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OBSERVATIONS ON DERMATOMYCOSIS IN PUERTO RICO

FURTHER REPORT ON THE ETIOLOGY OF EPIDERMOPHYTOSIS

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INTRODUCTION

In September, 1929, the author [1] reported on the fungus causing epidermophytosis of the *eczema marginatum* type—*tinea cruris* *—in Puerto Rico. The parasite was identified as *Trichophyton rubrum,* Sabouraud.

In a subsequent communication (March, 1930) [3] another organism associated with epidermophytosis of the feet was presented and described. The latter was identified as a variety of *Trichophyton gypseum* ** (Sabouraud)[4].

The failure of the author during many years of work to find, in this Island, cases of *tinea cruris* (*eczema marginatum*) or ringworm of the feet due to *Epidermophyton floccosum* (*E. inguinale*) was somewhat puzzling. Castellani [5], Sabouraud [6] and others have considered this species as the most common cause of *tinea cruris* in tropical as well as temperate regions. It is known, furthermore, that this organism occurs quite frequently in tinea of the feet. In 1932, Kesten *et al* [7] reported having isolated *E. floccosum* from a case of foot ringworm in San Juan (Puerto Rico). This moved us to further laboratory investigations in all cases where a mycotic infection of the glabrous skin was suspected, hoping that, some day, specimens of the fungus above mentioned would be encountered in these two forms of epidermophytosis. Our hopes have been partially fulfilled

* *Tinea cruris* is a misnomer as it conveys the idea of an eruption limited to the crural region. It is true that this disease shows predilection for the inguino-crural fold. However, other natural folds (the intergluteal, the axillary, the interdigital, etc.) are often affected just as well. In fact, the infection may, and in the tropics it usually does, spread to other parts of the skin, not even excepting the scalp, as shown by us in a recent publication [2].

** A more correct name for this organism is *Trichophyton mentagrophytes* (C. Robin, 1853) Blanchard, 1895.

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with the recent discovery of *Epidermophyton floccosum* in two cases of ringworm of the toes.

CASE REPORT

CASE No. I. *History*—G. E. M., a white, single, 23-year old Puerto Rican, engaged in the contracting business, came for a consultation on April 20, 1934.

He complained of an intensely pruriginous skin eruption affecting the interdigital spaces of both feet; also, of a peculiar thickening and discoloration of several nails. The disease had started 5 years before and had persisted with remissions throughout all that period. Involvement of the nails was noted after the third year.

Examination.—Upon examination, the interdigital spaces of both feet appeared red and desquamated, showing maceration in some areas and a yellowish thickening of the epidermis in others. These changes were more pronounced in the first (inner) interspace of each foot. Intense scratching was often followed by malodorous oozing. The anterior portion of both soles showed superficial desquamation in small zones as though groups of vesicles had ruptured and dried up leaving in their place white epidermal *collerettes* of varying sizes. A similar desquamation was present on the skin of the right palm.

The anterior half of the first and second toe nails of the left foot showed moderate thickening and a yellowish white discoloration. The superficial portion of the horny structures was apparently not involved, but the deeper layers were transformed into a dull white, soft, spongy mass. The first-toe nail of the right foot revealed similar changes over a small area. Here, the thickening was not so pronounced, but a dull white powdery substance could be obtained by scraping with a knife underneath the nail. A fourth and last onychial focus was discovered in the middle finger of the right hand. In this instance a certain portion of the nail plate was separated from the nail bed by a hollow space.

Laboratory findings.—Scrapings from the nails and interdigital spaces were treated with a 20 per cent solution of potassium hydroxide, set aside for an hour and then examined under the microscope. A part of the same material was inoculated on Sabouraud's glucose agar.

The direct microscopical examination of the squames revealed the presence of numerous, more or less undulating and septate mycelial elements. The filaments had a diameter of about 4 microns, and many of them were characteristically divided into short articles by the transverse septa.

In cultures, two different fungi were isolated. One of them, identified as *Trichophyton rubrum*, grew from the scrapings of the finger of the right hand and also from one of the toe nails. The other, *Epidermophyton floccosum*, was obtained from a second toe nail and from one of the interdigital spaces of the feet.

CASE No. II.—*History.*—F. D., a male, white Puerto Rican, aged 28, working as a stenographer in San Juan, came for medical advice on June 6, 1934.

He complained of itching, maceration and oozing between the toes. This eruption had been present for six months, but the patient had suffered from previous attacks. No history of *tinea cruris*.

Examination.—On examination, the first, third and fourth interdigital spaces of the left foot and the last interspace of the right foot appeared red, eroded, moist and covered in places by white, macerated epidermis. A few tiny vesicular

lesions were seen scattered over the affected areas. The eruption was not confined to the interdigital folds, but extended over the lower surface of the toes invading slightly the plantar aspect of the foot. The nails showed nothing abnormal.

Laboratory findings.—The microscopical examination of material from the lesions showed numbers of mycelial threads similar to the septate forms noted in Case No. 1, while cultures on Sabouraud's medium revealed the presence of only one organism: *Epidermophyton floccosum.*

MYCOLOGY

Of the three strains of fungi gathered from the above cases, one was classified as *Trichophyton rubrum* (See plate I). As already stated, this species was described in a previous publication [1] and, therefore, will not be discussed here. The other two have been identified as *Epidermophyton floccosum.* A study of the latter species as represented by the two strains just mentioned follows. In the course of this description, the parasite isolated from Case No. 1 will be referred to as strain "A"; that obtained from Case No. 2, as strain "B".

Methods of Study.—Immediately after isolation, the fungi were inoculated into Petri plates filled with *milieu d'epreuve* to a depth of about a quarter of an inch. In preparing this medium, Dr. Sabouraud's formula was carefully followed, Chassaing peptone and crude maltose being imported directly from France. The cultures were examined every day and photographed at weekly intervals.

The microscopical characters were studied by the hanging-drop culture method in plain broth and corn meal infusion; also, in slide cultures on corn meal agar. Growth was observed daily for over two weeks and photomicrographs and camera lucida drawings were made to illustrate the various characters noted.

Macroscopical Study of Cultures.—Although strains "A" and "B" differed morphologically in some respects (See plates II and III), there were certain fundamental common features, both macroscopical and microscopical, which linked them together. On Sabouraud's medium, the fungus generally reached its full development at the end of the third week. By this time it formed a fairly circular growth about 2¼ inches in diameter. The cultures could be arbitrarily divided for the purpose of description into three zones. The central portion, more or less raised over the rest of the

growth, appeared intricately corrugated in all directions, was powdery in structure, greyish in color and showed numerous minute brownish cracks on its surface. In some instances a crateriform depression was present at the center. Around this zone there was a second, more on a level with the surface of the medium, showing not so intricate but somewhat irregular radial folding, and colored in greenish grey or dull greenish yellow. In the marginal zone, about ¼ of an inch wide, the cultures were flat, shallow, less powdery, more filamentous and white.

Pleomorphic changes occurred within a comparatively short period after the isolation of the parasite. Laboratory transplants made during the first month showed practically no duvet until the colonies were four or five weeks old. However, in subsequent generations, pleomorphism was in evidence quite frequently as early as the second week. The duvetous growth would form prominent, small, white islands scattered over the surface of the cultures.

As already stated above, strains "A" and "B" were not exactly alike. In the former, the rate of growth was somewhat faster, the thallus was thicker and more filamentous, and the surface more irregular. In the latter (strain "B"), the growth formed a characteristic, thin, membranous structure over the medium. In strain "A", furthermore, the central zone was usually more elevated, acuminate and sometimes crateriform, making it closely similar to the cultures of this species as presented by Sabouraud [6]. The latter features were not nearly as marked in strain "B". Finally, strain "B" was apt to reproduce practically the same morphological pattern in successive generations, while strain "A" was rather inconsistent in this respect. Indeed, the various differential points just enumerated in cultures of these two strains were often so pronounced that a superficial examination of the cultures might throw some doubt as to their inclusion in the same species. Under the present conception of microbic variation, however, their separation into different species would not seem justifiable.

Microscopical Characters.—Microscopically, the two strains were indistinguishable from each other and the picture was so characteristic that a glimpse of a single field under low power magnification would have been sufficient to

identify the species as *Epidermophyton floccosum*. The thallus consisted exclusively of mycelial elements, pluriseptate macroconidia and a few clamydospores (see plate IV). Unicellular conidia and spirals were absent. It is well known that *Epidermophyton floccosum* is the only dermatophyte that would reveal such a picture under the microscope.

In hanging drops and in slide cultures, growth was comparatively active, germination being often noticeable several hours after inoculation. Germinating tubes would emerge from any of the cells forming a macroconidium, but most frequently they developed from one or both of its terminal elements (see plate VI). The cultures, which were usually exuberant after the fourth day, showed a thick growth of undulating and branching mycelium. The hyphae averaged about three microns in width ranging between 2.5 and 4.2 microns. Septa occurred at intervals varying between 8.3 and 36.6 microns. Highly refringent protoplasmic condensations, as well as clamydospores of both the intercalary and terminal types measuring from 6.7 to 16.7 microns in diameter, were often encountered. However, the most characteristic feature to be noted in cultures was the presence of great numbers of macroconidia (pluri-septate fusiform organs). These organs showed variations as to size and morphology. Their length ranged between 20 and 47 microns; their width, between 7 and 12 microns. Some were club-shaped, others were elipsoid, still others were more or less irregular in contour due to engorgement of the constituent cells. Transverse septa divided the structures into a number of compartments: usually 4 or 5; less often 2 or 3; exceptionally 6. When examined under the oil immersion lens, the limiting walls were found to consist of a double membrane, the cells containing a rather granular protoplasmic substance.

The macroconidia developed either singly or in characteristic bunches of two or more, from the tip or along the sides of the fertile hyphae (see plate VII). Their basal element was usually attached to a corresponding groove on the surface of the filaments from which they originated. In some instances this basal element would give origin to a secondary macroconidium (See plate V).

SUMMARY AND COMMENT

Trichophyton rubrum would seem to be the only fungus related to *tinea cruris* (*eczema marginatum*) in Puerto Rico. This impression is derived from a previous laboratory study of not less than 150 cases from different parts of the Island over a period of several years. Of course, further research along this line might reveal that, exceptionally, some other fungus may be the cause of infection. On the other hand, tinea of the feet seems to be associated with at least three different species, namely, *Trichophyton rubrum,* a variety of *Trichophyton mentagrophytes* and *Epidermophyton floccosum.*

In this communication, two cases of tinea of the toes are presented, one of them showing involvement of the hand, a finger nail and several toe nails. *Epidermophyton floccosum* was isolated in both instances from the interdigital spaces and, in Case No. 1, from an affected toe nail. Although the two strains described here are unquestionably identical as to species, their cultural characters on Sabouraud's medium differed in certain respects.

The infection of a toe nail with *Epidermophyton floccosum* in Case No. 1 would seem to be of significance.* Another interesting feature about this case was the concurrence of a second pathogen, namely, *Trichophyton rubrum,* which was isolated from a finger nail and from a toe nail. The writer had never encountered a mixed infection of this type before.

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* Although Castellani and Chalmers[3] have stated that "Cases of *tinea unguium*. . . . . are generally due to the same fungi producing "dhobie itch" (*tinea cruris*), we have been unable to find in the literature any report on nail infections caused by *Epidermophyton floccosum.*

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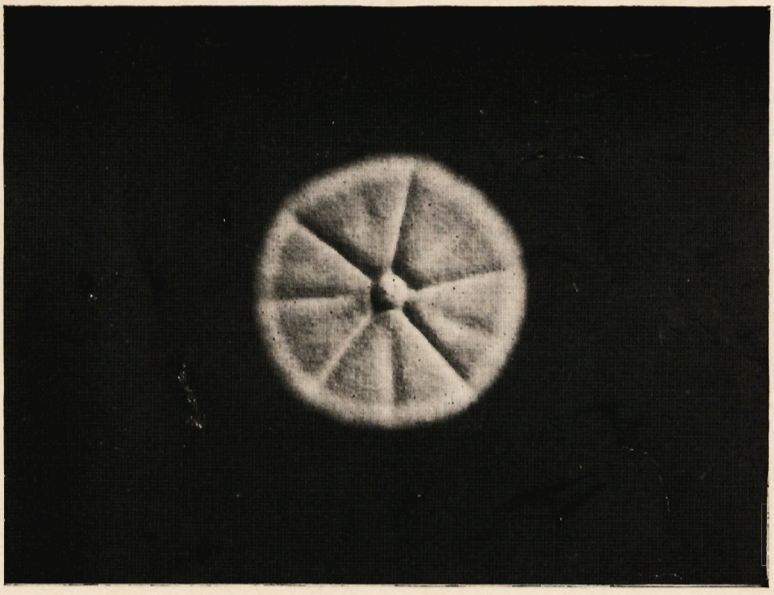
PLATE I

Trichophyton rubrum (Case No. 1). Cultures two and three weeks old on Sabouraud's maltose agar.



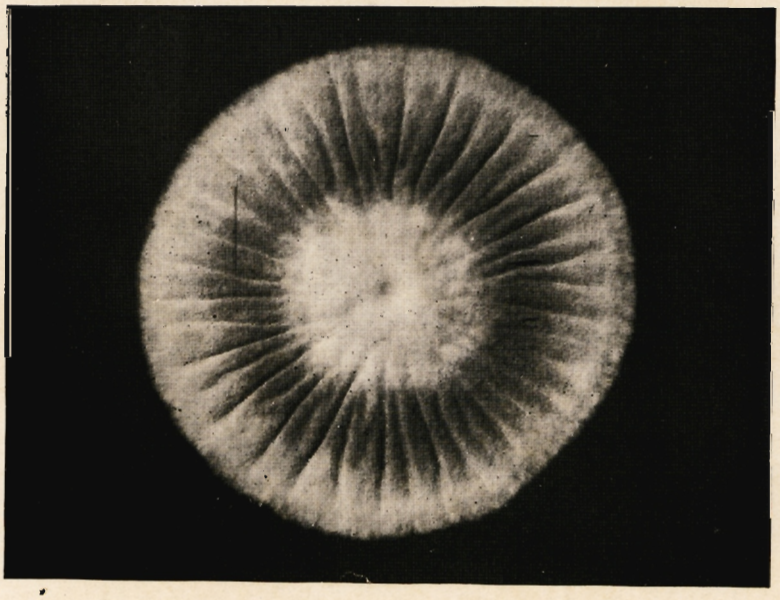


PLATE II

Epidermophyton floccosum (strain "A"). Cultures two and three weeks old on Sabouraud's maltose agar.