EVIDENCE FOR A HUMORAL MECHANISM WHICH PREVENTS GROWTH OF DERMATOPHYTES*

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In view of the ability of dermatophytes to thrive on even extremely simple nutrient substrates and under relatively wide ranges of temperature, pH, illumination, and oxygen tension, it is of interest to ask why they should fail to invade living tissues below the water-electrolyte barrier of the skin. The observations of others pertinent to this question, notably those of Saeves, Kogoj, Smolka, Jadassohn, Sulzberger, and Newcomer, Wright, and Sternberg have been cited in a previous communication (1). That this failure to invade living tissue does not result from lack of essential nutrients or of sufficient oxygen is indicated by the often demonstrated ability of these fungi to thrive in serum as well as on all sorts of non-keratinous internal tissues after death including the corium of human skin even when kept at low oxygen tensions.

The question of the existence of some type of circulating antibody in the blood serum which is fungistatic against dermatophytes has been the subject of considerable controversy. In 1934, Ayers and Anderson (2) reported that serum taken from patients with dermatophytids when added in ten per cent concentration to Sabouraud's medium completely inhibited the growth of dermatophytes isolated from such patients, whereas serum from normal individuals had no such effect. They further stated that this antibody was rapidly destroyed at room temperature. Lewis and Hopper (3) on attempting to repeat this work were unable to confirm it and found only irregular partial inhibition of the growth of dermatophytes in the presence of serum taken from individuals with dermatophytosis. Furthermore, such inhibition was also noted by them in a few instances where serum from persons without dermatophytosis was used. Attempts to demonstrate agglutinins, precipitins, or complement fixing antibodies in the sera of patients with superficial mycoses have uniformly failed (4).

This present report concerns some of our investigations on the nature of the defense mechanisms possessed by living tissues which so strictly keep dermatophytes from invading them.

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This work was supported in part by a grant from the National Institutes of Health, P.H.S. No. E-1444 (C).
Presented at the Eighteenth Annual Meeting of The Society for Investigative Dermatology, Inc., New York, N. Y., June 2, 1957.

MATERIALS AND METHODS

Trichophyton mentagrophytes was the representative dermatophyte used in all of the experiments. Inocula consisted of either pin-head size bits of fungal growth teased from a fresh colony grown on Sabouraud's agar, or opalescent suspensions in sterile distilled water prepared from relatively powdery colonies by shaking mycelial fragments with glass beads.

Millipore filter chambers of the HA type were prepared as described by Algire, Weaver and Prehn (5) and these were sterilized prior to use by immersion in 70 per cent alcohol for fifteen minutes followed by three rinses with sterile distilled water. The largest pores of these filters are about 0.3 microns in diameter and thus they exclude the passage of cells but do not interfere with the free penetration of proteins and tissue fluids.

Dialysis bags were prepared from Visking cellulose casing 1.0 cm. in diameter and were sterilized by boiling for fifteen minutes in water. After such boiling these bags were demonstrated to essentially retain their impermeability to serum proteins by the following experiment. A series of such bags boiled and non-boiled were filled with 3.0 ml. quantities of human serum and suspended in separate tubes in 20.0 ml. volumes of distilled water for three days. At the end of this period maximum protein loss from any of the bags was less than 0.4 per cent as determined by biuret assay.

In order to avoid contamination of the outsides of dialysis bags with fungal inocula the following procedure was followed in some of the *in vitro*

experiments. Glass melting point tubes were filled with the opalescent fungal suspension and then sealed by flaming their ends. These sealed tubes were placed into concentrated nitric acid for fifteen minutes and subsequently rinsed several times with sterile sodium bicarbonate solutions and distilled water. They were then with the use of sterile technic slipped into the sterile dialysis bags. After tying the bags, the thin glass tubes were broken to release their contents inside the bags.

The mice used in these experiments were normal adult animals of either dba or Swiss albino strain.

EXPERIMENTAL PROCEDURES, RESULTS, AND COMMENTS

Initially we sought to establish whether primarily cellular or humoral mechanisms were involved in preventing the growth of *T. mentagrophytes* in living tissues. In one experiment, millipore chambers containing bits of *T. mentagrophytes* colonies were implanted intraperitoneally into three mice. After three weeks these filters were removed from the mice and opened. In none was there the slightest evidence of growth of the original inoculum. Similar control chambers were placed on Sabouraud's medium and from all vigorous growth of *T. mentagrophytes* was obtained.

In a second experiment, six mice were given inoculations of *T. mentagrophyte* suspension into the anterior chambers of their eyes. After two to six weeks observation in none of the eyes did grossly visible fungal growth or cellular infiltration appear. One of the mice was killed two weeks after inoculation and cultures of both eyes readily yielded colonies of *T. mentagrophytes* on Sabouraud's medium even though there was no grossly visible fungal growth.

In both of these experiments striking fungistatic effect on *T. mentagrophytes* was demonstrated *in vivo* in areas out of contact with direct cellular defense mechanisms.

In order to obtain further information about the nature of the noncellular dermatophyte-suppressing mechanism in question the following further experiment was undertaken. Small inocula of *T. mentagrophytes* were placed inside sterilized dialysis bags which were then implanted intraperitoneally into ten mice. At intervals of one, two, three, four, five and twelve weeks, respectively one, one, three, one, two, and two of the bags were removed and examined. There was no evidence of severe tissue reaction around any of the bags and in none of them was there the slightest evidence of growth of the fungal inocula. Similar control dialysis bags prepared at the start of the experiment and placed into either Sabouraud's medium or normal human serum all showed vigorous fungal growth in less than two weeks. Even after as long as the twelve week stay in the peritoneal cavities of mice, when the original inocula were removed from the dialysis bags and planted on Sabouraud's medium, vigorous growth of the fungus occurred. This experiment indicated that the humoral factor involved in the suppression of dermatophytic growth in the living tissues of the mouse was dialyzable and hence not ordinary protein antibody either natural or acquired.

At this point, two explanations for these observations involving a dialyzable humoral factor still seemed possible. One was the existence in tissue fluids during life of a dialyzable, fungistatic substance. The other was the possible production by the fungus of a dialyzable, autocatalytic material necessary for fungal growth which was too rapidly removed by excretory or metabolic mechanisms of the living host. This latter rather unlikely possibility was eliminated by an experiment in which it was shown that wide variations in volume of liquid Sabouraud's medium kept continuously stirred failed to cause variations in the rate at which equal small inocula of *T. mentagrophytes* confined inside of dialysis bags grew out. Thus, growth in a bag suspended in 25 mls. of such constantly stirred medium appeared exactly equally and as rapidly as in bags in 150 and 600 mls. of medium.

Attempts were next made to demonstrate a dialyzable antifungal factor in human serum. It was felt that such antifungal material must be relatively unstable in view of the eventual growth of *T. mentagrophytes* in human serum.

A series of dialysis bags containing suspensions of *T. mentagrophytes* was prepared by the glass melting point tube technic already described and placed into a series of ten small sterile test tubes. Sterile serum was obtained from a healthy adult donor and 1.5 mls. were placed into each of the tubes. In five of the tubes the serum was removed daily and replaced with fresh serum from the same donor. All tubes were kept on a shaker to keep their contents in circulation. After three to four days, fungal growth appeared in all five tubes where the serum was not changed. In the five tubes where the serum was changed daily, growth failed to

occur in three tubes even after three weeks while in two it appeared on the fifth day and was slow and inhibited for another three days.

This experiment was repeated with serum from a second normal donor but 5.0 ml. amounts of serum were used in each tube. In three control tubes where the serum was not changed, growth appeared grossly within three days, whereas in two tubes where the serum was replaced with fresh serum each day, growth was delayed for three additional days.

An attempt was also made to repeat this experiment on a larger scale with still larger volumes of pooled normal human serum which was frozen within 24 hours after collection and kept at −20.0° C. until use. Twice daily replacement of ten ml. volumes of serum in six of a series of twelve tubes with freshly thawed batches of the same pooled serum resulted in no differences in the time of appearance of fungal growth in the two sets of tubes.

On the basis of these *in vitro* experiments, the demonstration of an antidermatophytic, dialyzable substance in human serum is still slightly equivocal and further more definitive studies are needed. Nevertheless, it is felt that there is highly suggestive evidence for the existence of some dialyzable, water-soluble, unstable substance in serum and tissue fluids which suppresses the growth of dermatophytes and hence permits them to thrive only in those areas of the host which are not reached by such fluids.

SUMMARY

The nature of the defense mechanism possessed by living tissues which keeps dermatophytes from invading them was investigated. The growth of *T. mentagrophytes* was found to be inhibited intraperitoneally in mice even under conditions where host cells as well as tissue fluid proteins were excluded by means of cellulose dialysis membranes. Additional evidence is also presented to strongly suggest the existence of some dialyzable, water-soluble, unstable substance in serum and tissue fluids which suppresses the growth of dermatophytes and thus permits them to thrive only in places in the host not reached by such fluids.

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