

Mycotic Diseases

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OBJECTIVE: The objective of these studies is to gather information on the prevalence and distribution of mycoses in this area of the world. In addition to these survey activities, one study was carried out to determine the *in vitro* effects of griseofulvin on morphological changes of selected dermatophytes isolated in Thailand and another was done to identify the bacterial components of dermatologic infections.

DESCRIPTION: Survey studies were prompted by the major medical problems the dermatophytic fungi can present to the military in times of stress. Specimens were usually collected by a member of this department from patients with dermatologic problems. Cultures were prepared by cleansing the lesion with 70 percent ethanol and transferring material (hair, skin, nail) directly to 2 plates of Sabouraud—Cycloheximide—Chloramphenicol medium. The plates were sealed with paper tape to prevent contamination, and periodically examined during a 21 day incubation at 25°C. Blood agar plates were also inoculated and incubated at 37°C when clinical appearance of the lesion suggested bacterial infection.

Thirteen recent isolates of dermatophytes were exposed to concentrations of griseofulvin ranging from 0.1 to 30 mcg/ml in Sabouraud—Cycloheximide—Chloramphenicol agar or broth. Colonial and microscopic changes were observed at 5, 10 and 15 days after inoculation and incubation at 25°C.

In the study of the bacterial component of dermatologic infections, culture sites for bacteria and fungi included the site of the lesion, canal of the right ear, right nostril, beneath the index fingernail of the right hand, perineum and the fourth toeweb of the right foot. Procedures listed above were used for fungus cultures and a battery of culture media designed to favor different genera were used for bacterial cultures.

PROGRESS: During the period covered by this report 755 routine clinical specimens were received for mycological examinations. Included were 212 from Women's Hospital, and 36 from the U.S. Embassy Medical Unit and the 5th Field Hospital. Results in Table 1 show that the organisms most frequently isolated from Thai patients were Trichophyton rubrum and Candida albicans. Five of 36 dermatologic specimens from U.S. personnel and dependents were positive for C. albicans; two were positive for T. rubrum and 1 was positive for Trichophyton mentagrophytes (Table 2). In specimens from other than human sources there were 5 isolates of Microsporium canis from 6 gibbons and 5 isolates of Microsporium gypseum from 246 soil samples (Table 3).

The study of the interaction(s) of dermatophytes and griseofulvin was undertaken because of concern to clinicians about the likelihood that griseofulvin—resistant strains of dermatophytes would emerge and the possibility that resistant forms would have different characteristics which would complicate their identification. This study was concerned with sequential macroscopic and microscopic changes of recently isolated dermatophytes in the presence of increasing concentrations of griseofulvin.

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The presence of griseofulvin in media in which the dermatophyte cultures grew resulted in distinctive changes of growth rates of the organisms in both liquid and solid media. As expected the growth rates progressively decreased with increasing concentrations of the drugs. Minimum inhibition concentrations (MIC) in fluid media paralleled results obtained with solid media but in most instances the MIC levels were lower in the former. It is assumed that this finding resulted from the organisms in the liquid media being in more intimate contact with the drug. Other morphological changes were those of texture, topography, and particularly, pigmentation which varied with different species. T. rubrum and T. mentagrophytes cultures developed increasing numbers of white tufts on the surface as the drug concentrations were increased. At 1.0 mcg/ml or more the colonies appeared as white and cottony; Trichophyton concentricum cultures changed from velvety to glabrous; M. canis changed from yellowish-brown with white cottony centers to light brown and glabrous throughout and the topography and pigmentation of Epidermophyton floccosum changed from flat, olivegreen colonies with white centers to pale olive or light brown. Only M. gypseum failed to show marked changes in texture. The inocula of all species were macerated at inhibitory concentrations of griseofulvin.

Although there were variations in the concentrations of griseofulvin eliciting morphological changes, the patterns of macroscopic and microscopic alterations were similar for all dermatophytes studied. In absence of griseofulvin the mycelium were long, septate with straight, rarely-branched hyphal tips and contained homogenous cytoplasm. After exposure to increasing concentrations of griseofulvin the following sequential changes occurred in the mycelium of all isolates. The earliest changes were the appearance of lateral branches, curling of advancing hyphae and appearance of granular cytoplasm in mature areas of hyphae. At higher concentrations these were followed by increased branching, increased curling of the lateral branches at the ends of new mycelium and the presence of only granular cytoplasm.

At concentrations almost inhibiting new growth, branching and curling had progressed to the point of stunting and distortion and cytoplasm was granular and vacuolated. At inhibitory concentration of griseofulvin there was autolysis, vacuolated cytoplasm, and subsequent loss of mycelial contents. Griseofulvin incorporated in the culture medium resulted in fewer microconidia but their shapes and sizes were unaffected. Similar decreases of macroconidia were noted but morphologic changes were discernible even at low concentrations. These changes ranged from a slight bending at low concentrations to the appearance of distorted and ghost cells at inhibitory concentrations.

The similarity of the sequential changes in the presence of increasing griseofulvin concentrations suggests—but does not prove—similar mode of action of this drug for each of these organisms. The observation that the low concentrations first affected new growth and that the drug is mycostatic rather than mycocidal is in keeping with therapy experiences which show that griseofulvin appears to produce a favorable clinical effect by concentrating at the point of infection. There it inhibits the fungi and causes them to be shed with the normal outward growth and desquamation of the skin. Although the exact mode of action of griseofulvin is not known, it appears to interfere with fungal mitosis, impairs synthesis of protein and nucleic acids and causes the breakdown of intracellular organelle membranes.

The interpretation of in vitro sensitivity determinations in terms of predictability of clinical effectiveness of griseofulvin is difficult. The usual criterion of relating microorganism sensitivities to blood levels does not pertain because these organisms are capable of in vitro growth at much higher concentrations of griseofulvin than is found in the blood after parenteral administration of the drug. It is concluded that therapeutic response to the drug depends on its concentration at the site of infection rather than in blood levels. A comparison of in vitro sensitivities of the strains used in this study to those reported in the literature suggests that even when one allows for differences of assay procedures, the dermatophytes isolated in Thailand were somewhat more resistant than those found elsewhere.

This study does not preclude the possibility that bizarre, mutant dermatophytes resulting from in vivo exposure to griseofulvin will not create diagnostic problems in the future. However the finding that morphologic changes of most of the griseofulvin-induced morphologic changes of these dermatophytes were completely reversible on subsequent subculturing is encouraging from the standpoint of the clinical laboratory.

Reports of therapeutic failures with griseofulvin are appearing. The finding of griseofulvin-resistant dermatophytes in Thailand indicates that griseofulvin will become less effective as more fungi become resistant following exposure to the drug. To preclude unnecessary exposure to sensitive dermatophytes it is recommended diagnoses be confirmed by laboratory procedures prior to institution of griseofulvin therapy. Ideally there should be repeated cultures during therapy because the only criterion that can determine the required dosage levels and duration of treatment with griseofulvin and the final attainment of a biological cure are laboratory findings obtained by the medical mycologist.

Incomplete studies on the bacterial ecology of adult Thai females with dermatologic infections indicate that Staphylococcus epidermidis, Corynebacterium spp., Micrococcus spp. were found at most culture sites of patients infected with T. rubrum and of normal controls. Bacteria found less frequently at all culture sites included Staphylococcus aureus, Streptococcus fecalis, Pseudomonas spp. and alpha hemolytic streptococci. Conspicuous by its relative scarcity was Escherichia coli. This study will be completed by 30 June 1970.

SUMMARY: Studies on patients with dermatologic problems indicate that pathogenic fungi are present in Thailand and could represent a major problem to the military in times of stress. Pathogenic dermatophytes isolated most frequently were T. rubrum and C. albicans. Macroscopic and microscopic changes resulting from exposure to graded concentrations of griseofulvin were observed for 31 recent isolates of dermatophytes. Most isolates were exposed to different concentrations of griseofulvin ranging from 0.1–30.0 mcg/ml in Sabouraud–Cycloheximide–Chloramphenicol Agar in petri dishes or Sabouraud–Cycloheximide–Chloramphenicol Broth in test tubes. Colonial and microscopic changes of cultures were observed at 5, 10 and 15 days at 25° C after inoculation. The results obtained indicated that griseofulvin created morphological changes of every isolate, especially on the microscopic structures of the mycelium. Discernible effects occurred at every concentrations of the drug. The first change was the production of short, lateral hyphae from the primary mycelium followed, as the drug concentration increased, by curling, distorting and stunting of lateral branches and new mycelial growth. The cytoplasm of the mycelium was gradually changed from homogenous to granular to vacuolar and, at sub-inhibitory concentrations, the cytoplasm partially disappeared from the mycelium. Large, round chlamydospores germinated from the mature hyphae of most isolates at sub-inhibitory concentrations of griseofulvin. At inhibitory concentrations large round "ghost" cells or cells devoid of content occurred in most areas of the mycelium. The changes of shape and size of microconidia were little affected but they were reduced in number and they became poorly developed at high drug levels. Numbers of macroconidia decreased as the drug concentrations were increased and their shape became distorted in high concentrations. The macroscopic and microscopic changes of most isolates caused by griseofulvin appeared to be temporary. Most dermatophytes reverted to normal when sub-cultures of viable pleomorphic colonies were made on griseofulvin-free medium. The bacterial flora of adult Thai females with or without T. rubrum infections consisted predominantly of gram-positive cocci and rods. Pseudomonas spp. was the gram-negative organism found most frequently.

Table 1. Mycology Specimens from Patients at Women's Hospital, Bangkok, Thailand
1 April 1969–31 March 1970

Body areas	Total Specimens Examined	Negative for Fungus	Non-pathogenic Fungi Isolated	Positive Cultures
Body (Trunk, Face, Arms, Legs)	75	46	13	Trichophyton rubrum 9 Epidermophyton floccosum 1 Trichophyton mentagrophytes 3 Candida albicans 2 Pityrosporum orbicularae 1
Feet	54	24	26	Candida albicans 1 Trichophyton rubrum 3
Hands	22	15	3	Trichophyton rubrum 1 Candida albicans 3
Head	16	8	6	Trichophyton tonsurans 1 Trichophyton rubrum 1
Nails	16	4	4	Candida albicans 7 Trichophyton rubrum 1
Groin	14	1	1	Trichophyton rubrum 2 Trichophyton mentagrophytes 4 Epidermophyton floccosum 1 Candida albicans 4 Microsporum gypseum 1
Buttocks	8	3	1	Trichophyton rubrum 4
Vagina	3	—	3	—
Ear	2	2	—	—
Axilla	2	1	—	Trichophyton rubrum 1
Total	212	104	57	51

Table 2. Routine Mycology Specimens from American Nationals
1 April 1969-31 March 1970

Body areas	Total Specimens Examined	Negative for Fungus	Non-Pathogenic Fungi Isolated	Positive cultures
Body (Trunk, Face, Arms, Legs)	6	3	2	Trichophyton mentagrophytes 1
Feet	5	3	—	Trichophyton rubrum 1 Candida albicans 1
Hand	1	1	—	—
Head	1	1	—	—
Hair	1	—	1	—
Nails	8	5	1	Trichophyton rubrum 1 Candida albicans 1
Sputum	10	4	3	Candida albicans 3
Lymph node tissue	1	—	1	—
Pus	1	—	—	Candida species 1
Cerebrospinal fluid	1	1	—	—
Lung aspirate	1	1	—	—
Total	36	19	8	9

Table 3. Mycology Specimens from Miscellaneous Sources
1 April 1969—31 March 1970

Sources	Total Specimens Examined	Negative for Fungus	Non-Pathogenic Fungi Isolated	Positive cultures
Dog's skin	1	1	—	—
Dog's ear	1	—	1	—
Gibbons' hair	6	—	1	<i>Microsporum canis</i> 5
Sheep blood	1	1	—	—
Soil	246	225	16	<i>Microsporum gypseum</i> 5
Water	194	163	31	—
Bats' lungs	55	42	13	—
Culture media	2	—	2	—
Human globulin immune serum	1	1	—	—
Total	507	433	64	10