LABORATORY EXPERIENCES WITH GRISEOFULVIN

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Griseofulvin, an antibiotic derived from penicillium species has elicited unusual interest because of its distinctive activity against dermatophytes, both *in vitro* and *in vivo* (1, 3, 5, 6). The following studies were undertaken to further evaluate the *in vitro* inhibitory effects and the possible toxic effects of this new remedy.

TABLE I

*Sensitivity studies with Griseofulvin**

	Griseofulvin μg/ml of Sabouraud Glucose Agar										Griseofulvin Plus Penicillinase*							
	80	40	20	10	5	2.5	1.3	.65	.3	.15	80/160	40/120	20/80	10/60	5/30	2.5/15	1.3/7	.65/3.5
M. audouini	−	−	−	−	−	−	−	−	−	+	/	/	/	/	/	/	/	/
M. canis	−	−	−	−	±	±	+	+	/	/	−	−	−	−	±	±	+	+
M. gypseum	−	−	−	−	+	+	+	+	/	/	−	−	−	−	+	+	+	+
T. rubrum	−	−	−	−	−	±	±	+	/	/	−	−	−	−	±	±	±	+
T. mentagrophytes	−	−	±	+	+	+	+	+	/	/	−	−	+	+	+	+	+	+
T. tonsurans	−	−	−	−	−	−	±	+	/	/								
Penicillium sp	+	+	+	+	+	+	+	+	/	/								
Aspergillus sp	+	+	+	+	+	+	+	+	/	/								
H. capsulatum	+	+	+	+	+	+	+	+	/	/								
B. dermatitidis	+	+	+	+	+	+	+	+	/	/								
S. schenckii	+	+	+	+	+	+	+	+	/	/								
N. asteroides	+	+	+	+	+	+	+	+	/	/								
N. brasiliensis	+	+	+	+	+	+	+	+	/	/								
Mucor sp.	+	+	+	+	+	+	+	+	/	/								

Key: * $\frac{80}{160}$: means 80 μg of Griseofulvin and 160 units of Penicillinase

− = complete inhibition

± = partial inhibition

\+ = no inhibition

/ = test not performed

* Variation in the sensitivity of different dermatophytes in our experiments and in comparison with the data obtained by other authors are related to the poor solubility of griseofulvin and the inherent difficulty of a constant and reproducible distribution of the antibiotic in solid media.

The readings in this table were recorded after 10 days observation.

MATERIAL AND METHODS

1. The antibiotic (which is almost insoluble in water) can be incorporated into solid media but when serial dilutions are made the calculated concentration is probably greater than the actual content. Therefore the minimal inhibitory concentration given in this paper can only approximate, and represents a truly "minimal" value.

2. The curling of hyphae under the influence of griseofulvin was studied by placing small amounts of the antibiotic several cms. from already developed fungus colonies on the surface of the agar plates.

3. Feeding experiments were made in order to establish the influence of the antibiotic on the development of infantile mice. Griseofulvin was incorporated into the animal food in different concentrations and the weight of the animals was established at regular intervals.

4. Brain, heart, lungs, liver, spleen, adrenals,

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kidneys, testicles or ovaries were examined microscopically after the animals were sacrificed.

5. Penicillinase was added to griseofulvin containing media to study the influence on the antibiotic's effect.

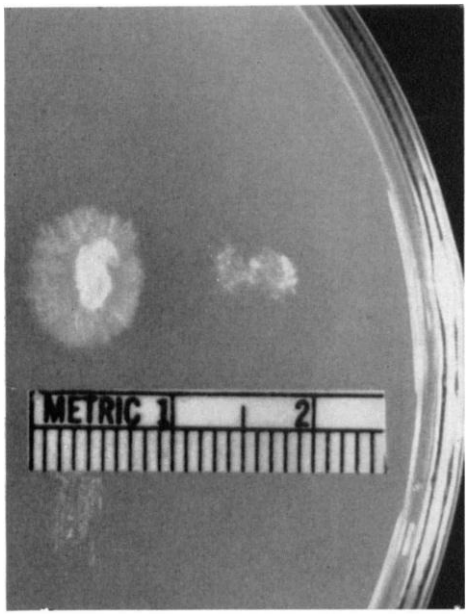


FIG. 1. Griseofulvin is placed on the agar close to the already developed colony of *M. canis*.

FIG. 2. The terminal hyphae of the "normal" colony are straight 53×.

FIG. 3. Few hours after addition of griseofulvin the hyphae become tortuous 53×.

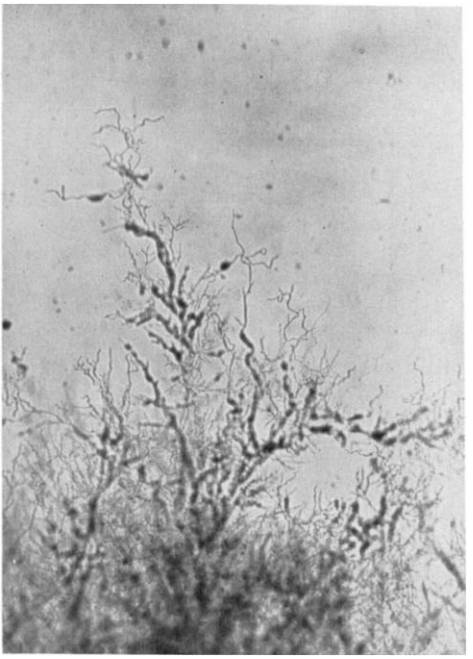


FIG. 4. This increases, producing distortion of the whole pattern. Notice the numerous lateral branches 53×.

RESULTS

Our results are in general agreement with the experience of others (1) demonstrating selective inhibition of dermatophytes and essential absence of influence upon the growth of other pathogenic fungi.

FIG. 5. Clumping together of the mycelium and fungistasis 53×.

TABLE II

Code Letter	Absolute Weight, grams			% Weight Increase in Relation to Initial Weight		Mg. Griseofulvin Intake per kg. of Actual Weight per day
	Initial	After 10 days	After 20 days	After 10 days	After 20 days	
G	16	17.2	22.3	7.5	39	0
H	16.2	18.1	22.9	12	42	0
F	17	22.2	23	30.6	35	15
K	15.4	21	22.7	36	47	16
L	15.5	21	22.5	36	45	29
M	15.8	20	22.1	36	40	66
A	18.8	21.3	22	13	17	125
C*	16.9	18.2	19.6	7	16	125
B	17.6	17.3	17.6	0	0	1350
E	17.2	17	19.8	0	15	1320
D*	18.1	18.7	19.4	0.5	7	1350

* Female, all other groups were males. Each group consisted of 25 animals. G & H are control groups getting normal (Rockland) mouse food.

Statistically differences of the initial weight of the animals were proven not to be significant.

The weight increase on low dosage of griseofulvin after ten days was significantly larger than 5.05 (F = 33.2); this was true even after twenty days albeit to lower degree (F = 16.4). "F values" from Dixon, W. J. and Massey, F. J. Introduction to statistical analysis. McGraw-Hill Book Company, New York 1951.

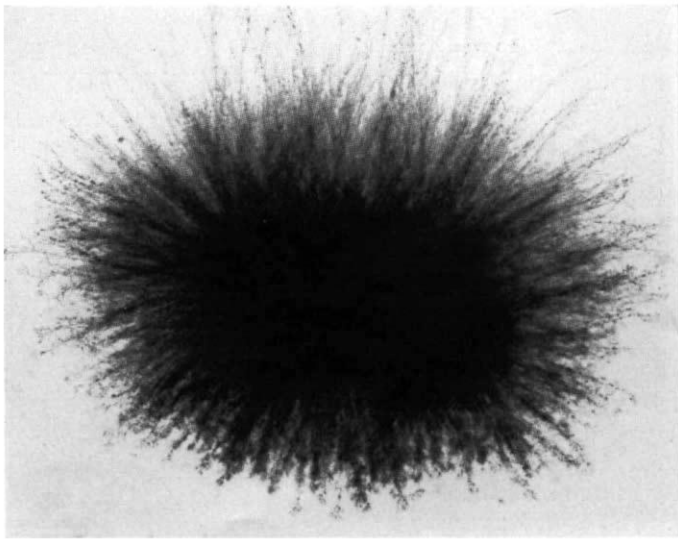
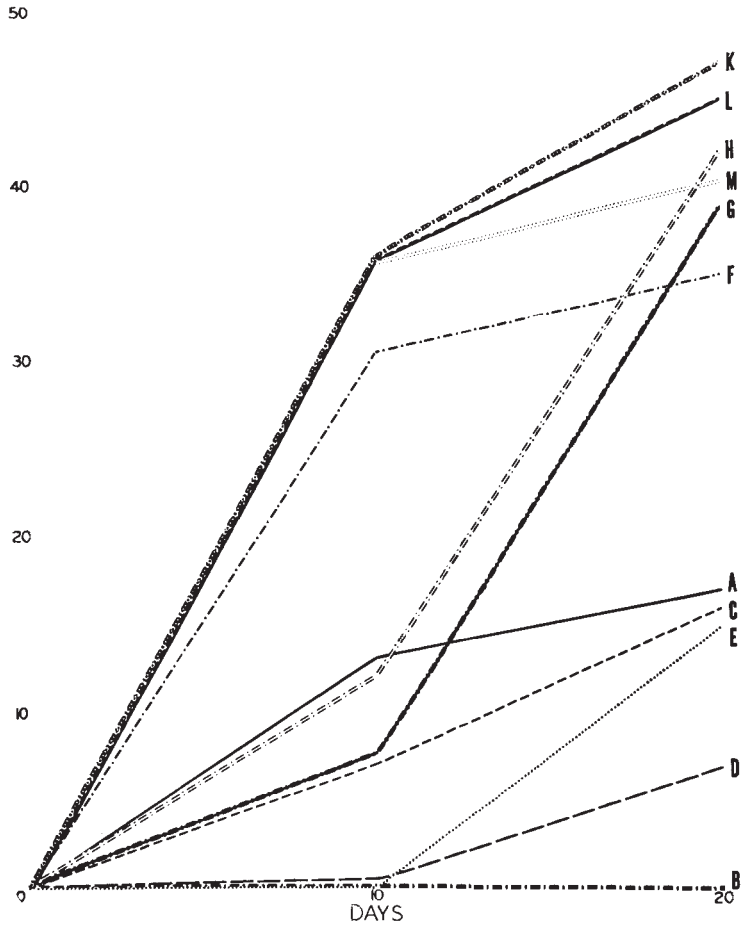


FIG. 6. The colony shows completely degenerated hyphae (bottom) close to the griseofulvin. The distant part of the colony (top) shows "curling" 7.9×.



GRAPH 1. The percentual increase of weight in infantile mice was plotted after 10 and 20 days feeding with griseofulvin (see Table II).

Two to three hours after griseofulvin was placed on agar plates in the vicinity of a well developed colony of *M. canis* the straight hyphae which were growing in the periphery of the colony became slightly undulated, developed curved short lateral branches and finally were so curled that any resemblance with the original morphology disappeared. The final state was a real degeneration and clumping of the mycelial growth. (Figs. 1–6).

Small amounts of griseofulvin given by mouth increased considerably the weight after ten days in infantile mice, whereas large amounts had definite inhibitory effect on the development.

Intake under 1 gram of griseofulvin per kg. of body weight per day had no recognizable effect on spermatogenesis. If 1.3 g. per kg. per day was given for 18 to 24 days, considerable decrease, or complete suppression of spermatogenesis resulted. The ovaries did not show comparable

TABLE III

	Spermatogenesis					
		Suppression				
	Normal	Slight	Moderate to severe	Severe	Almost complete	Complete
Low diet 19–24 days (19 animals examined)*	14	5				
High diet 12–19 days (6 animals examined)*			3	1		2
High diet 24 days (11 animals examined)†	2		3	1	2	3

* 125 mg./kg. per day
† 1.349 g./kg. per day

damage and looked as healthy as the rest of the organs even in series with heavy daily intake, 1.3 g. per kg.

If penicillinase was added to the griseofulvin-containing media no effect of the penicillinase was noted.

DISCUSSION

Griseofulvin promises to be a major factor in the treatment of dermatophytes because of its exquisite fungistatic activity especially against *T. rubrum*, which was so difficult to cure in the past. Microsporum species and other trichophytons show also impressive inhibition of growth in presence of micrograms of the antibiotic.

The morphologic changes induced by griseofulvin, and named "curling factor" (2, 4) can be studied within a few hours with the simple technic described above. Developed colonies show the curling in more spectacular form than can be seen incorporating griseofulvin into the media prior to inoculation.

The weight increase in infantile mice fed with doses of griseofulvin which were comparable to therapeutic levels in men was more prominent than in control animals. Factors similar to the ones obtained by feeding certain broad spectrum antibiotics came to mind.

No toxic effects were observed in animals eating less than 1 gm. griseofulvin per kg. per day. Toxic levels seemed to be reached over 1 gm. per kg. per day. Minimal or no weight increase occurred in those animals.

The only morphologically detectable lesions after feeding griseofulvin to mice up to 1.3 gm. per kg. per day were found in the form of partial to total suppression of spermatogenesis.

Penicillinase does not destroy the action of griseofulvin *in vitro*.

SUMMARY

The antibiotic griseofulvin is *in vitro* inhibitory for dermatophytes even in minimal concentrations.

Toxicity after oral intake of the antibiotic seems to be limited to influence on spermatogenesis and occurs only at levels 60 times larger than the recommended therapeutic dose in men.

ACKNOWLEDGMENT

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