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LABORATORY STUDIES OF MILITARY MYCOSES  
FINAL PROGRESS REPORT

By:

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and

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~~July 1978~~

(For the period 1 October 1968 to 31 March 1971)

Supported by:

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701  
in cooperation with the Commission on Cutaneous Diseases  
of the Armed Forces Epidemiological Board

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University of Miami  
Miami, Florida 33152

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Representative strains of dermatophytes from Vietnam and stateside installations were assayed for sensitivity to griseofulvin and for production of proteolytic enzymes. A new selective medium for the isolation and differentiation of dermatomycetes (DTM) from saprobic fungi was developed and tested in the field with great success. The principle employed was the inherent capacity of keratinolytic fungi to alkalize medium. The addition of Dieldrin to our routine Sabourauds medium proved of value in controlling mycophagous mites. A serum-substitute for the identification of <u>Candida albicans</u> by the production		

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20. (continued) A pseudogerm-tubes was designed and proved its value in extensive testing in the field situation. A selective and highly differential medium for the specific isolation and identification of C. albicans was developed. Its selectivity is dependent upon use of a specific carbon source, a critical pH, a high concentration of cycloheximide, and an elevated incubation temperature. The value of the drug Pimaricin, a polyene antifungal antibiotic, was established for the treatment of mycotic corneal ulcers. An isolation and transport medium for streptococci occurring in pyodermas in tropical environments was developed and proved to be a reliable and valuable field procedure. Two well-accepted diagnostic mycology manuals, dealing with dermatophytes and medically important yeasts, and compiled from data and observations derived, in part, from the studies supported by this contract were published and still enjoy wide use.

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This contract (DADA 17-69-C-9039) was initially granted to augment and secondarily support that of Blank and Taplin (DA-49-193-MD-2236) by providing diagnostic and mycologic research in conjunction with the Vietnam operation. The contract-supported work at the University of Miami consisted of the identification of fungi and yeast-like organisms isolated from service personnel in Vietnam theater as well as those obtained from the Elgin Ranger School, Fort Benning and WRAIR. These isolates, numbering over 1200 strains, were catalogued and representative growth forms were tested for sensitivity to griseofulvin, assayed for production of elastase and collagenase, and used for defining generic and species taxonomic limits.

Partial support from this contract of a research scientist (Gerbert Rebell) and supportive technicians (L. Lansdell, L. Jaynes, M. Gomez, and A. Denavare) contributed to the success of this study and those of Blank and Taplin. In that these individuals were involved with many interrelated aspects of the total laboratory effort in Miami, their activities and accomplishments are primarily recorded in the Annual Reports to the Armed Forces Epidemiological Board of the Commission on Cutaneous Diseases, 1967 to 1971 by D. Taplin, H. Blank and A. Allen.

As the Vietnam effort began to diminish, a number of related research studies in military mycology and bacteriology were instituted. Of paramount interest were the clinical laboratory aspects of fungus and bacterial infections of the skin in the tropics.

A total of 228 dermatophytes comprising Trichophyton rubrum, T. mentagrophytes and Epidermophyton floccosum were assayed for sensitivity to griseofulvin.

These fungi were largely those isolated in Vietnam but a representative number of stateside strains from recalcitrant cases of *tinia pedis* and *cruris* were included. The range of minimal inhibitory concentrations at 72 hours of incubation ranged from 0.12 to 0.48 mcg/ml, all of which are considered to be within the normal sensitivity range. In that no isolate demonstrated significant resistance to the antifungal drug, treatment failure, when observed, must be due to factors other than developed resistance to griseofulvin by the dermatomycete. In all cases tested, the drug was limited to fungistatic activity and no true fungicidal effects were observed. No correlation with severity of lesions, chronicity of infection, or zoophilic or anthropophilic nature of the mycotic agent was evidenced. These findings are in accord with those of others which indicate that development of a major magnitude drug-resistance by dermatomycetes to griseofulvin has not yet been observed and reported.

Many of the Vietnam dermatomycetes were employed to evaluate a new diagnostic medium for the selective isolation and differentiation of these keratinophilic pathogenic fungi. This new mycologic medium, named Dermatophyte Test Medium (DTM), is made selective and different according to its formula which includes a mold inhibitor -- Actidione, two antibacterial antibiotics -- chlor-tetracycline and gentamicin, and a pH indicator -- phenol red. The fact that dermatomycetes change the pH to the alkaline range during growth is a distinctive metabolic attribute among the fungi. Hence, aringworm fungus growing on DTM will change the medium from its original yellow hue to a bright and distinctive red. Since its introduction this medium has been enthusiastically accepted by clinical microbiologists throughout the world and it is now routinely employed for the

isolation and presumptive identification of these pathogenic keratinolytic cutaneous mycotic agents.

Four shipments of dermatophytic cultures prepared in Vietnam and shipped to Miami via surface mail or delayed in transit were found to be heavily infested with mycophagous mites. These fungus-eating arachnids, besides destroying the aerial portion of the dermatophyte's thallus, introduce diverse bacterial and mycotic contaminants into the originally pure culture by fecal and body contaminants from the mites. A study was undertaken to compound a medium which would control this serious problem. A survey of available insecticides and miticides was undertaken. The best control was secured with the pesticide Dieldrin (Velsicol) at a concentration of 20 parts per million in Mycosel medium with gentamicin. This formulation has largely eliminated this scourge and has proven invaluable in maintaining the fungi in our large culture collection.

The relative simplicity and high order of accuracy of the Germ Tube Induction Test for the specific identification of the pathogenic yeast, Candida albicans, as conducted in human serum, prompted the search for a more readily available medium. Under field conditions or in areas with rudimentary laboratory facilities, sterile serum is usually difficult to obtain. A serum substitute was formulated which performed as well as human serum and a tissue culture medium (TCM-199) and was simple enough to prepare even under relatively restricted laboratory conditions. The semi-synthetic germ tube induction fluid (GTF) was composed of 2.6 per cent Oxoid beef extract, 0.16 per cent dextrose in physiological saline and was sterilized by autoclaving. The specificity of the germ tube test with the GTF was established with 61 species of yeast-like growth forms representing 11 difficult genera. This formulation has been widely accepted and is being used in many diagnostic laboratories, including the C.D.C. in Atlanta.

The need for a primary selective and differential medium for the isolation of Candida albicans from clinical materials grossly contaminated with bacteria, filamentous fungi, and other species and genera of non-pathogenic yeast-like growth forms has long been recognized. The frequent occurrence of this opportunistic pathogen in clinical skin samples from Vietnam and stateside military situations has prompted us to endeavor to design a medium for the selective and differential isolation of C. albicans. Following a prolonged study with specimens from the oral cavity and diverse skin sites a medium was devised which has proven to be quite successful. The selective basis of this formulation is due to several interrelated components and physical conditions: (1) a differential source of carbon -- trehalose; (2) a pH that favors growth of C. albicans -- 4.8; (3) a temperature which is selective -- 39°C; (4) the addition of excess biotin -- 5.0 mcg/ml; (5) the presence of both gentamicin and chloramphenicol to inhibit contaminant bacteria; and finally, (6) a concentration of cycloheximide (1.2 g/L) which inhibits most of the man-associated yeast-like fungi but permits the more resistant C. albicans to grow in the environment established by the listed factors. This medium has been used with limited success in the culturing of primary-treated sewage, polluted fresh and brackish waters, and sediments. A single taxon, C. tropicalis, has on occasion broken through after a longer period of incubation (72 hours) than employed in the routine procedure (36 hours). The medium has not only demonstrated its selective growth promoting qualities for C. albicans but has served well as a differential test when used in conjunction with the germ tube test as described.

Preliminary studies of skin infections in Vietnam had shown that hemolytic streptococci were a dominant cause of pyoderma. There was an urgent need for a

reliable method for the isolation and transport of these streptococci from Vietnam to the central laboratory in Miami. A highly reliable medium and method were developed. Swabs of skin infections were rapidly dehydrated by placing them in sterile silica gel in an aluminum foil package. The best medium was found to be Trypticase Soy Agar with crystal violet and sheep blood. The results were conclusive: every positive lesion of the skin yielded hemolytic strep 3 to 4 weeks later by the silica gel method and use of the selective medium as described. The procedure has proved to be the most reliable one for conducting accurate epidemiological studies of streptococcal infections of the skin and throat in any area of the world without the need for preparation of culture media in the field.

The polyene antifungal antibiotic, Pimaricin, was employed successfully in the treatment of corneal ulcers due to Fusarium solani. These results proved the superiority of this polyene over amphotericin B in that treatment failure and undesirable side effects were much higher with the latter drug. This study established that a five per cent suspension of pimaricin was the drug of choice in the initial therapy in fusarial keratitis.

Data and findings derived from support furnished by this contract and that of Blank and Taplin (DA-49-193-MD-2236) contributed substantially to the contents of two diagnostic mycology manuals, Dermatophytes--Their Recognition and Identification, 1970, University of Miami Press, and A Guide to the Yeasts and Yeast-Like Fungi of Medical Significance, 1971. The latter manual was scheduled for publication by the University of Miami Press but unfortunately that enterprise ceased operation in 1973 just prior to publication of the manual. Publication of this work is currently being negotiated with Charles C. Thomas. Ten copies of this manual were previously sent to the Commission in 1972 and an additional copy is now included with this report. This study, although it has been given limited distribution, has enjoyed an enthusiastic reception and usage and has been cited by several authors for its value in the clinical laboratory.

Publications resulting from the direct or indirect support of  
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