EXPERIMENTAL INOCULATION OF MONKEYS (Silenus rhesus) AND GUINEA PIGS WITH TWO DERMATOPHYTES AND ONE BLASTOMYCOIDES.

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In a recent study by Kesten (1), conducted in one of the largest clinics in Porto Rico, it was determined that (omitting banal pigment anomalies) fungus infections formed fifty-one per cent of the skin lesions seen in the Island. That fungus infections of the skin are very common in Porto Rico has seemed apparent for some time but no exact figures have been available until the work of Kesten mentioned above. The problem has seemed of such importance that the writers felt that efforts should be made to study it in a more fundamental way. We accordingly planned experiments involving the experimental inoculation of animals in order to determine if certain of the fungi could be made to infect such laboratory animals as guinea pigs and monkeys, and to see what could be learned regarding the mechanism of immunity in diseases of this nature. In this paper we are reporting the results of the first part of our work.

Working with the dermatophytes, Block (2) in 1908 stated that the subcutaneous or intravenous methods of infection with fungi do not immunize or reproduce the disease in animals. Lombardo (3) also failed to produce any reactions to the Trichophyton when this fungus was introduced into animals in any other way than through the skin. Mililiary granulations, however, were described in the lung of a rabbit following the subcutaneous and intravenous injection of Trichophyton and Oospora canina in a series of experiments performed by Sabrazés (4). Similar lesions were also found by Bukowsky (9) with Achorion. Saeyes (5) produced cutaneous lesions in a guinea pig with Trichophyton gypseum and Achorion quickeanum by intracardial injection in animals which had been previously shaved on the abdomen. This work was confirmed and further extended by Kogo (7). In a recent paper Fried and Segal (8) summarize the literature and report the successful infection of rabbits with Trichophyton gypseum. These authors shayed the skin of the animals and

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then scarified with sandpaper. The rabbits were then injected intravenously with from 2 to 5 cc. of an emulsion of *Trichophyton gypseum*. Of twenty-nine animals injected, eleven, or thirty-eight per cent developed cutaneous lesions confined to the shaved and scarified areas only. The authors state that the bulk of the fungus disappears from the circulation within the first twenty-four hours.

Since *Trichophyton asteroides* is a variety of trichophyton which affects chiefly lower animals (horses and cattle) and gives rise to inflammatory lesions with folliculitis and the formation of kerion, we thought this fungus would be a most favorable one to work with for the purpose we had in mind. In addition we have had available an interesting *Blastomycoides (Coccidio-ides) aurea* recently isolated from a subcutaneous abscess in a negro who was presented at one of our clinics by Dr. García Cabrera. The patient presented severe ulcerative lesions and subcutaneous collections of pus all over the inner aspects of the thighs and buttocks with extensions into the groin. Pus was withdrawn with a sterile hypodermic syringe from a closed deep abscess and planted on Sabouraud’s glucose medium. Golden-orange colonies appeared in from four to five days which were very translucent, round, convex, and of a beeswax consistency and appearance, without duvet. For our third fungus *Sabouraudites rubra* (Castellani) Langeron has been employed.

The *trichophyton* was sown in several tubes of four per cent glucose broth (pH 7) and upon Sabouraud’s glucose agar in giant culture. After a ten-day growth had been obtained the fluffy deposit and superficial growth in the glucose-broth tubes were removed and ground with sterile sand in a mortar. The ground material was then suspended in physiological saline. Microscopical examination of the emulsion revealed an abundance of spores and mycelia. The *Blastomycoides* and cultures of *Sabouraudites rubra* were treated in the same fashion.

Experiments with *Trichophyton asteroides gypseum*.

Several methods were devised to infect monkeys. In each animal three methods were employed at the same time with the hope that at least one would succeed. On November 9, 1929, 2 cc. of Sabouraud’s glucose agar was melted, cooled to 43 degrees C., and injected hypodermically in monkeys 1 and 2 in each armpit and the right groin. Into the nutrient mass was immediately injected 1 cc. of an emulsion of *Trichophyton asteroides gypseum* prepared as described above. On the left side of the shaven abdominal wall a pocket was dissected subcutaneously and a piece of the growth in giant culture
approximately one inch square was inserted therein, the wound being closed thereafter with collodion. Finally, on the right side of the abdomen, the skin was brusquely rubbed with sandpaper over an area of one and a half inches by three inches until a sero-sanguinous exudation had left the surface humid. Into this area 1 cc. of the *Trichophyton asteroides* emulsion was rubbed with sandpaper.

Twenty days later, Monkey 1 presented a mass about the size of a small chestnut in the right groin at the site of inoculation. This mass was rounded, indurated, and smooth, with the feel of an enlarged lymph gland. The skin over this mass was slightly reddened. A similar mass about the size of a pea was noted in the left axilla at the site of the previous inoculation. No other lesions were present. Monkey 2 showed slight induration in the region of the pocket on the left side of the abdomen where the fragment of a giant culture was planted. A small indurated smooth mass was also noted in the right groin. No other lesions were present in this animal.

On the twenty-sixth day following inoculation, Monkey 1 showed no further change but Monkey 2 presented on the right side of the abdomen, where the culture had been rubbed into the skin with sandpaper, a large, irregularly-shaped, festooned patch, about three-quarters to one and a half inches in extent. The lesion was slightly elevated, indurated, and presented a dull red center. The periphery was clearly delimited from the surrounding skin by a slightly elevated ridge and showed a little desquamation. In addition there were secondary and smaller patches in the vicinity of the main lesion. Skin scrapings were soaked in forty per cent potassium hydrate solution and were found to be heavily invaded by *Trichophyton asteroides*. This was further demonstrated to be the case by cultures (Figures 1 and 2). In three days, plate cultures of the squames showed some thirty stellate, cream-colored, translucent colonies of a silky sheen. There were only one or two contaminating bacterial colonies. On the next day, by direct examination of these plate colonies with the high, dry lens, each was found to be composed of a mass of mycelial filaments, radiating at the periphery. A few aleurys, a few loose spirals, and a very few chlamydespores were noted. There were no fusiform bodies. The mycelium was usually straight, at times undulating. Some of the original scales were soaked in alcohol for half an hour and the original scales were soaked in Sabouraud’s glucose agar. All gave the same type of colonies, but there were no contaminating bacteria.

On the thirty-fourth day, Monkey 1 showed no changes from the
conditions expressed for the twentieth day but the skin lesions of Monkey 2 had become accentuated.

On the seventy-first day Monkey 1 also showed definite skin lesions on the right side of the abdomen where the culture had been rubbed into the skin with sandpaper. These lesions were precisely identical to those described for Monkey 2. The lesion of Monkey 2 had persisted but the squames were more abundant and gave a pearly cast to the eruption. No other lesions were found.

On the ninety-ninth day after inoculation the lesions on both monkeys had practically disappeared.

On the fifty-second day of this experiment, ten guinea pigs, whose abdomens had been shaven twenty-four hours previously, were inoculated with cultures obtained from Monkey 2 by sandpapering the abdomen and rubbing the emulsion into the skin. Twenty-three days later five of the guinea pigs were living and two of the animals which had died of pneumonia were all found to have lesions at the site of inoculation identical to those produced in the monkeys. Scrapings from the lesions revealed spores and mycelia on direct examination and sub-cultures proved to be Trichophyton asteroides. The other three guinea pigs had died before lesions had time to appear. Autopsies of the two dead guinea pigs with lesions of the skin revealed no other lesions save those of pneumonia. Fragments of lung, spleen, liver and kidney were negative for Trichophyton asteroides. Experiments with Sabouraudites rubra (Castellani) Langeron.

Exactly the same methods and technique were employed in the inoculation of monkeys with Sabouraudites rubra as have been described for the Trichophyton above. Monkeys 3 and 4 were inoculated on the same day as Monkeys 1 and 2. Twenty days later Monkey 3 presented only an enlarged gland in the right groin at the site of inoculation. No other lesions were visible. Twenty-six days after inoculation no change was noted in Monkey 3. Monkey 4 had escaped at the time of the first examination but on this occasion showed no lesions whatsoever. Thirty-four days and seventy-two days after inoculation no lesions were visible in either monkey. It should be stated that all of the animals were examined daily in their cages by one of us but were only removed for special examination as indicated when the cage examination revealed anything suspicious which demanded closer survey. One hundred and eleven days after inoculation Monkey 3 died. The findings at autopsy were unimportant since it was found that the animal died of pneumonia and tuberculosis. Cultures from the lungs, liver, spleen, kidney and skin at
the site originally inoculated were all negative for *Sabouraudites rubra*.

Experiments with *Blastomycoïdes* (*Coccidioides*) *aurea*.

This organism was sown and emulsified, as were the *Trichophyton* and *Sabouraudites rubra*, and two monkeys were inoculated in the same manner as described before. (Monkeys 5 and 6.)

Twenty days following inoculation Monkey 5 presented a mass in the right groin. The overlying skin was reddened. Similar but smaller masses were noted under each axilla at the sites of inoculation. The pocket excavated in the abdominal wall showed induration and the wound was found to be gaping, revealing grayish, necrotic material within. The area on the right side of the abdomen showed some thickening and induration. In addition, the general condition showed sepsis. The animal was definitely ill and depressed. One cubic centimeter of glucose broth was injected into the groin lesion and withdrawn immediately thereafter without disturbing the needle. This fluid was sown in plates.

Monkey 6 showed lesions identical with those of Monkey 5. On the twenty-sixth day both monkeys showed improvement over the sandpapered area which, however, had never been the seat of any distinct eruption. The pocket in the abdomen had closed and the glandular lesions remained as before. Fluid was again injected, withdrawn and plated.

On the seventy-first day, Monkey 5 revealed no lesions and Monkey 6 only an enlarged gland in the right groin which was excised, emulsified in an aseptic mortar and sown on Sabouraud's glucose agar. All cultures from these glands were doubtful as far as the original organism was concerned. Yeast-like bodies were found in abundance but could not be separated from a contaminating coccus and were finally lost. But the colonies, from the first, were brownish-yellow and seemed to promise from this cultural likeness a positive subculture.

The morphology and cultural characteristics of this fungus will be reported in a subsequent paper by one of us (A). The outstanding features, however, are many mycelia, many arthrospores and some large, very heavy-shelled round bodies containing spore-like bodies.

**CONCLUSIONS.**

These studies indicate the experimental transmission of infection in monkeys and guinea pigs with the *Trichophyton asteroides gypseum* by at least one of the methods employed. Negative results were
Figure 1. Dermatomycosis experimentally produced by sand-papering abdomen of monkey and inoculating Trichophyton asteroides gypseum both directly and hypodermically.

Figure 2. Organism found in squames after clearing with 40 per cent potassium hydrate solution.
obtained with *Sabouraudites rubra* in monkeys with the same methods. The pathogenicity of *Blastomyces aureus* falls short of proof from the failure to secure positive cultures, but this question is still open.

It seems apparent that experimental transmission of infection with the *Trichophyton* cannot be accomplished by introducing the fungus under the skin. Infection is successful when the fungus is introduced in the superficial layers of the skin and it would seem a safe statement to make that this fungus possesses definite dermotropic properties. Also it is known that greater growth of this fungus is obtained in an environment rich in oxygen(2) but its selective preference for the skin cannot be explained entirely upon this basis since it also grows to some extent when the environment is comparatively limited in oxygen. Fried and Segal have reported the successful transmission of this fungus infection to rabbits but of chief interest to us is their statement that in over a third of cases of rabbits inoculated by marginal ear vein with this organism, a typical dermatomyositis was only obtained on the previously abraded skin. The analogy is all the more striking in that lesions appeared on the skin in their animals at about the same periods of incubation as we have reported in our animals. These authors suggest that infection following intravenous injection takes place after injury to the skin because the fungus is able to migrate from the injured papillary capillaries into the skin where it develops its pathogenic properties. This is a quite logical explanation of the phenomena observed but we would also suggest that the dermotropism of the fungus is also an important factor and that to some extent the oxygen supply available is also a contributing factor favoring infection in the skin.

REFERENCES.