulvin–phenobarbital interaction is a formulation-dependent phenomenon and a result of reduced absorption. A drug–drug interaction mediated by enzyme induction or a change in the blood flow would be expected to be formulation independent for drugs with a high extraction ratio.

Griseofulvin formulation in a 100% polyethylene glycol 600 preparation noticeably changed the gut appearance. Therefore, polyethylene glycol 600 itself might have affected peristalsis and membrane permeability. The absorption properties of griseofulvin possibly might have been altered by the vehicle in both control and test rats. To clarify this point, two different griseofulvin dosage forms containing 100 mg of drug/kg in either 70% polyethylene glycol 600 or 70% polyethylene glycol 300 in distilled water were prepared and their effects on the gut were tested.

Single doses of 100 mg of griseofulvin/kg in either 70% polyethylene glycol 300 or 70% polyethylene glycol 600 (1 ml) were administered to two separate groups of five rats. One rat from each group was sacrificed at 1, 3, 5, 8, or 12 hr postdosing; after an abdominal incision, the gut was examined for any abnormalities.

While the 70% polyethylene glycol 600 preparation showed a noticeable increase in the volume of gut fluid, the 70% polyethylene glycol 300 preparation exerted no effect on the gross appearance of the gut. Therefore, 70% polyethylene glycol 300 was chosen as a vehicle to administer griseofulvin orally at two different concentrations (20 and 100 mg/kg). In addition, the effect of phenobarbital pretreatment on the plasma griseofulvin levels was studied. The mean plasma griseofulvin levels following administration of 20 and 100 mg of griseofulvin/kg in 70% polyethylene glycol 300 are plotted as a function of time in Figs. 4 and 5, respectively. Pretreatment with phenobarbital did not reduce the plasma griseofulvin concentrations of either of the 70% polyethylene glycol 300 preparations (Tables IV and V). This result was due, perhaps, to the wetting and solvent properties of polyethylene glycol.

Administration of 20 and 100 mg of griseofulvin/kg in 70% polyethylene glycol 300 yielded a rapid rise in the plasma concentration similar to that observed after griseofulvin in 100% polyethylene glycol 600. This observation is consistent with the rapid absorption of the dissolved fraction of drug in the suspension dosage forms. Attainment of approximately the same C_{max} following administration of both preparations to control rats (4.2–6.3 and 3.1–5.3 μg/ml for 20- and 100-mg/kg doses, respectively) supports this suggestion (Tables IV and V). The remaining fraction of the drug (undissolved fraction), however, was absorbed slowly and incompletely. As shown in Fig. 6, at equilibrium, 3.2 mg of griseofulvin was

Table III—Area under Plasma Concentration–Time Curves, *AUC*, and Peak Plasma Concentrations, C_{max}, following Oral Administration of 50 mg of Griseofulvin/kg in Polyethylene Glycol 600 (Solution) to Control and Phenobarbital-Pretreated Rats

	Control Rats		Phenobarbital-Pretreated Rats	
	AUC, μg hr/ml	C_{max}, μg/ml	*AUC*, μg hr/ml	C_{max}, μg/ml
	37.6	15.32	43.8	15.30
	55.5	12.01	24.4	8.53
	30.1	6.09	35.7	18.25
	41.3	12.33	35.3	7.68
	28.9	8.12	41.4	14.46
	32.6	9.23	35.2	9.36
Mean	37.7	10.52	36.0	12.26
SE	4.0	1.36	2.7	1.76
CV, %	26.3	31.7	18.7	35.2
Statistical difference[a]			NS	NS

[a] Determined by the Student *t* test (two tailed); $\alpha = 0.05$; NS = not significant.

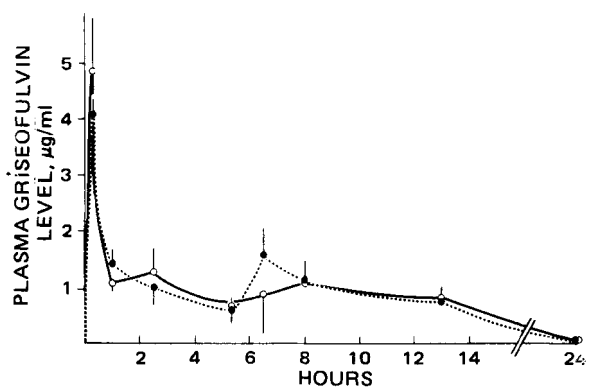


Figure 5—*Mean plasma griseofulvin levels* versus *time following administration of single doses of 100 mg/kg in 70% polyethylene glycol 300 to control* (○, n = 4) *and phenobarbital-pretreated* (●, n = 4) *rats. Error bars represent the standard error of the mean.*

Table IV—Area under Plasma Concentration–Time Curves, *AUC*, and Peak Plasma Concentrations, C_{max}, following Oral Administration of 20 mg of Griseofulvin/kg in 70% Polyethylene Glycol 300 to Control and Phenobarbital-Pretreated Rats

	Control Rats		Phenobarbital-Pretreated Rats	
	AUC, μg hr/ml	C_{max}, μg/ml	*AUC*, μg hr/ml	C_{max}, μg/ml
	9.3	3.77	16.8	9.95
	13.1	4.47	8.6	6.76
	12.3	5.32	11.4	4.63
	10.3	3.30	10.4	3.72
Mean	11.2	4.21	11.8	6.27
SE	0.8	0.44	1.7	1.38
CV, %,	15.7	20.8	29.8	44.2
Statistical difference[a]			NS	NS

[a] Determined by the Student *t* test (two tailed); $\alpha = 0.05$; NS = not significant.

dissolved in 1 ml of 70% polyethylene glycol 300 at 37°. Therefore, both suspensions had the same drug concentration in the supernate (3.2 mg/ml) but were different in their total griseofulvin concentration (5 or 25 mg/ml). Thus, the increase in the *AUC* from 11 to 17–18 μg hr/ml following administration of the higher dose, although small, may be related to the absorption of the undissolved fraction in the dosage form following dissolution.

The appearance of a second plasma concentration peak after 6–8 hr (Figs. 4 and 5) may also result from absorption of drug from the undissolved fraction of griseofulvin in the dosage form. Phenobarbital apparently did not reduce the absorption of either the dissolved or the undissolved fraction of griseofulvin when given in 70% polyethylene glycol 300. These observations further suggest that the observed griseofulvin–phenobarbital interaction may result from reduced dissolution rather than increased gut motility. If the gut motility had increased following phenobarbital pretreatment, it should have affected the absorption of the undissolved fraction of the dose. Because of the large fraction of undissolved drug in the preparation containing 100 mg of griseofulvin/kg in 70% polyethylene glycol 300, this effect should have been most profound. The undissolved fraction of the dose, due to the very slow dissolution rate, requires a longer residence time in the absorptive site to be dissolved and subsequently absorbed. Therefore, a reduced residence time is likely to reduce the total griseofulvin absorption (*i.e.*, as reflected by the *AUC*).

Absorption studies in this investigation revealed that griseofulvin availability in polyethylene glycol, unlike that of griseofulvin in polysorbate 80, is high. Once the drug was dissolved in polyethylene glycol, it was absorbed very rapidly (T_{max} = 20 min), while the griseofulvin absorption from polysorbate 80 was relatively slow. Bloedow and Hayton (18) reported a T_{max} of 8 hr following administration of griseofulvin in pure polysorbate 80 (solution). In this investigation, T_{max} values after administration of a suspension of griseofulvin in 0.5 or 2% polysorbate 80 were about 6 hr. A delayed T_{max}, coupled with the observed reduced *AUC* on increasing the concentration of polysorbate 80, indicates that the griseofulvin absorption rate decreases with an increasing polysorbate 80 concentration.

Phenobarbital pretreatment reduced the plasma griseofulvin levels when the drug was administered in tablet (in humans) (9, 11) or suspension forms containing polysorbate 80 (in the rat) (12). These dosage forms, unlike polyethylene glycol preparations, share the property of being very slowly absorbed. *In vivo*, bile salts probably accelerate the griseofulvin dissolution or release rate from these solid preparations. Preparations containing polyethylene glycol, however, may not, due to the solvent effect of the vehicle, be affected by this change.

Table V—Area under Plasma Concentration–Time Curves, *AUC*, and Peak Plasma Concentrations, C_{max}, following Oral Administration of 100 mg of Griseofulvin/kg in 70% Polyethylene Glycol 300 to Control and Phenobarbital-Pretreated Rats

	Control Rats		Phenobarbital-Pretreated Rats	
	AUC, μg hr/ml	C_{max}, μg/ml	*AUC*, μg hr/ml	C_{max}, μg/ml
	15.8	4.99	12.2	3.44
	17.8	3.73	25.6	4.37
	26.4	7.54	11.7	3.14
	14.2	3.11	18.3	5.32
Mean	18.5	4.84	16.9	4.07
SE	2.7	0.98	3.2	0.49
CV, %,	29.5	40.4	38.5	24.3
Statistical difference[a]			NS	NS

[a] Determined by the Student *t* test (two tailed); $\alpha = 0.05$; NS = not significant.

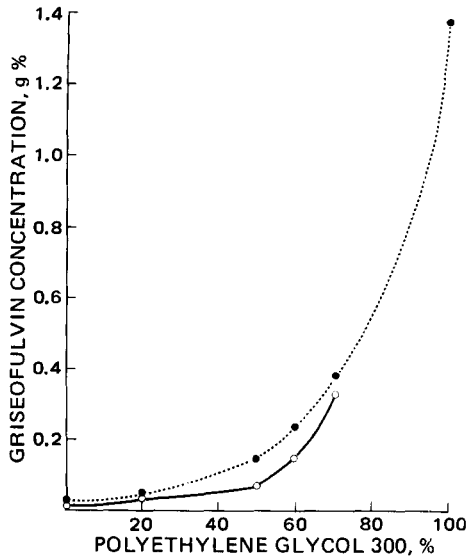


Figure 6—*Equilibrium solubility of griseofulvin in aqueous solutions containing different concentrations of polyethylene glycol 300 at room temperature (○) and 37° (●). Each point represents the average of two measurements.*

Griseofulvin solubility in an aqueous solution sharply increased after an increase in the concentration of the simulated intestinal bile salt mixture above the critical micelle concentration (17). This result is attributed to a micellar solubilization phenomenon. Klaassen (13) reported an increase in the bile flow accompanied with a decrease in the concentration of the bile salts following treatment with phenobarbital in the rat. Therefore, the observed reduction in the concentration of the bile salts (13) resulting from phenobarbital treatment may have reduced the solubility and, hence, the absorption of griseofulvin.

Recently, Lin and Symchowicz (10) observed that, *in vitro*, phenobarbital treatment was capable of increasing the maximum velocity, V_{max}, for griseofulvin metabolism in the rat and mouse. The treatment, however, did not alter the enzyme–substrate dissociation constant, K_m, for the reaction. The failure of phenobarbital to reduce the griseofulvin level after administration in polyethylene glycol contradicts the suggestion by Lin and Symchowicz (10) that the barbiturate may also induce griseofulvin metabolism *in vivo*.

Griseofulvin is eliminated almost exclusively *via* metabolism. The observed griseofulvin biological half-life in the rat is approximately 2 hr (15), an indication of very rapid elimination and rapid metabolism. This rate is, perhaps, due to a large capacity of the liver enzymes to metabolize this drug. In *in vitro* experiments, a given quantity of the drug is directly exposed to the enzymes. Therefore, an increase in the metabolizing capacity of the liver mediated by phenobarbital would yield a higher extent of metabolism *in vitro* (10, 12).

However, the mechanism may be different *in vivo*. The metabolism rate of griseofulvin may be limited by its access to the liver rather than by its metabolism *per se*. Therefore, *in vivo*, the inducing effect of phenobarbital on the liver enzyme, however profound, might exert no effect on the griseofulvin metabolism rate. This evidence supports the statement that *in vitro* observations may not necessarily be extrapolated to *in vivo* conditions. Therefore, it is suggested that the griseofulvin–phenobarbital interaction results from reduced availability and/or diminished dissolution caused by a reduction in the bile salt concentration rather than by induced metabolism or a decreased transit time in the absorption region.

Phenobarbital has been observed to reduce plasma concentrations of many other drugs, such as phenytoin, dicumarol (3), and digoxin (4). All these drugs, like griseofulvin, show low solubility in aqueous media but, unlike the latter, lack highly lipophilic properties. The shorter biological half-life observed in phenobarbital-pretreated subjects suggests that induced metabolism is responsible for the reduced plasma dicumarol

levels. However, the reduced plasma dicumarol level may also be partly due to reduced absorption. Considering the low dicumarol solubility, it is possible that a more complex mechanism consisting of simultaneous increased metabolism and reduced absorption may be involved in this interaction. Aggeler and O'Reilly (19) noted that heptabarbital appears to induce dicumarol metabolism while simultaneously reducing anticoagulant absorption.

Barbiturates interact with many drugs. This effect is usually attributed to the acknowledged ability of these compounds to induce the metabolism of other drugs. Less importance, however, has been given to another possible mechanism by which barbiturate administration may alter drug disposition, *e.g.*, an increase in bile flow with a simultaneous decrease in bile salt concentration. In this work, the influence of these changes was not directly studied. However, the evidence suggests that these mechanisms could be involved in the interactions between phenobarbital and poorly soluble drugs.

REFERENCES

(1) S. Symchowicz and B. Katchen, *J. Pharm. Sci.*, **57**, 1383 (1968).

(2) A. H. Conney, C. Davison, R. Gastel, and J. J. Burns, *J. Pharmacol. Exp. Ther.*, **130**, 1 (1960).

(3) S. A. Cucinell, A. H. Conney, M. Sansur, and J. J. Burns, *Clin. Pharmacol. Ther.*, **6**, 420 (1965).

(4) A. Kaldor, G. Somogyi, L. A. Debreczeni, and B. Gachalyi, *Int. J. Clin. Pharmacol.*, **12**, 403 (1975).

(5) J. E. Axelson and F. Jamali, presented at the Research Symposium and Research Conference of the Association of Faculties of Pharmacy of Canada, Ottawa, Canada, May 1974.

(6) F. Jamali and J. E. Axelson, presented at the Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, Apr. 1975.

(7) J. Nimmo, R. C. Heading, P. Tothill, and L. F. Prescott, *Br. Med. J.*, **1**, 587 (1973).

(8) G. Levy, M. Gibaldi, and J. A. Procknal, *J. Pharm. Sci.*, **61**, 798 (1972).

(9) D. Busfield, K. J. Child, R. M. Atkinson, and E. G. Tomich, *Lancet*, **2**, 1042 (1963).

(10) C. Lin and S. Symchowicz, *Drug Metab. Rev.*, **4**, 75 (1975).

(11) S. Riegelman, M. Rowland, and W. L. Epstein, *J. Am. Med. Assoc.*, **213**, 426 (1970).

(12) D. Busfield, K. J. Child, and E. G. Tomich, *Br. J. Pharmacol.*, **22**, 137 (1964).

(13) C. D. Klaassen, *J. Pharmacol. Exp. Ther.*, **176**, 743 (1971).

(14) V. P. Shah, S. Riegelman, and W. L. Epstein, *J. Pharm. Sci.*, **61**, 634 (1972).

(15) F. Jamali, Ph.D. dissertation, University of British Columbia, Vancouver, British Columbia, Canada, 1976.

(16) W. A. Duncan, G. MacDonald, and M. J. Thornton, *J. Pharm. Pharmacol.*, **14**, 217 (1962).

(17) T. R. Bates, M. Gibaldi, and J. L. Kanig, *J. Pharm. Sci.*, **55**, 191 (1966).

(18) D. C. Bloedow and W. L. Hayton, *ibid.*, **65**, 328 (1976).

(19) P. Aggeler and R. O'Reilly, *J. Lab. Clin. Med.*, **74**, 229 (1969).

ACKNOWLEDGMENTS

Presented in part at the Research Symposium and Research Conference of the Association of Faculties of Pharmacy of Canada, Ottawa, Canada, May 20, 1974, and at the Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, April 1975.

Supported by M.R.C. Grant MA-5358.

The analytical support of Mr. W. Campbell, Jr., of Hewlett-Packard, Ltd., is gratefully acknowledged.

Micro and Macro GLC Determination of Ethambutol in Biological Fluids

CHING S. LEE * and LESLIE Z. BENET ˣ

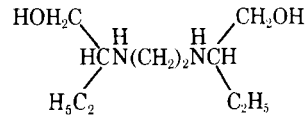
Received December 13, 1976, from the *Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94143.* Accepted for publication July 5, 1977. *Present address: College of Pharmacy, University of Florida, Gainesville, FL 32610.

Abstract □ Previously available GLC methods for ethambutol may only be used to measure quantities of drugs much greater than those found in biological fluids such as plasma and dialysate. A previously published GLC method for plasma samples is extended to measure ethambutol in dialysate. A second GLC method, involving derivatization with bis(trimethylsilyl)trifluoroacetamide and subsequent quantitation using a flame-ionization detector, is described for urine samples. With a dual-column and dual-detector gas–liquid chromatograph, simultaneous micro (plasma and dialysate) and macro (urine) determinations of ethambutol are possible.

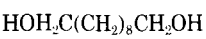
Keyphrases □ Ethambutol—GLC analysis in biological fluids □ GLC—*analysis*, ethambutol in biological fluids □ Tuberculostatic antibacterials—ethambutol, GLC analysis in biological fluids

A previous publication (1) indicated that the available GLC methods may only be used to measure quantities of ethambutol[1] (I) much greater than those found in patient samples of blood, plasma, and dialysate. Pharmacokinetic study of I requires a sensitive and specific method of measuring the unchanged compound.

In this work, the GLC method previously used for plasma is extended to the measurement of I in dialysate. Ethambutol is largely excreted unchanged into the urine (2). To avoid the tedious and possibly erroneous serial dilution of the urine necessary for the GLC assay using an electron-capture detector, another GLC method for I, involving derivatization with bis(trimethylsilyl)trifluoroacetamide and subsequent quantitation using a flame-ionization detector, is described. Decanediol[2] (II) and *d*-2,2′-(ethylenediimino)-di-1-propanol[3] (III) are used as internal standards for the urine and the dialysate assay,



I



II

[1] Myambutol, Lederle Laboratories, Pearl River, N.Y.

[2] Aldrich Chemical Co., Milwaukee, Wis.

[3] Provided by Dr. Raymond Wilkinson, Lederle Laboratories, Pearl River, N.Y.