large enough, while for sulfamethazine the acetyl derivative will cause crystalluria (this can be seen from the equation for Case H). In order to have the largest total concentration of sulfonamides without crystalluria, the ratio of sulfadiazine–sulfamerazine–sulfamethazine should be 1:3.1:3.9 (on a sulfadiazine equivalent). This is the ratio of the terms in the equation for Case H when $t_{s1} = t_{s2} = t_{s3} = \infty$. The ratio in terms of the individual compounds instead of the sulfadiazine equivalent is sulfadiazine–sulfamerazine–sulfamethazine = 1:3.2:4.3. On the average this ratio should have the least chance for crystalluria, if the kinetic constants are the best average ones. Since the kinetic constants in some cases were only estimated from blood level curves in the literature, they may not be the best.

It should be noted in Fig. 2 that the numerical values for D_T are large. This is a result of using the best ratio of sulfas for the given set of conditions and equations which assume a constant urine volume. The effect of varying urine volume or flow rate was not considered because the objective was only to calculate the best ratio of triple sulfas which gives the least chance of crystalluria. Case H will always be limiting whatever the flow rate.

The solubilities of the sulfonamides depend on pH. Above pH 7 the solubilities increase rapidly. The solubilities at pH = 6 of the three sulfonamides in question were chosen because this approaches the normal human urine pH, and the ratio of the solubilities of the three sulfonamides is almost constant over the pH range 5.6–6.5 (3). Therefore, the calculated ratio of 1:3.2:4.3 for the three sulfonamides should be the best ratio over the pH range 5.6 to 6.5.

As the solubility and/or the biological half-life of a drug increases, the incidence for crystalluria will become less. If the three sulfonamides in a combination have similar long biological half-lives, similar half-lives of acetylated derivative production and elimination, and similar fractions absorbed and volumes of distribution, then the ratio of the sulfonamides in the formulation should be the ratio of their solubilities.

The calculations indicate that kinetic aspects can be important in the simultaneous administration of drugs. The triple sulfas was used as an example for the application of kinetics and limiting solubilities to obtain a formulation with the best margin of safety. To show this increased margin of safety, a large-scale clinical test would be needed.

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Keyphrases

Drugs, simultaneous administration—kinetics
Sulfadiazine-sulfamerazine-sulfamethazine—co-administration
Absorption-elimination rates—simultaneous administration
Solubility, drug—simultaneous administration
Kinetic equations—multiple drug dosage forms

Griseofulvin Absorption in Man After Single and Repeated Treatments and Its Correlation with Dissolution Rates

By SAMSON SYMCHOWICZ and BERNARD KATCHEN

Absorption of griseofulvin from six different preparations was studied in man. Plasma levels were followed for 1 day after a single oral 500-mg. dose, and for 7 days after daily 500-mg. doses. A high correlation was found between dissolution rates in simulated intestinal fluid and absorption during 0–25 hr., 49–173 hr., and 0–173 hr.

Griseofulvin is widely used for treating fungal infections in man and animals. Its relatively high dose and meager water solubility (15 mcg./ml. at 37°) might contribute to its poor and variable absorption. Because certain minimal drug-plasma levels are needed for an effective cure (1–3), griseofulvin preparations must be carefully designed to ensure maximum absorption.

The authors have shown previously (4) that

Received February 27, 1968, from the Schering Corporation, Departments of Physiology and Biochemistry and Pharmaceutical Research, Bloomfield, NJ 07003
Accepted for publication May 2, 1968.
The authors thank Mr. M. Miller for the statistical analysis and Miss La Reine La Mar for her excellent technical assistance.

dissolution rates in simulated intestinal fluid correlate well with drug absorption following a single 500-mg. oral dose. The present communication shows there is also a correlation between dissolution rate and absorption following 7 daily 500-mg. doses of griseofulvin. Thus, the absorption characteristics of griseofulvin preparations can be conveniently predicted on the basis of dissolution rate data.

EXPERIMENTAL

In the first part of this study, four different griseofulvin preparations (No. 1–4) were given at weekly intervals to eight normal humans in a replicated Latin square crossover design (5). Before breakfast, each subject ingested one 500-mg. tablet (preparation 2) or four 125-mg. tablets (preparations 1,3,4).

In the second part of this study, three different griseofulvin preparations (No. 2,5,6) were given to 18 normal subjects. Each subject ingested one 500-mg. tablet (preparation 2) or two 250-mg. tablets (preparations 5,6). Preparation 5 has the same composition as 1, and preparation 6 the same composition as 3.

All subjects were told to avoid foods and drugs which interfere with the griseofulvin plasma assay (6). Blood was drawn at specified time intervals (Tables I, IV), and plasma samples were assayed fluorometrically (7) in duplicate. All plasma levels were corrected for their 0-hr. values.

Mean plasma level, which is the absorption index, was calculated by dividing the area under the plasma level curve (calculated by the trapezoidal rule) by the appropriate time interval.

Dissolution rates (Table III) were measured in simulated intestinal fluid as described previously (4). Single 125-mg. tablets, half of the 250-mg., and one quarter of the 500-mg. tablets were assayed.

RESULTS AND DISCUSSION

Single-Dose Study—Table I shows the individual plasma levels at different times following ingestion of the four preparations. Plasma levels vary widely, but, in general, peak at 4 hr. There are large variations in drug absorption between subjects on all preparations. This agrees with the findings of other investigators (1, 8).

Table II—Analysis of Mean Griseofulvin Plasma Levels by Duncan's Multiple-Range Statistic

Treatment[a]	Area/25
1	1.02
4	0.93
2	0.83
3	0.74

[a] Values not connected by a solid line differ significantly. $1 > 3$ ($p < 0.05$); $1 > 2$ ($p < 0.10$); $4 > 3$ ($p < 0.10$).

An analysis of variance procedure for Latin square crossover designs (5), applied to the plasma levels in Table I, shows that treatments, time, and the treatment × time interaction are statistically significant factors ($p = 0.05$). Duncan's multiple-range test, applied to the area/25 values in Table I, shows (Table II) that preparation 1 is significantly better than preparations 2 and 3, and that preparation 4 is significantly better than 3.

Figure 1 shows a good correlation between the mean plasma levels (area/25) in Table I and the logarithm of the 30-min. dissolution values in Table III. The correlation coefficient is 0.94 ($p = 0.04$). The 15- and 10-min. dissolution values have correlation coefficients of 0.96 ($p = 0.03$) and 0.92 ($p = 0.05$), respectively. These results agree well with the authors' previous single-dose study (4).

Because griseofulvin therapy requires prolonged drug administration, the correlation between dissolution rates and drug absorption during prolonged treatment was studied. This was also of interest

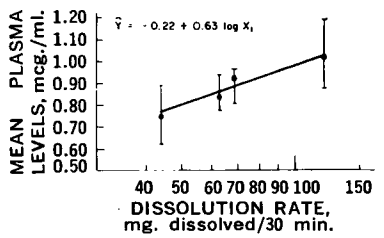

Fig. 1—Correlation of dissolution rate and mean griseofulvin plasma levels. Correlation coefficient = 0.94 (p = 0.04). Brackets enclose 95% confidence intervals of regression line.

Table I—Griseofulvin Plasma Levels (mcg./ml.) Following an Oral 500-mg. Dose

Preparation	Time, Hr.	Subject 1	2	3	4	5	6	7	8	Average	Area/25
1	2	1.73	2.20	1.23	1.23	0.62	1.13	0.35	1.00	1.18	1.02
	4	1.46	1.60	1.58	1.18	1.18	1.51	0.87	1.13	1.17	
	8	0.70	1.15	1.45	0.83	0.82	2.32	0.94	0.84	1.13	
	25	0.71	1.06	0.77	0.49	0.99	1.00	1.11	0.86	0.86	
2	2	0.72	1.07	0.29	0.82	0.43	1.49	0.29	0.75	0.73	0.83
	4	0.82	1.08	1.62	1.13	0.59	1.58	0.53	0.83	1.02	
	8	0.64	0.94	2.20	0.58	0.60	0.86	0.77	0.80	0.92	
	25	0.78	0.62	0.58	0.85	0.65	0.59	1.00	1.03	0.76	
3	2	0.29	0.67	0.49	0.56	0.47	0.75	0.35	0.48	0.51	0.74
	4	0.76	0.71	1.40	0.79	0.52	1.46	0.80	1.00	0.93	
	8	0.96	0.54	1.17	0.68	0.51	1.10	0.51	1.08	0.82	
	25	0.66	0.61	0.53	0.76	0.94	0.69	0.82	0.79	0.72	
4	2	1.00	0.66	0.94	0.64	0.15	1.07	0.50	0.27	0.65	0.93
	4	1.81	0.97	1.58	1.00	0.84	1.20	1.02	1.52	1.24	
	8	1.71	0.71	1.38	1.01	0.93	0.93	0.93	1.10	1.09	
	25	0.84	0.64	0.68	0.54	1.03	0.58	1.00	1.00	0.79	

TABLE III—DISSOLUTION RATES OF GRISEOFULVIN PREPARATIONS

Time, min.	% Dissolved Preparation[a] 1	2	3[b]	4	5	6
5	24	17	7	13	21	11
10	54	30	15	31	48	17
15	77	37	24	42	70	22
30	97	50	35	55	94	33
45	99		45	65	98	42
60	99	65	52	75	100	44

[a] Preparations: 1, 3, 4—125-mg. tablets; 5, 6—250-mg. tablets; 2—500-mg. tablets. [b] This preparation is the same as preparation 2 in the authors' previous study (4).

in view of a report (9) urging caution when interpreting data from a single-dose study with griseofulvin.

Multiple-Dose Study—Table IV shows the individual griseofulvin plasma levels obtained with three preparations, in 18 subjects treated daily for 7 days with 500 mg. of griseofulvin. Table V compares the mean plasma levels in the same eighteen subjects. The data in Table IV show that the peak and residual plasma levels for each treatment group and also within most of the subjects are remarkably constant after the second or third dose. Griseofulvin mean plasma drug levels plateau after the third daily dose with all preparations (Table V).

Analysis of variance of the mean plasma levels in Table V does not show any statistically significant differences between preparations. This is probably due to the small number of subjects, large intersubject variation, and lack of a crossover design. Despite the lack of demonstrable statistically significant differences between preparations, de-

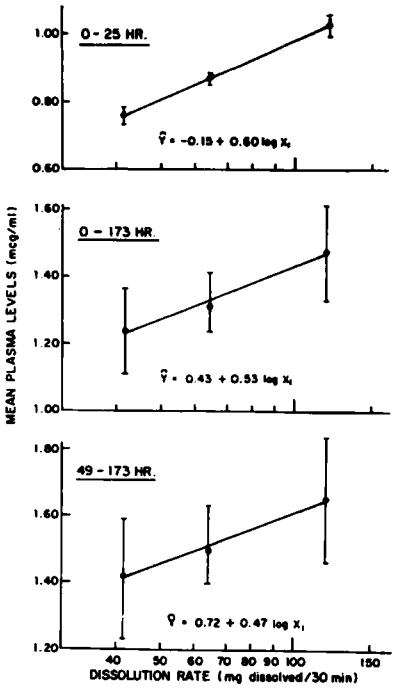

Fig. 2—Correlation of dissolution rate and mean griseofulvin plasma level. Correlation coefficients are: 0.999 (p = 0.015) for 0–25 hr., 0.992 (p = 0.077) for 0–173 hr., and 0.995 (p = 0.056) for 49–173 hr. Brackets enclose 95% confidence intervals of regression lines.

TABLE IV—GRISEOFULVIN PLASMA LEVELS (mcg./ml.) FOLLOWING AN ORAL 500-mg. DOSE DAILY FOR 7 DAYS[a]

Preparation	Subject	Time, hr. 2	4[b]	8	25[c]	49[c]	53[b]	97[c]	101[b]	169[c]	173[b]
2	1	0.97	0.92	0.78	0.74	0.89	1.60	0.96	1.82	1.16	2.16
	2	0.49	1.68	1.12	0.61	0.85	1.44	1.03	2.44	0.97	1.86
	3	0.54	0.71	0.65	0.43	0.96	1.65	0.85	1.79	1.12	2.20
	4	0.41	1.68	1.12	0.92	1.29	2.11	1.34	2.48	1.03	2.40
	5	0.76	1.87	1.42	0.53	0.76	2.49	0.74	2.30	0.85	2.39
	6	0.45	1.31	1.03	0.87	1.09	1.87	1.15	1.60	0.94	1.37
	Average	0.60	1.36	1.02	0.68	0.97	1.86	1.01	2.07	1.01	2.06
5	7	1.43	1.14	0.98	0.90	1.19	1.85	1.23	2.35	1.30	1.96
	8	1.19	0.97	0.83	0.52	0.81	1.69	0.77	1.54	0.75	1.47
	9	0.90	1.36	0.97	0.83	0.83	1.26	1.20	1.98	1.10	1.72
	10	1.27	1.54	1.17	0.93	1.55	2.66	1.57	2.14	1.44	2.33
	11	2.08	2.22	1.89	1.23	1.35	2.58	1.99	2.98	1.35	2.41
	12	0.81	1.14	0.56	1.14	1.27	1.77	1.25	1.65	1.30	1.88
	Average	1.28	1.39	1.07	0.92	1.16	1.97	1.33	2.11	1.21	1.96
6	13	0.41	0.70	0.63	0.80	1.12	1.17	1.38	1.79	1.18	1.61
	14	1.20	1.90	1.75	0.82	1.25	2.70	1.36	2.38	1.36	3.00
	15	0.68	0.51	0.68	0.61	1.18	1.23	0.97	1.43	0.93	1.04
	16	0.44	0.98	0.62	0.36	0.84	1.38	0.85	1.46	0.69	1.32
	17	0.67	0.80	0.77	0.74	0.84	2.31	0.91	2.27	0.88	1.99
	18	0.78	1.07	0.69	0.60	0.77	2.01	0.75	2.15	0.65	1.16
	Average	0.70	1.00	0.86	0.65	1.00	1.80	1.04	1.91	0.95	1.68

[a] Drug was given at 0, 25, 49, 73, 97, 121, 149, and 169 hr.; plasma was analyzed at the indicated time intervals. [b] Peak plasma levels. [c] Residual plasma levels (blood drawn just prior to drug ingestion).

TABLE V—MEAN GRISEOFULVIN PLASMA LEVELS, mcg./ml.[a]

Preparation	Hr. 0–25	25–49	49–53	53–97	97–101	101–169	169–173	49–173	0–173
2	0.87	0.83	1.42	1.44	1.54	1.54	1.54	1.50	1.31
5	1.03	1.05	1.57	1.65	1.72	1.67	1.58	1.65	1.48
6	0.76	0.84	1.40	1.42	1.47	1.43	1.32	1.42	1.24

[a] Areas under curve divided by periods as indicated.

tailed inspection of the data reveals some interesting points. For example, the ranking of preparations based on mean plasma levels during the first 25 hr. (single dose) remains the same throughout the entire experiment (except for the 25–49-hr. period). Also, if the residual plasma levels are considered (values 25 hr. after the first dose, and 24 hr. after the second, fourth, and seventh doses), the following statistically significant differences emerge: at 25 hr., preparation 5 > 6 (p = 0.1, least significant difference), at 97 hr., preparation 5 > 6 (p = 0.1, Duncan's multiple-range statistic), and 5 > 2 (p = 0.05, Duncan's multiple-range statistic). Residual plasma levels may be important in griseofulvin therapy since a minimum level of 1 mcg./ml. appears to be needed for an effective cure (1).

When the dissolution values (Table III) are correlated with the mean plasma levels (Table V), a good correlation is seen (Fig. 2) between the logarithm of the 30-min. dissolution values and mean plasma levels after 25 hr. (effect of a single dose), 49–173 hr. (the plateau region), and 0–173 hr. (total experimental period). The correlation coefficients for the 3 time periods are 0.999 (p = 0.015), 0.992 (p = 0.077), and 0.995 (p = 0.056). The slope of the 0–25-hr. regression line compares favorably with the slope of the single-dose treatment (0.60 in Fig. 2 *versus* 0.63 in Fig. 1). The slopes for the later time intervals (Fig. 2) are lower than the 0–25-hr. slope because differences between the mean plasma levels of the preparations are smaller for the 49–173-hr. period.

Since dissolution rates correlate well with griseofulvin absorption after a single dose, as well as after repeated drug administration, they should serve as an effective tool for selecting therapeutically useful griseofulvin preparations.

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Keyphrases

Griseofulvin absorption—human
Absorption-dissolution rate correlation—griseofulvin
Plasma levels, griseofulvin—single, multiple doses
Fluorometry—plasma griseofulvin analysis

Further Applications of Diazotized 4-Amino-6-chloro-*m*-benzenedisulfonamide

Colorimetric Determination of Pyridoxine Hydrochloride, 17β-Hydroxy-7aα-17α-dimethyl-B-homo-A-norestrane-3,6-dione and Morphine Sulfate

By TIBOR URBÁNYI and SUSAN BUDAVARI

The diazotization product of 4-amino-6-chloro-*m*-benzenedisulfonamide has been found to be a useful reagent for the colorimetric determination of a vitamin, steroid, and alkaloid. A general method, with slight modification, can be applied to those compounds which contain a phenolic or similar-to-phenolic hydroxyl group. Pyridoxine hydrochloride, 17β-hydroxy-7aα-17α-dimethyl-B-homo-A-norestrane-3,6-dione, and morphine sulfate were used as coupling components in this study. The optimum reaction conditions for coupling and color formation have been determined and the applicability of the method in the presence of commonly used ingredients is demonstrated.

A REPORT ON the colorimetric determination of estrogenic hormones by coupling with diazotized 4-amino-6-chloro-*m*-benzenedisulfonamide reagent has already been published (1). The purpose of the present investigation was to extend the application of that method to the quantitative determination of a phenolic alkaloid,

Received March 25, 1968, from the Analytical Research and Development Division of CIBA Pharmaceutical Company, Summit, NJ 07901
Accepted for publication May 10, 1968.