within-laboratory variance for the ADS-12 data was significantly lower than the average for the 17 participating laboratories.

CONCLUSION

The described computerized automated dissolution testing system conforms to the basic requirements of the USP dissolution test. The system, which can be adapted to almost any type of tablet agitation technique, can test 12 solid dosage forms simultaneously under either sink or nonsink conditions and can test products requiring any type of chemistry amenable to an automatic analyzer. Total run time is reduced essentially to sample dissolution time since sampling and analysis are performed simultaneously with sample dissolution. A complete dissolution profile report, with or without graphs, is obtained within minutes after the required sample dissolution time.

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Use of Rabbits for GI Drug Absorption Studies: Relationship between Dissolution Rate and Bioavailability of Griseofulvin Tablets

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Abstract □ The correlation between the dissolution rate and bioavailability of griseofulvin tablets was studied in stomach-emptying-controlled rabbits and in humans. Three different test tablets, each consisting of two dose levels (62.5 or 125 mg) of griseofulvin, were used. The dissolution rates in 0.5 hr were ~75, 40, and 12%. With oral administration at 62.5 mg/rabbit, the ratio of peak plasma level, C_{max}, was 1.00:0.66:0.40 and that of the area under the curve (AUC) was 1.00:0.73:0.46 for the three tablets. The corresponding C_{max} ratio was 1.00:0.74:0.34 and the AUC ratio was 1.00:0.72:0.33 in humans at the dose level of 500 mg. A good correlation was observed for the rank order of C_{max} and AUC between rabbits and humans, but such a correlation was not seen between *in vivo* data and *in vitro* data at a larger dose of 125 mg/rabbit. This finding was attributable to the dose, which exceeded the GI drug dissolution or absorption capacities. These results suggest that the stomach-emptying-controlled rabbit is useful for evaluating oral dosage forms for human use and that dose level selection is important in the bioavailability study of a barely water-soluble drug.

Keyphrases □ Models, animals—rabbits, use in GI drug absorption studies, griseofulvin tablet dissolution rate and bioavailability, compared to humans □ Griseofulvin—dissolution rate and bioavailability, rabbits compared to humans □ GI tract—drug absorption, griseofulvin, rabbits compared to humans □ Antifungal agents—griseofulvin, dissolution rate and bioavailability, rabbits compared to humans

Use of human subjects in bioavailability studies for dosage forms provides the most appropriate results; but in new drug development and preformulation studies, such use presents economic, ethical, analytical, and statistical difficulties.

The availability of adequate animal models has been desired for estimating the bioavailability of dosage forms in humans. The beagle dog has been used as a model animal in some studies (1–3). Although rabbits are easy to breed, have a mild temper, and are a low-cost laboratory animal, they have not been considered useful for drug absorption studies because of the difficulty in obtaining an empty stomach (4). However, control of the stomach emptying rate increased the usefulness of rabbits in GI drug absorption studies and produced a good correlation in GI drug absorption between rabbits and humans (5). Since the earlier work was performed on powdered drugs using griseofulvin, indomethacin, and nalidixic acid, further investigation on oral dosage forms was desired. This paper describes the usefulness of rabbits in dosage form bioavailability studies.

While the dissolution test has been widely used as an *in vitro* test for solid dosage forms, efforts have been made to obtain good correlations between *in vitro* data and *in vivo* data (6–8). In this study, three groups of griseofulvin tablets were prepared using three grades of polyvinyl alcohol as binders for granulation so that the dissolution rate of each group was different. With these tablets, bioavailability tests were performed in stomach-emptying-controlled rabbits, and the correlation between *in vitro* dissolution tendency and *in vivo* absorption characteristics was examined. Bioavailability studies were also conducted in humans, and the suitability of the stomach-emptying-controlled rabbit as an animal model for dosage form evaluation was reviewed.

EXPERIMENTAL

Materials—Griseofulvin USP was micronized to <5 μm using a fluid energy mill. The granule was prepared by the conventional wet granulation method. The granule contained, by weight, 83.3 parts of micronized griseofulvin, 6.7 parts of calcium carboxymethylcellulose JP, 8.0 parts of lactose USP, and 1.5 parts of polyvinyl alcohol as the binder.

To control drug release from the tablet, three grades of polyvinyl alcohol[1], prepared by partial or full saponification of polyvinyl acetate, were used as the binders for granulation: grade L for Formula L, grade H for Formula N, and grades M and H (2:1) for Formula M (Table I). After 99.5 parts of the granules was mixed with 0.5 part of magnesium stearate USP

[1] Gohsenol, Nippon Gohsei Kagaku Kogyo Co., Tokyo, Japan.

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Table I—Properties of Polyvinyl Alcohol [a]

Grade	Degree of Polymerization	Degree of Saponification, mole %	Viscosity[b], cps
L	1000	86.5–89	5.3 ± 0.5
M	1000–1500	86.5–89	18 ± 2
H	1500	99.4–100	39 ± 4

[a] Polyvinyl alcohol manufactured by partial or full saponification of polyvinyl acetate. [b] A 40% aqueous solution at 20°.

as a lubricant, the mixture was compressed with a single-punch machine into 6.3- and 8-mm diameter flat tablets, containing 62.5 and 125 mg of griseofulvin, respectively.

Dissolution Rate Study—A slightly modified literature method (9) was used. Ten liters of distilled water was poured into a glass bath (30 cm in length, 23 cm in width, and 30 cm in depth) and maintained at 37 ± 2°. The test tablet was placed in a plastic cylinder (22 × 77.5 mm) covered at each end with a 42-mesh screen. The cylinder was moved up and down in the water at 30 ± 2 strokes/min. The test fluid was stirred continuously with a six-blade turbine-type propeller rotating at 200 ± 3 rpm. At a predetermined time, an aliquot was removed using a 0.45-μm pore membrane filter; the dissolved drug was assayed spectrophotometrically[2].

***In Vivo* Studies in Rabbits**—The stomach emptying rate of white male rabbits, 2.5–3.2 kg, was controlled by the method reported previously (5). Six rabbits were employed first. The rabbits were given one of the three kinds of tablets (Formula L, M, or N) containing 125 mg of griseofulvin. Administration of the other two kinds of tablets followed in a strict crossover manner after 2-week washout periods. Similarly, six other rabbits were given tablets of Formulas L, M, and N containing 62.5 mg of griseofulvin in turn in a strict crossover manner. Blood samples were collected by cardiac puncture periodically up to 10 hr.

***In Vivo* Studies in Humans**—The four male volunteers, 29–46 years of age and 55–67 kg in weight, were all in good health. Each subject took a standard breakfast at 7:00 am and received 500 mg of griseofulvin (four 125-mg tablets) with a glass of water at 9:00 am. At 1-week intervals, three different tablets of griseofulvin (Formulas L, M, and N) were given to these four subjects in a crossover manner. Blood samples were taken from the cubital vein at the predetermined time for analysis.

Analytical Method and Equipment—Griseofulvin was extracted with ether from aqueous or plasma samples and was determined by a gas chromatograph equipped with a ^{63}Ni-electron capture detector[3]. Diazepam was used as an internal standard, and the procedure of Shah *et al.* (10) was followed. The column was a 1.5-m × 3-mm glass tube packed with 10% SE-30 on Chromosorb W. The column temperature was 270°, the injection port temperature was 300°, and the detector block temperature was 310°. The carrier gas (99.999% nitrogen) flow rate was 60 ml/min.

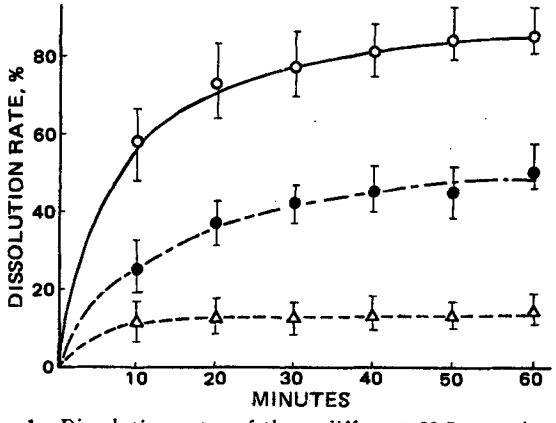

Figure 1—*Dissolution rates of three different 62.5-mg griseofulvin tablets in distilled water at 37°. Each point represents the average and range of triplicate experiments. Key: ○, Formula L; ●, Formula M; and △, Formula N.*

[2] Shimadzu spectrophotometer UV-200.
[3] Shimadzu GC-5APTFE.

Table II—Plasma Level–Time Parameters following Oral Administration of 62.5-mg Griseofulvin Tablets in Stomach-Emptying-Controlled Rabbits

Parameter	Formula L	Formula M	Formula N
C_{max}[a], μg/ml	1.12[b,c]	0.55	0.40
	(1.00)[d]	(0.49)	(0.36)
C'_{max}[e], μg/ml	1.21[b,c]	0.80[c]	0.48
	(1.00)	(0.66)	(0.40)
T_{max}[f], hr	2	2	3
	(1.00)	(1.00)	(1.50)
T'_{max}[g], hr	2.2	2.5	3.0
	(1.00)	(1.14)	(1.36)
AUC[h], hr μg/ml	5.64[c]	4.14[c]	2.57
	(1.00)	(0.73)	(0.46)

[a] Average plasma level peak. [b] Significantly different from Formula M at $p < 0.05$. [c] Significantly different from Formula N at $p < 0.05$. [d] Each value in parentheses means relative ratio of parameters among Formulas L, M, and N. [e] Average of individual peak plasma levels. [f] Time of the average plasma level peak. [g] Average of individual peak times. [h] Area under the plasma level–time curve to 10 hr calculated by the trapezoidal rule.

RESULTS AND DISCUSSION

To conduct dissolution rate studies on a dosage form containing a barely water-soluble drug, it is important to select an appropriate experimental method. In a preliminary study, the dissolution rates of three tablet types (Formulas L, M, and N), each containing 125 mg of griseofulvin, were estimated according to Dissolution Test Method II of NF XIV, using 1 liter of distilled water as the medium. As a result, the dissolution rate was very low, only ~12% in 1 hr for Formula L and M tablets with no significant difference between the two. Since griseofulvin solubility is very limited (11, 12), only ~10% of 125 mg of griseofulvin present in the tablet could be dissolved in 1 liter of water at 37°. In fact, the drug concentration seemed to have reached saturation for Formula L and M tablets in ~20 min, and no difference was observed thereafter between the two.

In a subsequent dissolution test, the method of Katchen (9) was employed with 10 liters of water as the medium. The dissolution rate–time curves for tablets of Formulas L, M, and N containing 62.5 mg of griseofulvin are shown in Fig. 1. The average dissolution rates at 0.5 hr after initiation of the experiment were 77.4, 41.5, and 13.2%, respectively, and a difference of ~30% was observed between L–M and M–N. Similarly, the average dissolution rates at 0.5 hr after initiation of the experiment for the 125-mg tablets, which were prepared using the same granules as the 62.5-mg tablets, were 73.1, 38.2, and 10.7% for L, M, and N, respectively. There was no significant difference in dissolution rates between the 62.5- and 125-mg tablets. Furthermore, good reproducibility was seen in the dissolution rates obtained with this method (Fig. 1). To examine the relationship between *in vitro* and *in vivo* data, tablet dissolution properties must be estimated adequately; these results demonstrated that the selected method was satisfactory.

Bioavailability studies were carried out on the tablets of Formulas L, M, and N to determine how the described *in vitro* dissolution charac-

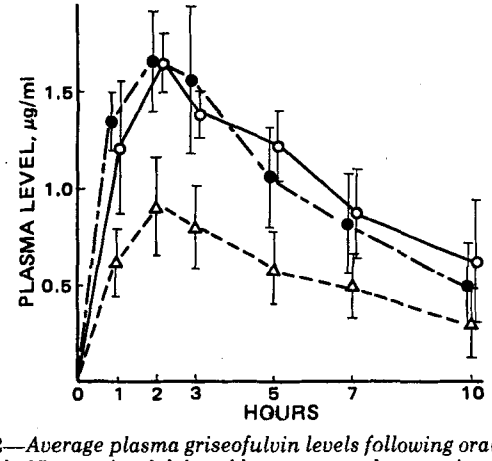

Figure 2—*Average plasma griseofulvin levels following oral administration of 125-mg griseofulvin tablets to stomach-emptying-controlled rabbits. Each point represents the average ± SE in six animals. Key: see Fig. 1.*

Table III—Plasma Level–Time Parameters following Oral Administration of 500 mg of Griseofulvin (125-mg Tablet × 4) in Humans

Parameter	Formula L	Formula M	Formula N
C_{max}[a], μg/ml	1.61[b,c] (1.00)[d]	1.13[c] (0.70)	0.55 (0.34)
C'_{max}[e], μg/ml	1.61[b,c] (1.00)	1.19[c] (0.74)	0.55 (0.34)
T_{max}[f], hr	3 (1.00)	3 (1.00)	5 (1.67)
T'_{max}[g], hr	3 (1.00)	4 (1.33)	5 (1.67)
AUC[h], hr μg/ml	11.70[c] (1.00)	8.40[c] (0.72)	3.91 (0.33)

[a] Average plasma level peak. [b] Significantly different from Formula M at $p < 0.05$. [c] Significantly different from Formula N at $p < 0.05$. [d] Each value in parentheses means relative ratio of parameters among Formulas L, M, and N. [e] Average of individual peak plasma levels. [f] Time of the average plasma level peak. [g] Average of individual peak times. [h] Area under the plasma level–time curve to 10 hr calculated by the trapezoidal rule.

teristics were reflected in the stomach-emptying-controlled rabbits. Since the minimum active ingredient content of ordinary griseofulvin tablets for clinical use is 125 mg/tablet (NF XIV and USP XIX), the 125-mg tablets of Formulas L, M, and N first were administered to six rabbits in a strictly crossover manner; the results obtained are shown in Fig. 2. Both the peak of the average plasma level, C_{max}, and the area under the average plasma level–time curve up to 10 hr, AUC, showed an order of L ≃ M > N. The plasma level after administration of the 125-mg Formula L tablet did not increase in parallel with its dissolution rate as expected.

Among the Formula L, M, and N tablets, no evident correlation was found between the dissolution rate and C_{max} or AUC. These results were attributable to griseofulvin overdose. As already mentioned, the water solubility of griseofulvin is extremely low (11, 12); its limited solubility has been the rate-limiting factor in drug absorption (13–15). These facts suggested that *in vitro* and *in vivo* correlation studies should be conducted under the dose level where a dose–response should be seen in the drug absorption.

To avoid the overdose problem, the griseofulvin dose level was reduced by one-half in subsequent studies in rabbits. The 62.5-mg tablets of Formulas L, M, and N were administered to six rabbits in a strictly crossover manner, and the plasma level–time curves and relevant data are shown in Fig. 3 and Table II. For 62.5-mg tablets, a good correlation was found between dissolution rate and bioavailability indexes such as the rate and extent of drug absorption. The C_{max} values for Formulas L, M, and N were 1.12, 0.55, and 0.40 μg/ml, respectively, in a ratio of 1.00:0.49:0.36. The AUC values were 5.64, 4.14, and 2.57 hr μg/ml, and the ratio was 1.00:0.73:0.46. Thus, both the C_{max} and AUC values were consistent with the dissolution rate order.

Although the times of peak average plasma level, T_{max}, for Formulas L, M, and N were 2, 2, and 3 hr, respectively, showing no difference between L and M, the averages of the individual peak times, T'_{max}, were 2.2, 2.5, and 3.0 hr, which were consistent with the dissolution rate order. Not only the average data but also the area under the individual rabbit plasma level–time curves showed the order L > M > N. These results were in contrast with the report of Fischer and Riegelman (16), who pointed out large individual variations when griseofulvin was given orally to rabbits fasted by the conventional method.

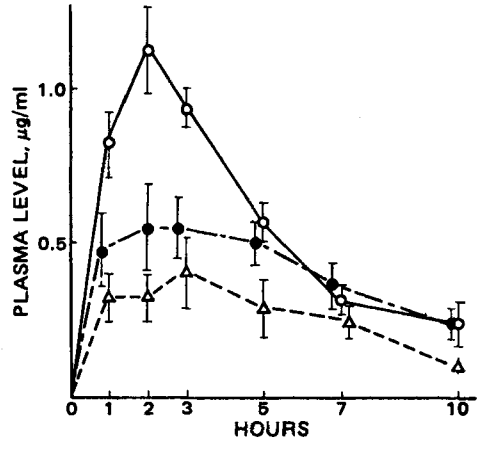

Figure 3—*Average plasma griseofulvin levels following oral administration of 62.5-mg griseofulvin tablets to stomach-emptying-controlled rabbits. Each point represents the average ± SE in six animals. Key: see Fig. 1.*

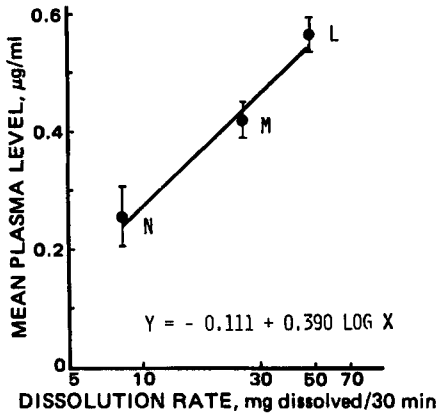

Figure 4—*Correlation of dissolution rates and mean plasma griseofulvin levels in stomach-emptying-controlled rabbits for Formulas L, M, and N. Correlation coefficient = 0.988 (p < 0.10).*

The average plasma level after administration of Formulas L, M, and N was examined statistically by the Student t test. The plasma level of Formula L differed significantly from that of Formula M at 2 and 3 hr ($p < 0.05$) and from that of Formula N at 1, 2, 3, and 5 hr ($p < 0.05$). There was no significant difference between the plasma levels of Formulas M and N. According to the method of Katchen (9), the mean plasma level, C_m, was the value of one-tenth of the AUC up to 10 hr, and this C_m was plotted against the logarithm of griseofulvin dissolved in 0.5 hr in the *in vitro* study (log $D_{0.5}$). A very close correlation was obtained between C_m and log $D_{0.5}$, with a correlation coefficient of 0.988 ($p < 0.10$) (Fig. 4). All of these data show that the best correlation was obtained between the dissolution rates and bioavailability of griseofulvin tablets by using stomach-emptying-controlled rabbits and selecting an adequate dose level.

Further studies were undertaken to examine the relationship between the stomach-emptying-controlled rabbits and human subjects. Four 125-mg tablets, corresponding to 500 mg of griseofulvin, of Formulas L, M, and N were given orally to four human volunteers in a crossover manner. The plasma level–time curves and relevant data are shown in Fig. 5 and Table III. The correlation between tablet dissolution rates and bioavailability was very high. The C_{max} values for Formulas L, M, and N were 1.61, 1.13, and 0.55 μg/ml, respectively, and their ratio was 1.00:0.70:0.34. The AUC values for Formulas L, M, and N were 11.70, 8.40, and 3.91 hr μg/ml, respectively, and the ratio was 1.00:0.72:0.33. This AUC ratio was in good agreement with that of rabbits administered 62.5 mg of the drug. Although the T_{max} for Formulas L, M, and N were 3, 3, and 5 hr, showing no difference between L and M, the individual peak time averages, T'_{max}, were 3, 4, and 5 hr for L, M, and N, which suggested a faster absorption of the tablet with superior dissolution (Table III).

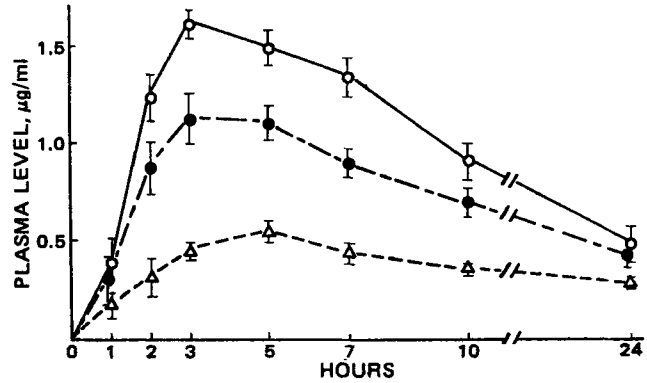

Figure 5—*Average plasma griseofulvin levels following oral administration of 500 mg of griseofulvin (125-mg tablet × 4) in humans. Each point represents the average ± SE in four men. Key: see Fig. 1.*

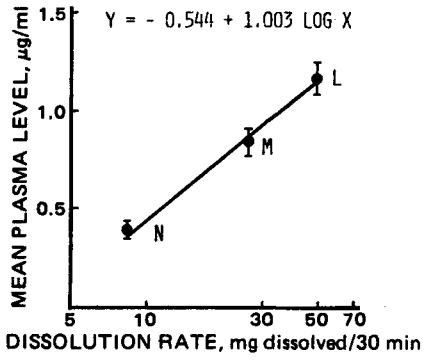

Figure 6—*Correlation of dissolution rates and mean plasma griseofulvin levels in humans for Formulas L, M, and N. Correlation coefficient = 0.995 (p < 0.10).*

While individual data are not shown, both the area under the plasma level–time curve and the peak plasma level were in the order L > M > N in all four subjects. The average plasma level of Formula L was significantly higher than that of Formula M at 3, 5, and 7 hr and than that of Formula N at 2, 3, 5, and 7 hr ($p < 0.05$). Similarly, the average plasma level of Formula M was significantly higher than that of Formula N at 2, 3, 5, 7, and 10 hr ($p < 0.05$).

The relationship between the mean plasma level, C_m, and the logarithm of griseofulvin dissolved in 0.5 hr ($\log D_{0.5}$) is shown in Fig. 6. As in the rabbit study, high correlation between C_m and $\log D_{0.5}$ was confirmed in humans, with a correlation coefficient of 0.995 ($p < 0.10$). These results support the view of previous investigators (13–15) that the dissolution process is the rate-determining step in griseofulvin absorption.

To examine the relationship between the stomach-emptying-controlled rabbit and humans, the C_m values after administering the tablets of Formulas L, M, and N to humans were plotted on the ordinate and those of the rabbit were plotted on the abscissa (Fig. 7). While the straight-line gradient suggested that the C_m of human subjects was a little more sensitive to the dissolution rate than that of the rabbit, a high correlation existed between rabbits and humans, with a correlation coefficient of 0.997 ($p < 0.05$). Although the absolute values of T_{max} and T'_{max} seemed slightly smaller in the rabbit, the ratio for Formulas L, M, and N was fairly compatible between rabbits and humans for both T_{max} and T'_{max} (Tables II and III).

Although the dose levels of 500 mg/human and 62.5 mg/rabbit corresponded to ~10 mg/kg for humans and 20 mg/kg for rabbits, being twice as large in the rabbit, both the C_{max} and the AUC were larger in humans than in rabbits. This result probably comes from the difference in the drug absorption capacity of the GI tract or in the metabolism in the two species. This difference makes it difficult to compare the absolute values of bioavailability indexes. However, these findings suggest that the stomach-emptying-controlled rabbit is a useful animal model for examining comparative bioavailability properties for oral dosage forms.

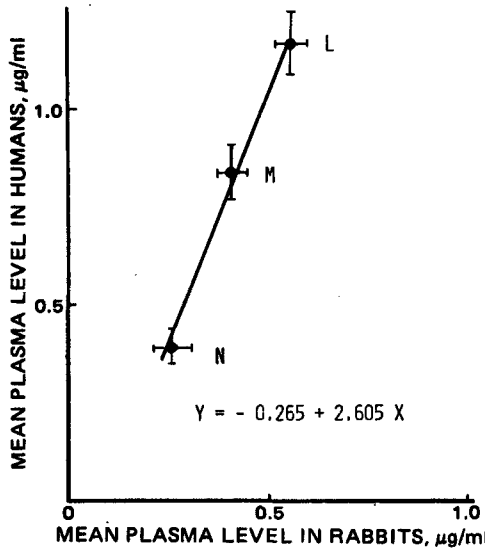

Figure 7—*Correlation of mean plasma griseofulvin levels in humans and in stomach-emptying-controlled rabbits for Formulas L, M, and N. Correlation coefficient = 0.997 (p < 0.05).*

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