A COMPARISON OF THE PATHOGENICITY OF *ALTERNARIA ALTERNATA* AND ITS ANTIBODY PRODUCTION WITH *TRICHOPHYTON MENTAGROPHYTES* AND THEIR SYNERGISM ON THE SKIN OF GUINEA PIGS

by

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INTRODUCTION

Alternaria alternata is a well known plant pathogen, and has been frequently detected in routine atmospheric sampling throughout the world. Although *A. alternata* is often isolated from normal and diseased skin and occasionally from deep tissues, its role as a pathogen remains an enigma.

This study included evaluation of lesions produced by topical application of *A. alternata* to normal skin of guinea pigs and the subsequent antibody production. *Trichophyton mentagrophytes*, a well-known pathogenic fungus of the skin, was studied in the same fashion as was the synergistic activity of both fungi.

LITERATURE REVIEW

Alternaria alternata is an ubiquitous fungus frequently detected in routine atmospheric sampling throughout the world (Papavassilious 1975, Street 1976, Tuchinda 1976). It is commonly found as a saprophyte in soil and house dust (Lumpkins 1976). Alternaria sp. has been isolated from the normal skin of man (Yu 1965) and animals (Kelley 1976), the conjunctival sac of normal man (Hammeke 1960), is a common laboratory contaminant and a well known plant pathogen.

Kramer (1959) studied fungi in the air on the Kansas State University campus and found that Alternaria sp. comprised approximately 13% of the total population of air-borne fungi.

Kelley (1976) studied the prevalence of Alternaria sp. on normal horse skin in rural areas and in the city. In this work, 103 horses were surveyed and Alternaria sp. were recovered from 96%.

Although Alternaria is generally considered non-pathogenic for man and animals, it has been isolated frequently from diseased men and animals.

Hopkins et al. (1930) reported a case of asthma due to Alternaria tenuis, later (1930), he reported a case of chronic eczema involving the hands and feet which was extremely sensitive to contact with Alternaria. Borsook (1933) isolated the organism from a skin infection on the hand of a woman.

Ohashi (1960) isolaged Alternaria from the urine of an 11 year old boy with pollakiuria, hematuria and urodynia. He further reported on 37 cases which he referred to as involving infection with Alternaria. Some had hematuria, others cholecystopathy and he isolated Alternaria spores from cerebrospinal fluid, bile and sputum. He suggested that the patients became infected by drinking contaminated water. Ohashi (1960) studied the pathogeneity of Alternaria in animals by injecting spores intraperitoneally in guinea pigs. Five of seven guinea pigs died in eight days. Alternaria spores were found in the lung, liver, kidneys and the adrenal gland as well as in the bile.

English (1965) demonstrated, in vitro, the ability of Alternaria to disintegrate the cortex of cattle and human hairs by means of boring hyphae.

Comstock (1974) associated chronic wheezing and subsequent respiratory illness with the presence of Alternaria in the sputum of several men.

Azar (1975) reported a case of keratomycosis from which a species of Alternaria was isolated. The patient was treated with pimaricin for ten days withoutssuccess. Forster (1975) reported a case of keratitis caused by A. alternata.

Cutaneous Alternaria infection in man and animals has been reported by several investigators. Hubalek (1974) isolated Alternaria alternata from 6 house sparrows that had

dermatomycoses. Reddy (1974) induced Alternaria infection of the skin of rabbits and guinea pigs by scarifying the skin prior to inoculation. Delacretaz et al. (1970) reported two cases of onychosis and one case of acute skin infection on the back of the hand from which Alternaria tenuis was isolated.

Bone (1971) reported that Alternaria comprised 25% of the fungal isolates from hair samples from cats and dogs presented for treatment of a skin condition. In addition, Alternaria has been frequently isolated from hair samples and scrapings of horses and dogs received at the Mycology Laboratory, College of Veterinary Medicine at Kansas State University. Cutaneous alternariosis was reported by Farmar (1976). He isolated A. alternata from multiple ulcerated hemorrhagic cutaneous lesions of an old man with acute lymphocytic lymphoma. Pedersen (1976) isolated A. alternata from the skin lesions of two patients who had primary debilitating diseases. The lesions were characterized by multiple non-healing ulcers covered with a dry crust. Higashi (1973) reported a case of cutaneous alternariosis, which was characterized by crusted ulcerated lesions in exposed areas.

Salkin (1974) isolated A. alternata from a lesion on the ear of a White-tailed Deer. No other fungus was isolated. The lesion was characterized by scaling and hair loss. In 1975 he isolated A. alternaria and Dermotophilus congolensis from lesions on the ear, back, flanks, and around the eyes. The main lesions were scaly, crusty and alopetil. On microscopic examination, hyphal elements of A. alternata were

observed in the epidermis and extended deep within the dermis.

Jand (1975) isolated Alternaria from the udder of buffaloes with subclinical mastitis.

Allergenicity of A. alternata spores for man was reported by Buisseret (1976). He found that the most common fungal spore causing seasonal allergy was A. alternata. This occurred, in spite of the higher concentration of other fungal spores in the air.

The antigenic properties of A. alternata were studied by Michael (1976). He found considerable biochemical variability among culture filtrates. There were differences between isolates and between culture filtrates from the same isolate. There was extensive antigenic cross-reactivity among the isolates.

Trichophyton mentagrophytes

Trichophyton mentagrophytes is a zoophilic dermatophyte with soil as its reservoir. Animals commonly affected include: dogs, cats, rabbits, chinchillas, guinea pigs, mice and rats. Infection may be clinically inapparent. It affects the hair follicle and causes irregularly defined areas of hair loss with considerable scaling. Heavy crusts may form. Occasionally pustules appear at the edges of lesions and suppuration beneath the crusts. In dermatophyte infection, the fungus remains confined to the stratum corneum, while the pathologic changes are produced in the deeper layers of the epidermis and dermis (Jungerman, 1972). This suggests that the deeper

lesions may be mediated by some diffusable products of the fungus or the interaction of the fungus and stratum corneum (Chattaway, Ellis and Barlow 1968; Weary 1943, and Canby 1967). They observed that T. mentagrophytes strains produce uniformly high levels of proteolytic enzymes, and it has been suggested that these enzymes may be related to the production of inflammatory reactions (Minocha 1972).

MATERIALS AND METHODS

Experimental Animals

Twenty-four English short-hair-Hartley albino guinea pigs of either sex, weighing between 300 and 500 gm were used.

All animals were marked for identification and each animal was housed in a separate cage. All animals received commercial rations[1] and water ad libitum during the experiment.

Pre-inoculation Procedure

The health of the guinea pigs was determined by checking general appearance, appetite and rectal temperatures. Hair samples were collected from each animal by picking up the hair from different parts of the body (head, back, sides), into separate sterile tubes. The hair samples were examined microscopically after clearing with 20% KOH and they were cultured. Each hair sample was inoculated into four tubes, two contained Sabouraud's Dextrose agar and two Mycobiotic agar. All tubes were incubated 14 days at 24-25° C and examined for the presence of A. alternata or T. mentagrophytes. These procedures provided assurance that none of the guinea pigs had disease or were previously infected with the fungi studied.

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[1]Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, Mo.

Inoculum

Alternaria alternata (QM 10,161)[2] grown 2 to 3 weeks on Potato Dextrose Agar (PDA)[3] in a Roux flasks was used for preparing the inoculum.

Preparation of the inoculum: Cultures were scraped from the agar surface using a heavy-gauge flammed nichrome loop. The growth was suspended in sterilized phosphate Buffered Saline (PBS)[4] pH 7.4. The suspension was ground in a tissue grinder,[5] and transferred to a test tube and mixed on a vortex[6] for 2 minutes.

Quantification of inoculum: The number of conidia and broken mycelia per milliliter were determined by direct count in a hemocytometer[7]. A suspension containing 8 x 10^4 conidia and mycelia per milliter was prepared based on the hemacytometar count. All spores and hyphae fragments in 80 of the smallest

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[2]QM 10,161 - culture collection of fungi (QM), Dept. of Botany, University of Massachusetts, Amherst, Mass. 01002.

[3]Bacto-Potato Dextrose Agar (Dehydrated), Difco Laboratories, Detroit, Mich.

[4]PBS - $Na_2 HPO_4$, 5.6 g, K_2HPO_4, 2.7 g; NaCl, 4.1 g; and distilled water, 1000 ml.

[5]Tissue grinder (Homogenizer), Pyrex Brand, Fisher Scientific Company.

[6]Vortex Genie Mixer, Scientific Products, General Offices, Evanston, Ill.

[7]Hemocytometer - Bright line, Improved Neubeuen, A.O. Spencer.

squares in the center portion of the hermacytometer were counted. This value was divided by 20 to give the number in millions per milliliter.

Trichophyton mentagrophytes var. granulosum ATCC 18748 grown 2-3 weeks on PDA in Roux flask was used for preparing the spore inoculum.

Preparation of inoculum: Twenty ml of sterile phosphate buffer saline with 0.1% Tween 40,wwas pipetted of the Roux flask and the growth removed by scraping with a heavy-gauge flamed nichrome loop. This suspension was poured into a sterile 250 ml flask containing sufficient No. 3000 (6 mm) glass beads to form a layer 3 beeds in depth. The culture bottle was rinsed twice with 10.0 ml of sterile PBS - 0.1% Tween 40 solution. The rinses were added to the flask. The flask was shaken on a laboratory rotator[8] for 30 minutes to homogenize the cultures and to free the spores. After shaking, the homogenized suspension was filtered through a glass wool column.[9]

The flask was rinsed twice with 10 ml of sterile PBS Tween 40 solution and added to the column. The filtrate was centrifuged at 15,000 *g* for 20 minutes at 20°C in a Sorvall[10]

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[8]Fisher Scientific Co., Eimer and Amend. St. Louis, Mo.

[9]Pyrex Brand Wool, Owens-Corning Fiberglass Corp., Corning Glass Works, Corning, N.Y.

[10]Ivan Sorvall Corp., Newton, CT, 06470.

RC_2-B centrifuge. After centrifugation supernatant fractions were decanted and the spores resuspended in equal volume of membrane-filtered (Millipore HA 0.45)[11] sterilized antiobiotic wash solution containing 300 mg cyclohexamide, 100 mg chloramphenicol, and 100 mg tetracycline HCl per liter of distilled water. The resuspended spore pellet was triturated 10 times and the spores recentrifuged and washed twice with antibiotic solution. Twenty ml of the washed spore suspension were transferred to a test tube and mixed on a vortex for 2 minutes. This suspension was allowed to stand for 30 minutes and then the top 10.0 ml of the spore suspension removed and transferred to another test tube. The spore suspension was mixed vigorously for 2 minutes on a vortex mixer[12], 1.0 ml was removed and serially diluted in a test tube containing membrane filtered (Millipore HA 0.45) saline. After vigorous shaking for 2 minutes, spores in the diluted suspensions were counted and the number of conidia verified and confirmed in the same procedure as for A. alternata.

Viability of the organisms for both cultures was determined by using standard plate count technique. Serial dilutions from 10^0 to 10^{-8} of the culture suspension were used. One ml of each dilution was spread uniformly over the surface

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[11]Millipore Filter Corp., Bedford, Mass., U.S.A.

[12]Vortex Genie Mixer, Scientific Products, General Offices, Evanston, Ill.

of PDA media with sterile flammed nichrome loop. The plates were incubated for 7 days at 25°C and the colonies enumerated. There were 890,000 per/ml colony forming units and in the A. alternata suspention and 930,000 per/ml the suspention of T. mentagrophytes, while 10 dilution plates were negative.

Inoculation procedure: All infections were initiated on normal glabrous skin; no abrasion, scarification, or epilation of the skin was carried out prior to inoculation.

The guinea pigs were shaved with an Oster[13] clipper with a #40 blade attachment. Four circular areas 20 mm in diameter (two on the back and one on each side) were shaved using a safety razor. The skin was washed with soap and water, dried by applying 95% alcohol and allowing it to evaporate. Areas to be infected were outlined and protected by a tape. Hollister[14] medical adhesive was sprayed onto the surrounding areas, allowed to dry for one minute, and resprayed. The protecting tape was removed and one milliliter of inoculum was applied to each site. A sterile circular gauze 20 mm diameter was positioned over the inoculation site and saturated with sterile PBS. A piece of Telfa[15] was placed over the gauze patch so that it adhered to the surrounding area. For further protection, a piece of aluminum foil was placed over the Telfa.

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[13]Model No. 2, John Oster Manufacturing Co., Milwaukee, Wisc.

[14]Hollister Inc., Chicago, Ill. 60611.

[15]Colgate-Palmolive Co., N.Y. 10022.

Elastoplast[16] was tightly wrapped around the guinea pig. The animal was replaced in its cage. The dressing was removed twice a week to examine the inoculation site. Hollister spray remover was used to remove the medical adhesive.

Lesion Evaluation

Lesions were evaluated according to: size, and amount of erythema, scale, crust or scar. The lesion was first measured across its greatest diameter and a second perpendicular measurement was made and the area calculated using the formula for an oval ($\pi r_1 \times r_2$ - where r_1 is 1/2 the large diameter and r_2 is 1/2 the small diameter). Erythema was graded on a subjective 1-4 basis, where 1 was pink, 2 rose, 3 red, and 4 deep red. Scale and crust were graded on 1-4 basis in the following manner: 1 from few punctate areas to 25% of the lesion covered; 2, 25-50% of the lesion was covered; 3, 50-80% of the lesion was covered; 4, 80-100% of the lesion was covered.

Lesions were cultured on days 11, 14, 18 and 21 described for the preinoculation studies.

Antigen Preparation

The antigen was prepared according to the modified technique of Thjotta et al. (1950) and described by Reddy (1974).

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[16]Beiersdorf, Inc., S. Norwalk, Conn. 06854 (5 cm width 2.75 meters slack).

The A. alternata and T. mentegrophytes were cultivated on Roux flasks on PDA media. After one week of growth the cultures were harvested with sterile PBS, pH 7.4 and pooled in a sterile flask. Formalin was added to the culture suspension to a final concentration of 0.5% and refrigerated at 4°C for 24 hrs; an equal amount of sodium bisulfate (meta) $Na_2S_2O_5$ was added to neutralize the formalin and again refrigerated for 24 hours. Sterility was checked using PDA media. The culture suspension was centrifuged at 3000 g and the precipitate dried in a dessicator. The dried fungus was ground in a sterile mortar until a fine homogenous mass was obtained. Two-hundred-fifty mg was suspended in 250 ml of sterile borate saline buffer[17], and sonified[18]. The material was mixed on a shaker for one hour and kept in a refrigerator 4°C over night. It was centrifuged for 30 minutes at 4000 g. The sediment was discarded and the supernatant liquid collected in sterile test tubes. This antigen was stored at -20°C until used.

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[17]Borate Saline Buffer Boric Acid, 6.184 g, Sod. tetraborate (Borax), 9.536 g; Sod. chloride, 4.384 g; and distilled water, 1000 ml. (adjust the pH to 7.4).

[18]Sonifier Cell Disruptor, Model W185, Heat Systems Ultrasonics, Inc., N.Y.

RESULTS

The general appearance and appetites of all animals, prior to inoculation, were satisfactory. Rectal temperatures were within normal range limits; direct examinations of the hair for dermatophytes prior to inoculation were negative; and pre-inoculation cultural examinations were negative for A. alternata and T. mentagrophytes. One tube contained an Alternaria species. All other isolates were common saprophytic fungi and bacteria (see Appendix 2).

Most guinea pigs, irrespective of the type of inoculum, had erythemia, scales, and crust but no scars. There were some variations according to the kind of inoculum and some slight lesion variation among those inoculated with the same fungus or combination of fungi (see Appendix 1).

Erythema reached a maximum on the eleventh day post-inoculation (Fig. 1). Scaling was at a maximum on the eighteenth day post-inoculation in the case of A. alternata, and on the fourteenth day in guinea pigs inoculated with T. mentagrophytes or with both fungi (Fig. 2). Maximum crustation was reached on day 18 with T. mentagrophytes but continued to increase in areas inoculated with A. alternata or with both fungi (Fig. 3, 5). The lesion size in guinea pigs infected with T. mentagrophytes was greatest on day 18 post-inoculation. Lesions produced by A. alternata and both cultures had their maximum size on day 21 (see Appendix 1).

Two sites (on two animals) inoculated with T. mentagrophytes did not develop obvious gross lesions, but were positive histologically. One site in each of two animals infected with both A. alternata and T. mentagrophytes developed erythema and scaling that disappeared on day 14.

Lesions caused by A. alternata infection were generally dry and the hairs were unaffected. With T. mentagrophytes, the lesions were moist, irregular to mummular plaques, sticky and had loose hairs. Infection with both A. alternata and T. mentagrophytes produced lesions that varied from irregular to ring shape, were moist and hairs were sticky, loose and easily detached. Frequently, hairs in the lesions appeared as a single mass. Some animals attempted to remove the bandage especially during the last week of the experiment. This was attributed to the development of pruritis. Vesicles were not observed and if present, probably were obscured by scales and crusts. The difference in the lesions produced by A. alternata alone, T. mentagrophytes alone and the combination of the two agents are compared in Figures 1, 2, 3 and 5.

Hair regrowth appeared normal but delayed in guinea pigs infected with T. mentagrophytes.

Microscopic examination of the epidermal scales scraped from the lesions produced by A. alternata alone revealed separated, branched hyphae and germinating conidia. Lesions infected with T. mentagrophytes alone had numerous microaleuriospores on the surface of the hair and mycelium within the hair (Fig. 4). Lesions infected with both cultures were

similarly positive. Scrapings inoculated on Sabourands dextrose agar media and Mycobiotic agar were all positive for the inoculated fungi by day 18.

The results of the tube precipitation tests are presented in Table 36. None of the animals were positive prior to inoculation while 10 of 21 were positive to A. alternata following infection as were 9 of 21 infected with T. mentagrophytes. All control animals remained serologically negative.

Histologic examination of the lesions revealed hyperkeratosis, lymphoid infiltration and the presence of fungal elements. Some tissues had slight to moderate leucocyte infiltration, which suggested a possible secondary bacterial infection. Trichophyton mentagrophytes were present in the epidermis, particularly the stratum corneum and invading the hair follicles (Fig. 6, 7). Alternaria alternata elements were detected in the keratinized layer and the epidermis of infected guinea pigs (Fig. 8).

Fig. 1. Comparison of average measurements of erythema in three groups of G. pigs infected with *Alternaria alternata*, *Trichophyton mentagrophytes* alone or in combination.

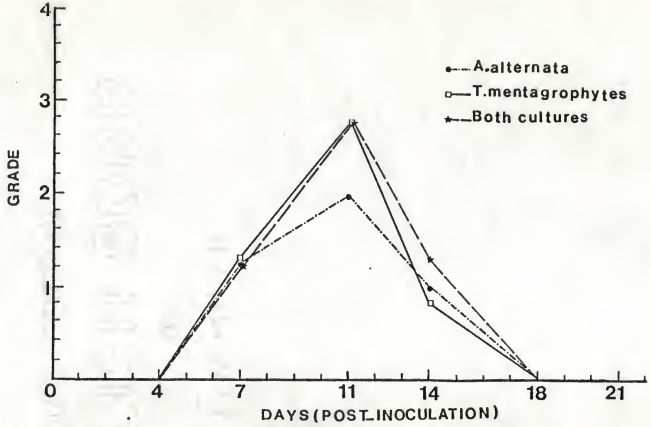

4
3
2
1
0
GRADE
A.alternata
T.mentagrophytes
Both cultures
4
7
11
14
18
21
DAYS (POST_INOCULATION)

Fig. 2. Comparison of average measurements of scales in three groups of guinea pigs infected with *Alternaria alternata* or *Trichophyton mentagrophytes* alone or in combination.

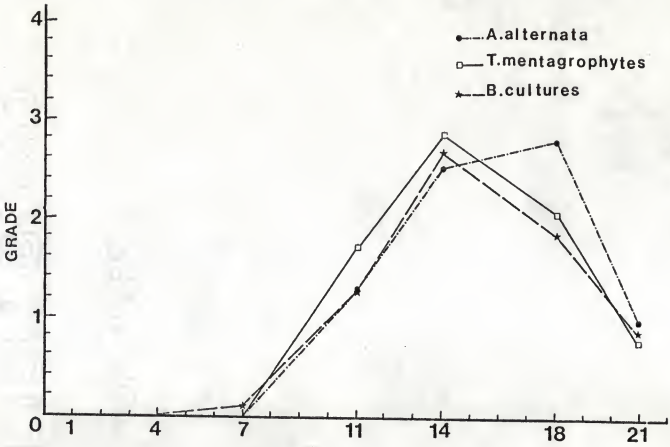

A.alternata
T.mentagrophytes
B.cultures
GRADE
0
1
2
3
4
1
4
7
11
14
18
21

Fig. 3. Comparison of average measurements of crusts in three groups of guinea pigs infected with Alternaria alternata or Trichophyton mentagrophytes alone or in combination.

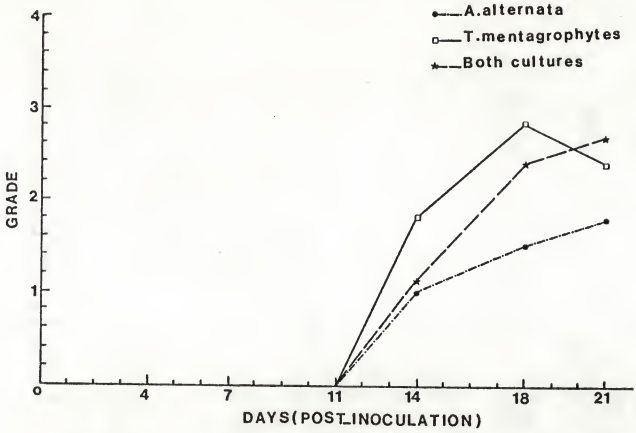

A.alternata
T.mentagrophytes
Both cultures
GRADE
4
3
2
1
0
4
7
11
14
18
21
DAYS(POST_INOCULATION)

Fig. 4. Hair from a lesion produced by Trichophyton mentagrophytes showing threads of mycelia running longitudinally within the hair.

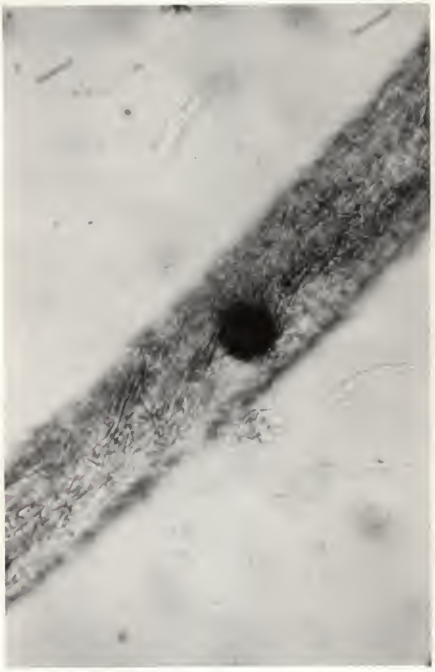


Fig. 5. Eighteen days post-infection with both cultures. This is a grade 2 scale and grade 2 crust.

Fig. 6. Ecothrix and hair follicle invasion by Trichophyton mentagrophytes (PAS).

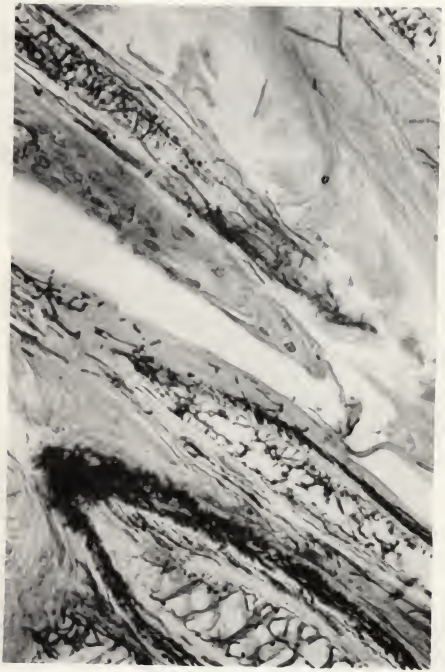


Fig. 7. Cross-section of hairs infected with Trichophyton mentagrophytes (Gridley's Stain). Note the ectothrix invasion with fungi within and outside the hair shaft.

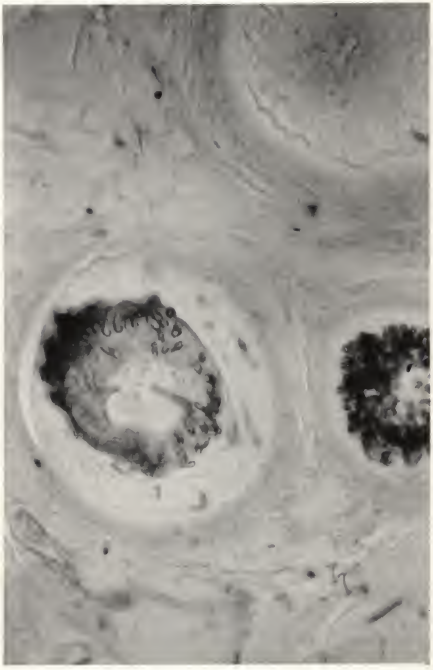
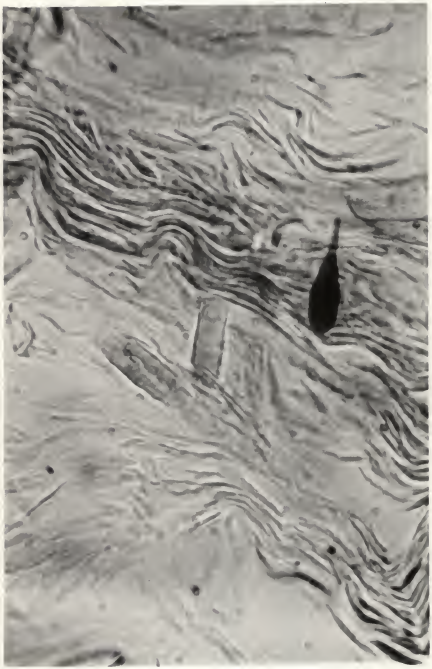


Fig. 8. Germinating macroconidium of Alternaria alternata in the hyperkeratinized layer of the skin (Gridley's Stain).



DISCUSSION

Inoculations were performed according to the technique described by Reinhardt (1974) with some modification. This procedure resulted in a high percentage of infection for T. mentagrophytes alone, A. alternata alone, and with both fungi. The result of this study supported the findings of Reddy (1974) and suggested that A. alternata is an "opportune agent" that can produce lesions when applied on the skin with continuous occlusion and humidity for 21 days.

Earlier work suggested that Alternaria spp. were capable of causing in vitro disentegration of the cortex of hair, nails and callus by means of their boring hyphae (English 1965). There was no evidence, in this research, that supported this observation.

The severity of erythema produced by T. mentagrophytes alone and by both fungi was similar, which suggested that erythema, initiated by both cultures, occurred mostly as a result of the action of T. mentagrophytes. Crustations in lesions produced by cultures and A. alternata alone continued to increase until the experiments were terminated. Perhaps A. alternata had a role in causing more epidermal cell degeneration. Histologic studies on the last biopsies (at day 21) revealed the presence of germinating Alternaria conidia. At this time, the organism may have secreted a substance which caused the epidermal cell degeneration.

Alternaria alternata can cause lesions in deep tissues. Ohashi (1960) isolated the conidia of this organism from the urine and cerebrospinal fluid of a patient. Other investigator supported this finding (Comstock 1974). In T. mentagrophytes infection, the fungal elements and lesions were limited to the superficial layer of the skin and did not survive in deep tissues (Jungerman 1972).

On histologic examination, conidia of A. alternata were seen in the keratinized layer of the skin and in the epidermis. Some, but not all, were germinating. Alternaria alternata may require more time than other fungi to germinate and make hyphal growth.

With T. mentagrophytes infection, most of the known macroscopic and microscopic lesions were detected. Macroscopically, erythema, scaling, crustation, were observed. Microscopically there was hyperkeratinization, lymphoid infiltration, and arthrospores and extensive hyphal growth were present in the stratum corneum, inside the hairs, and in the hair follicles. These were demonstrated in tissue sections stained with hematoxylin and eosin, PAS, and Gridley.

Circulating antibodies to A. alternata and T. mentagrophytes were detected only in the correspondingly infected guinea pigs. Animals inoculated with both fungi had antibodies for A. alternata and T. mentagrophytes. More guinea pigs had antibodies to A. alternata than for T. mentagrophytes. This might suggest that the fungal elements or secretions of A. alternata penetrated deeper in tissues and stimulated the

immune system more than did T. mentagrophytes, with its fungal elements restricted to the superficial layer of the skin.

In the present study, the number of subjects infected and the strain of the organisms used, together with the procedure applied suggest the need for further experiments. Prolongation of the experimental period might provide for an understanding of the role of A. alternata in the production of secondary infection in the internal organs as an extension of the primary skin infection.

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BIBLIOGRAPHY

Azar, P., Aquavella, J.B. and Smith, R.S. (1975) Keratomycoses due to an Alternaria sp. Am. J. of Ophthalm. 79(5):881-883.

Ainsley, R. and Smith, B. (1965) Fungal flora of the conjunctival sac in healthy and diseased eyes. Brit. J. Ophthalm. 49:505.

Bone, W.J. and Jackson, W.F. (1971) Pathogenic fungi in Dermatitis: Incidence in two small animal practices in Florida. Vet. Med. Small Animal Clinician 66:140-142.

Buisseret, P.D. (1976) Seasonal allergic symptoms due to fungal spores. British Med. J. 28 Aug.:507-508.

Borsook, M.E. (1933) Skin infection due to Alternaria tenuis with report of a case. Canad. Med. Ass. J. 29:479.

Comstock, G.W., Palmer, C.E., Stone, R.W. and Goodman, N.L. (1974) Fungi in the sputum of normal men. Mycopathologia and Mycologia Applicata 53(1):55-62.

Chattaway, F.W., Ellis, D.A. and Barlow, A.J.E. (1963) Peptidases of dermatophytes. J. Invest. Derm. 41:31-37.

Delacretaz, J., Grigoriu, D. and Grigoriu, A. (1970) Alternaria tenuis en Cutanee Humaine. Ann. de Dermatologie 97(1):15-20.

English, M.P. (1965) The saprophytic growth of non-keratinophilic fungi on keratinized substrata and a comparison with keratinophilic fungi. Trans. Brit. Mycol. Soc. 48:219.

Froster, R.K. (1975) The dematinaceous fungal keratitis, clinical isolates and managements. Br. J. of Ophthalm. 59(7):372-375.

Farmer, S.G., Komorowski, R.A. (1976) Cutaneous microabscess formation from A. alternata. Am. J. Clin. Path. 66(3):565-569.

Higashi, N., Asada. (1973) Cutaneous Alternariosis with mixed infection of Candida albicans. Arch. Dermatol. 108:558-560.

Hubalek, Z. (1974) Cutaneous staphylococcosis and secondary infection of house sparrow with the fungus Cladosporium herbarum. Folia Parasitologica 21(1):59-66.

Hammeke, J.C. and Ellis, P.P. (1960) Mycotic flora of the conjunctiva. Am. J. Ophthalmol. 49:1174.

Hopkins, J.G., Benham, R.W. and Kesten, B.U. (1930) Asthma due to a fungus Alternaria. J.A.M.A. 94:6-10.

Hopkins, J.G. (1930) Sensitization to saprophytic fungi in a case of eczema. Proc. Soc. Exp. Biol. Med. 27:342.

Jungerman, P.E. and Schwartzman, R.M. (1972) Veterinary Medical Mycology, Lea and Febiger, Philadelphia, page 6.

Jand, S.K. and Dhillon, S.S. (1975) Mastitis caused by fungi. Indian Vet. J. 52:125-128.

Kramer, C.L., Pady, S.M., Rogerson, C.T. and Duye, L.G. (1959) Kansas aeromycology. II. Materials, Methods and General Results. Trans. Kans. Acad. Sci. 63(3):184-199.

Kelley, D.C. and Al-Lebban, Z.S. (1976) Survey on the prevalence of Alternaria sp. on normal skin of horses (unpublished data). Dept. of Infectious Diseases, College of Vet. Medicine, K.S.U.

Lumpkins, E.D. (1976) Airborne fungi survey. II. Culture plate survey of the home environment. Annals of Allergy 36(1):40-44.

Minocha, Y., Pasrich, J.S., Mohapatra, L.N. and Kandhari, K.C. (1972) Proteolytic activity of dermatophytes and its role in the pathogenesis of skin lesions. Sabouraudia 10(1):79-85.

Michael, J., Schumacher, and Jeffery, S.E. (1976) Variability of Alternaria alternata, biochemical and immunological characteristics of culture filtrates from seven isolates. J. Allergy Clin. Immunol. 58(2):263-277.

Nema, H.V., Ahuja, O.P., Bal, A. and Mohapatra, L.N. (1966) Mycotic flora of the conjunctiva. Am. J. Ophthalmol 62:968.

Ohashi, Y. (1960) On rare disease due to Alternaria tenuisnees (Alternariasis). Tohoku J. Exper. Med. 72:78-82.

Pedersen, N.B., Mardh, P.A., Hallberg, T., Jonson, N.S. (1976) Cutaneous alternariosis. British J. of Dermatology 94(2): 201-209.

Papavassilious, J.T. and Bartzokas, C.A. (1975) The atmospheric fungal flora of the Athens Metropolitan area. Mycopathologia 57(1):31-34.

Reddy, B.D., Kelley, D.C., Minocha, J.C., Anthony, H.D. (1974) Pathogenicity of A. alternata and its antibody production in experimental animals. Mycopath. et Mycologia Applicata 54(3):385-390.

Reinhardt, J.H., Allen, A.M. and D. Gunnison. (1974) Experimental human Trichophyton mentagrophytes infections. J. Invest. Derm. 63:419-422.

Salkin, I.F., Gordon, M.A., Stone, W.B. (1975) Dual infection of a white tailed deer by Dermatophilus and A. alternata. JAVMA 167(7):571-573.

Salkin, I.F. (1974) Subcutaneous mycotic infection of a white-tailed deer. J. of Wildlife Dis. 10:34-38.

Street, D.H., Humburger, R.N. (1976) Atmospheric pollen and spore sampling in San Diego, California. I. Mentrological correlations and potential clinical relevance. Annals of Allergy 37(1):32-40.

Thjotta, T. et al. (1951) Preparation of fungus antigens for immunization and for serological reactions. Acta. Path. and Microbial Scand. 28:132-138.

Tuchinda, M. and Y. Theptaranon. (1976) Aeroallergens in Bangkok, Thailand. Annals of Allergy 37(1):47-54.

Weary, P.E. and Canby, C.M. (1967) Keratinolytic activity of Trichophyton schoenleinii, Trichophyton rubrum and Trichophyton mentagrophytes. J. Invest. Derm. 48:240-248.

Yu, H. (1965) Studies on fungi of the normal skin. Acta. Dermato. 60:126-174.

APPENDICES

APPENDIX 1

RECORDS OF OBSERVATIONS ON ALL EXPERIMENTAL GUINEA PIGS

Table 1. The size, degree of erythema, scales, crusts, scars and histologic results on Guinea pig Number 1 inoculated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	2	-	-	-	
	3	4.71	1	-	-	-	
	4	-	0	0	0	0	
11	(1)	-	-	-	-	-	
	2	15.7	3	2	-	-	
	3	9.43	3	2	-	-	
	4	0	0	0	0	0	
14	(1)	-	-	3	2	-	
	2	12.58	0	0	0	0	+
	3	24.57	1	4	2	-	
	4	0	0	0	0	0	
18	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	37.71	-	2	2	-	
	4	0	0	0	0	0	
21	(1)	-	0	0	0	0	-
	2	0	0	0	0	0	
	3	22.0	0	0	2	0	+
	4	0	0	0	0	0	

Table 1. (continued)

1 = Site on the anterior part of the back.

2 = Site on the posterior part of the back.

3 = Site on the left side.

4 = Site on the right side.

( ) = control site; 0 = biopsy was taken; - = no lesion.

$\overset{1}{0}, \overset{2}{0}, \overset{3}{0}$ = The biopsy was taken after reading the lesion.

* = Organism identified in tissues.

Table 2. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 2 isolated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	9.43	2	-	-	-	
	(2)	-	-	-	-	-	
	3	9.43	1	-	-	-	
	4	0	0	0	0	0	
11	1	15.71	4	2	-	-	
	(2)	-	-	-	-	-	
	3	12.58	3	2	-	-	
	4	0	0	0	0	0	
14	1	23.57	-	3	1	-	
	(2)	-	-	-	-	-	
	3	23.57	0	3	2	0	
		0		0	0		
	4	0	0	0	0	0	
18	1	37.71	-	2	3	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	1	42.42	0	1	4	0	+
		0		0	0		
	(2)	0	0	0	0	0	-
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 3. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 3 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	(3)	-	-	-	-	-	
	-	-	-	-	-	-	
7	1	0	0	0	0	0	
	2	4.71	1	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	11.79	3	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	
	2	18.86	1	2	-	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	-
18	1	0	0	0	0	0	
	2	49.5	-	3	-	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
21	1	0	0	0	0	0	
	2	44.0	0	2 0	0	0	+
	(3)	0	0	0	0	0	-
	4	0	0	0	0	0	

Table 4. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 4 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
7	1	4.71	1	-	-	-	
	2	4.71	1	-	-	-	
	3	0	0	0	0	0	-
	(4)	-	-	-	-	-	
11	1	11.79	3	-	-	-	
	2	9.43	3	1	-	-	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
14	1	11.79 0	1 0	3 0	1 0	-	+
	2	19.64	2	3	2	-	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
18	1	0	0	0	0	0	
	2	27.5	-	-	2	-	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
21	1	0	0	0	0	0	
	2	23.57 0	0	0	4 0	0	+
	3	0	0	0	0	0	
	(4)	0	0	0	0	0	-

Table 5. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 5 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	9.43	2	-	-	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	-
	4	-	-	-	-	-	
11	1	15.7	4	2	-	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
14	1	27.5	2	3	1	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	-
18	1	42.43	-	4	2	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
	1	died*	died	died	died	died	

*Died during anaesthesia by Ethyl Ether Anhydrous (Ether Absolute).

Table 6. The size, degree of erythema, scales, crusts, scars and histologic results of guinea pig Number 6 while kept as a control.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Nisto-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	0	0	0	0	0	-
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
		Died**	Died	Died	Died	Died	

**Died during anaesthesia.

Table 7. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 7 inoculated with Phosphate Buffer Saline.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	-	-	-	-	-	
	2	ō	ō	ō	ō	ō	-
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	-	-	-	-	-	
	2	0	0	0	0	0	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	-	-	-	-	-	
	2	0	0	0	0	0	
	3	0	0	0	0	0	-
	4	-	-	-	-	-	
18	1	ō	ō	ō	ō	ō	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
21	1	ō	ō	ō	ō	ō	-
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	4	ō	ō	ō	ō	ō	-

Table 8. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 8 inoculated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	2	-	-	-	
	3	4.71	1	-	-	-	
	4	0	0	0	0	0	-
11	(1)	-	-	-	-	-	
	2	9.43	2	1	-	-	
	3	4.71	2	1	-	-	
	4	0	0	0	0	0	
14	(1)	-	-	-	-	-	
	2	11.79	1	3	-	-	
	3	4.71	0	2	0	0	-
	4	0	0	0	0	0	
18	(1)	-	-	-	-	-	
	2	18.86	-	2	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	(1)	0	0	0	0	0	-
	2	9.43	0	1	0	0	+
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 9. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 9 isolated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	-	-	-	-	-	
	2	ō	ō	ō	ō	ō	-
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	-	-	-	-	-	
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	ō	ō	ō	ō	ō	-
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
18	1	0	0	0	0	0	
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
21	1	0	0	0	0	0	
	2	0	0	0	0	0	
	(3)	ō	ō	ō	ō	ō	-
	4	ō	ō	ō	ō	ō	-

Table 10. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 10 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	1	-	-	-	
	3	4.71	2	-	-	-	
	4	4.71 0	1 0	0	0	0	-
11	(1)	-	-	-	-	-	
	2	11.79	3	2	-	-	
	3	9.43	3	1	-	-	
	4	0	0	0	0	0	
14	(1)	-	-	-	-	-	
	2	11.79 0	2 0	2 0	1 0	0	+
	3	14.15	1	4	2	-	
	4	0	0	0	0	0	
18	Died**	Died	Died	Died	Died	Died	

**Died during anaesthesia.

Table 11. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 11 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	4.71	2	-	-	-	
	(2)	-	-	-	-	-	
	3	4.71	1	0	0	0	-
	4	4.71	1	-	-	-	
11	1	9.43	4	2	-	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	11.99	3	1	-	-	
14	1	18.86	1	4	2	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	14.15	0	3	1	0	+
18	1	31.43	-	2	4	-	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	1	55.0	0	0	4	0	+
	(2)	0	0	0	0	0	-
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 12. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 12 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	4.71	2	-	-	-	
	2	4.71	1	-	-	-	
	(3)	-	-	-	-	-	
	4	-	0	0	0	0	-
11	1	4.71	3	1	-	-	
	2	9.43	3	2	-	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
14	1	11.79	1	3	1	-	
	2	18.86	2	3	2	0	+
		0	0	0	0		
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
18	1	18.86	-	2	3	-	
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
21	1	31.43	0	2	3	0	+
		0		0	0		
	2	0	0	0	0	0	
	(3)	0	0	0	0	0	-
	4	0	0	0	0	0	

Table 13. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 13 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histopath Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
7	1	4.71	2	-	-	-	
	2	4.71	1	-	-	-	
	3	$4\frac{71}{0}$	$\frac{1}{0}$	0	0	0	-
	(4)	-	-	-	-	-	
11	1	11.79	3	2	-	-	
	2	9.43	3	1	-	-	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
14	1	15.71	1	3	2	-	
	2	$4\frac{71}{0}$	0	$\frac{3}{0}$	$\frac{1}{0}$	0	+
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
18	1	18.86	-	2	3	-	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
21	1	$37\frac{71}{0}$	0	$\frac{1}{0}$	$\frac{3}{0}$	0	+
	2	0	0	0	0	0	
	3	0	$\frac{0}{0}$	0	$\frac{0}{0}$	$\frac{0}{0}$	
	(4)	-		0			-

Table 14. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 14 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	ō	ō	ō	ō	ō	-
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	ō	ō	ō	ō	ō	-
	4	-	-	-	-	-	
18	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
21	(1)	ō	ō	ō	ō	ō	-
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	4	ō	ō	ō	ō	ō	-

Table 15. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 7 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histopath Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	2	1	-	-	
	3	4.71	1	-	-	-	
	4	4.71	2	0	0	0	-
11	(1)	-	-	-	-	-	
	2	9.43	3	2	-	-	
	3	4.71	2	1	-	-	
	4	0	0	0	0	0	
14	(1)	-	-	-	-	-	
	2	19.64	2	3	-	-	
	3	0	0	0	0	0	-
	4	0	0	0	0	0	
18	(1)	-	-	-	-	-	
	2	27.57	-	2	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	(1)	0	0	0	0	0	-
	2	19.64	0	1	0	0	+
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 16. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 16 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	4.71	2	$\bar{0}$	$\bar{0}$	$\bar{0}$	-
		0	0				
	(2)	-	-	-	-	-	
	3	4.71	2	1	-	-	
	4	4.71	2	1	-	-	
11	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	15.71	-	3	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	23.57	-	2	1	-	
	4	$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	-
18	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	31.34	-	1	2	-	
	4	0	0	0	0	0	
21	1	0	0	0	0	0	
	(2)	$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	-
	3	42.43	$\bar{0}$	$\bar{0}$	3	$\bar{0}$	+
		0			0		
	4	0	0	0	0	0	

Table 17. The size, degree of erythema, scales, crusts, scars and histologic results of guinea pig Number 17 inoculated with phosphate buffer saline.

Day Post-Inoculation	Site of Inoculation	Size of Lesion	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	ō	ō	ō	ō	ō	-
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	ō	ō	ō	ō	ō	-
18	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	0	0	0	0	0	
21	1	0	0	0	0	0	
	2	ō	ō	ō	ō	ō	-
	3	ō	ō	ō	ō	ō	-
	4	0	0	0	0	0	

Table 18. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 18 inoculated with phosphate buffer saline.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	0	0	0	0	0	-
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	0	0	0	0	0	-
	4	-	-	-	-	-	
18	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
21	1	0	0	0	0	0	
	2	0	0	0	0	0	-
	3	0	0	0	0	0	
	4	0	0	0	0	0	-

Table 19. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 19 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	4.71	2	-	-	-	
	(2)	-	-	-	-	-	
	3	4.71	2	-	-	-	
	4	$4_0^{.71}$	$\frac{1}{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	-
11	1	9.43	3	1	-	-	
	(2)	-	-	-	-	-	
	3	4.71	3	1	-	-	
	4	0	0	0	0	0	
14	1	$11._0^{73}$	$\frac{2}{0}$	$\frac{2}{0}$	$\bar{0}$	$\bar{0}$	+
	(2)	-	-	-	-	-	
	3	9.43	2	3	1	-	
	4	0	0	0	0	0	
18	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	11.73	-	2	3	-	
	4	0	0	0	0	0	
21	1	0	0	0	0	0	
	(2)	$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	-
	3	$27._0^{5}$	$\bar{0}$	$\frac{1}{0}$	$\frac{3}{0}$	$\bar{0}$	+
	4	0	0	0	0	0	

Table 20. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 20 inoculated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	0	0	0	0	0	-
	(2)	-	-	-	-	-	
	3	4.71	1	-	-	-	
	4	9.43	2	-	-	-	
11	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	9.43	3	1	-	-	
	4	11.73	3	2	-	-	
14	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	15.71	1	2	0	0	+
		0	0	0			
	4	22.0	-	3	1	-	
18	1	0	0	0	0		
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	-	-	2	3	-	
21	1	Died**	Died	Died	Died	Died	

**Died due to an accident.

Table 21. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 21 inoculated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	0	0	0	0	0	-
	2	4.71	1	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	9.43	2	2	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	
	2	27.5	1	3	1	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	-
18	1	0	0	0	0	0	
	2	42.43	-	2	3	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
21	1	0	0	0	0	0	
	2	33.0	0	1 0	3 0	0	+
	(3)	0	0	0	0	0	-
	4	0	0	0	0	0	

Table 22. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 22 inoculated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
7	1	4.71 0	2 0	ō	ō	ō	-
	2	12.57	1	-	-	-	
	3	4.71	1	-	-	-	
	(4)	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	9.43	2	1	-	-	
	3	9.43	3	1	-	-	
	(4)	-	-	-	-	-	
14	1	0	0	0	0	0	
	2	9.43 0	21 0	1 0	ō	ō	+
	3	18.86	2	3	-	-	
	(4)	-	-	-	-	-	
18	1	0	0	0	0	0	
	2	0	0	0	0	0	
	3	35.36	-	2	3	-	
	(4)	-	-	-	-	-	
21	1	0	0	0	0	0	
	2	0	0	0	0	0	
	3	33.0 0	ō	1 0	3 0	ō	+
	(4)	ō	ō	ō	ō	- 0	-

Table 23. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 23 inoculated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	ō	ō	ō	ō	ō	-
	4	-	-	-	-	-	
11	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
14	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	0	0	0	0	0	
	4	ō	ō	ō	ō	ō	-
18	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	(1)	ō	ō	ō	ō	ō	-
	2	ō	ō	ō	ō	ō	-
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 24. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 24 inoculated with Trichophyton mentagrophytes.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histopath Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	1	-	-	-	
	3	0	0	0	0	0	-
	4	-	-	-	-	-	
11	(1)	-	-	-	-	-	
	2	9.43	2	1	-	-	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
14	(1)	-	-	-	-	-	
	2	18.86	2	2	-	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	-
18	(1)	-	-	-	-	-	
	2	23.57	-	3	1	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	(1)	0	0	0	0	0	-
	2	27.5	0	3 0	1 0	0	+
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 25. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 25 inoculated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	ð	ð	ð	ð	ð	-
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	ð	ð	ð	ð	ð	-
	4	-	-	-	-	-	
18	1	0	0	0	0	0	
	(2)	-	-	-	-	-	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
21	1	0	0	0	0	0	
	(2)	ð	ð	ð	ð	ð	-
	3	0	0	0	0	0	
	4	ð	ð	ð	ð	ð	-

Table 26. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 26 inoculated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	-	-	-	-	-	
	2	0	0	0	0	0	-
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	4.71	2	2	-	-	
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	4.71	1	3	-	-	
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	-
18	1	9.43	-	3	-	-	
	2	0	0	0	0	0	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
21	1	15.71	0	1	0	0	+
	2	0	0	0	0	0	
	(3)	0	0	0	0	0	-
	4	0	0	0	0	0	

Table 27. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 26 inoculated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path-Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
7	1	4.71	1	-	-	-	
	2	4.71	1	-	-	-	
	3	0	0	0	0	0	-
	(4)	-	-	-	-	-	
11	1	4.71	2	1	-	-	
	2	-	-	-	-	-	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
14	1	9.43	1	2	-	-	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
18	1	12.75	-	3	-	-	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	(4)	-	-	-	-	-	
21	1	23.57	0	1 0	0	0	+
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	(4)	0	0	0	0	0	-

Table 28. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 28 inoculated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	ō	ō	ō	ō	ō	-
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	ō	ō	ō	ō	ō	-
	4	-	-	-	-	-	
18	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	4	-	-	-	-	-	
21	(1)	ō	ō	ō	ō	ō	-
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	4	ō	ō	ō	ō	ō	-

Table 29. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 29 inoculated with Alternaria alternata.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
7	1	0	0	0	0	0	-
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
11	1	0	0	0	0	0	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
14	1	0	0	0	0	0	
	2	0	0	0	0	0	-
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
18	1	0	0	0	0	0	
	2	0	0	0	0	0	
	3	-	-	-	-	-	
	(4)	-	-	-	-	-	
21	1	0	0	0	0	0	
	2	0	0	0	0	0	
	3	0	0	0	0	0	-
	(4)	0	0	0	0	0	-

Table 30. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 30 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	1	ō	ō	ō	-
	3	9.43	2	-	-	-	
	4	4.71	1	-	-	-	
11	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	15.71	3	1	-	-	
	4	15.71	3	-	-	-	
14	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	23.57	1	2	1	-	
	4	15.71	2	2	ō	ō	+
18	(1)	-	-	-	-	-	
	2	0	0	0	0	0	
	3	37.71	-	-	3	-	
	4	0	0	0	0	0	
21	(1)	ō	ō	ō	ō	ō	-
	2	0	0	0	0	0	
	3	60.5	ō	ō	3	ō	+
	4	0	0	0	0	0	

Table 31. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 31 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	(1)	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	(1)	-	-	-	-	-	
	2	4.71	2	-	-	-	
	3	9.43	2	1	-	-	
	4	ō	ō	ō	ō	ō	-
11	(1)	-	-	-	-	-	
	2	9.43	3	2	-	-	
	3	11.79	3	2	-	-	
	4	0	0	0	0	0	
14	(1)	-	-	-	-	-	
	2	18.86	1	3	1	-	
	3	18.86	1	3	ō	ō	+
		0	0	0			
	4	0	0	0	0	0	
18	(1)	-	-	-	-	-	
	2	62/86	-	2	3	-	
	3	0	0	0	0	0	
	4	0	0	0	0	0	
21	(1)	ō	ō	ō	ō	ō	-
	2	33.0	ō	ō	3	ō	-
					0		
	3	0	0	0	0	0	
	4	0	0	0	0	0	

Table 32. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 32 inoculated with both cultures.

Day Post-Inoculation	Site of Inoculation	Size of Lesion	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	(3)	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	9.43	2	-	-	-	
	2	9.43	2	-	-	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	-
11	1	15.71	3	1	-	-	
	2	12.57	3	2	-	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
14	1	22.0	1	2	0	0	+
		0	0	0			
	2	9.43	1	3	1	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
18	1	0	0	0	0	0	
	2	18.86	-	2	3	-	
	(3)	-	-	-	-	-	
	4	0	0	0	0	0	
21	1	Died**	Died	Died	Died	Died	

**Died due to an accident.

Table 33. The size, degree of erythema, scales, crusts, scars and histologic results on guinea pig Number 33 inoculated with phosphate buffer saline.

Day Post-Inoculation	Site of Inoculation	Size of Lesion mm^2	Erythema	Scales	Crusts	Scars	Histo-path Results*
4	1	-	-	-	-	-	
	2	-	-	-	-	-	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
7	1	-	-	-	-	-	
	2	0	0	0	0	0	-
	3	-	-	-	-	-	
	4	-	-	-	-	-	
11	1	-	-	-	-	-	
	2	0	0	0	0	0	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
14	1	0	0	0	0	0	-
	2	0	0	0	0	0	
	3	-	-	-	-	-	
	4	-	-	-	-	-	
18	1	Died**	Died	Died	Died	Died	

**Died due to anaesthesia.

A COMPARISON OF THE PATHOGENICITY OF ALTERNARIA ALTERNATA AND ITS ANTIBODY PRODUCTION WITH TRICHOPHYTON MENTAGROPHYTES AND THEIR SYNERGISM ON THE SKIN OF GUINEA PIGS

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The pathogeneity of Alternaria alternata had not been studied until 1974, when Reddy conducted his experiments on the pathogenicty of this organism. As early as 1930, Hapkins et al. confirmed A. alternata (tenuis) as a cause of asthma and demonstrated its allergenicity in man. Since then this organism has been isolated from many different sources in man (Ohashi, 1960; Forester, 1975; Delacretaz et al., 1970; Pedersen, 1976), and in animals (Hubalek, 1974; Bone, 1971; Hygushi, 1973; Salkin, 1974). Allergenicity of A. alternata spores to man was confirmed by Busseret (1976) who found that the commonest spores causing seasonal allergy was related to A. alternata.

As Alternaria is a common contaminent in the laboratory, and because of its prevalence on the normal skin of man and animals and in the air, it has generally been regarded as insignificant when cultured from clinical materials. It is not justifiable, solely on the basis of isolation of this organism from human and animal specimens, to conclude that it is pathogenic in man and animals.

Alternaria alternata may not always be nonpathogenic to man and animals. The frequent isolation of this fungus from clinical specimens, gave rise to a suspicion that A. alternata might not be a primary causative agent, but may act synergistically, with other agents to produce disease. With this in mind, this study was conducted with the following objectives: first, to determine the potential pathogenicity of A. alternata in normal skin of guinea pigs and to compare it with

T. mentagrophytes, as a well known dermatophyte; second, to evaluate the development of circulating antibodies against A. alternata, and T. mentagrophytes; and third, to determine the synergistic effect of both cultures on the skin of guinea pigs.

Experiments were performed on thirty-four English short hair-Hartley albino guinea pigs by standardizing the inocula. site of infection and the infection procedure. Alternaria alternata produced superficial mycoses but produced less severe lesion than T. mentagrophytes. The antibody response was positive for both A. alternata and T. mentagrophytes in the experimental animals infected with each culture alone, and positive in animals infected with both cultures.