OBSERVATIONS ON DERMATOMYCOSIS IN PUERTO RICO

REPORT ON A CASE OF CHROMOBLASTOMYCOSIS *

A. L. CARRIÓN
Of the Department of Mycology, and
E. KOPPISCH
Of the Department of Pathology
From the School of Tropical Medicine of the University of Puerto Rico under the auspices of Columbia University.

INTRODUCTION

Dermatitis verrucosa is the name given in dermatology to a fungous disease characterized by a more or less extensive, chronic, papillomatous eruption affecting ordinarily the lower extremities, particularly the feet. As a rule there is little if any impairment of the general health. The term has been more loosely applied to lesions due to a number of infectious agents such as leishmania, bacillus tuberculosis, a special type of spirochete (*Spironema noguchii*[1]), and certain species of fungi. In the case herein reported the etiologic factor was a fungus.

The role of parasitic fungi as a possible etiologic factor of dermatitis verrucosa was first suggested by A. Pedroso of Sao Paulo, Brazil, in 1911 [2a]. While studying the pathological reaction in certain patients who showed verrucous lesions of the skin, this author noted the presence in the tissues of dark, spherical bodies, sometimes included within giant cells, which were thought to be fungous in nature. By inoculating material from these lesions into proper media, he was able to isolate an organism producing dark colonies which, however, for the time being, was not subjected to further study. In view of the similarity of this organism, as observed in tissue sections, with the large, spherical, parasitic cells noted in cases of blastomycosis, the disease was denominated blastomycosis nigra, this term being also descriptive of the pigment-producing character of the supposed causative agent. Several years later, in 1922, O. da Fonseca and his colleagues pro-

* This paper will appear in Spanish in the Report of the Eighth Meeting of the Argentine Society of Regional Pathology of the North, 1933. (Octava Reunión de la Sociedad Argentina de Patología Regional del Norte, 1933.)
Received for publication, August 20th, 1933.

posed the name chromoblastomycosis for those cases of dermatitis verrucosa due to fungi [3], and, since then, this latter name has gained considerable popularity.

Chromoblastomycosis has many points in common with North American blastomycosis both clinically and histopathologically. There are certain fundamental differences, however, which are worth mentioning here. The etiologic fungi of the former disease would seem to multiply by cellular division rather than budding in the tissues and, in stained pathological sections, they take a dark brown color, these features being in marked contrast with what happens in blastomycosis. In cultures, the morphology of the organisms is still more markedly different both macroscopically and microscopically. Last of all, the prognosis in blastomycosis is generally considered more serious, since this disease has shown a certain tendency to invade the general circulation producing metastatic lesions in the internal organs with a fatal termination, a happening that has not been reported as yet in chromoblastomycosis.

CLINICAL REPORT

HISTORY: J. R., male, white laborer, aged 50, was admitted to the University Hospital on January 27, 1931. He was born in the town of San Sebastián, and had always lived there.

The patient stated that about the year 1915 he had had a sore on his penis which was treated with local applications. After that, however, he never suffered from suspicious symptoms and at the time of admission there was no clinical evidence of a luetic infection, the Wassermann and Kahn tests having been negative several times for the blood. His habits in general were good and there was nothing else of importance in the past history.

The first manifestations of the present disease appeared 15 years ago. At that time the patient was employed on a coffee plantation where he worked barefooted. The eruption began as a small, itchy papule situated on the posterior third of the dorsum of the second toe of the left foot. This papule was cauterized after which a crust formed. According to the patient the condition of the skin beneath this crust improved somewhat, but the skin surrounding it became inflamed and pruriginous. This reaction continued to extend eccentrically, and the lesion was eventually transformed into a small, ringworm-like patch. Shortly afterward, new efflorescences became visible higher up in the dorsum of the foot; later on, the region of the ankles became invaded, and after this, the disease gradually but steadily extended its limits year by year until the time he was examined, when it reached up to the middle of the thigh (Plates I and II).

EXAMINATION: The eruption as a whole was quite remarkable in appearance, its characters varying strikingly in different regions. For the purpose of better

description we shall refer to: I, the lesions on the lower thigh and knee; II, lesions on the leg; III, lesions on the foot.

I. Lesions on the lower thigh and knee: In the lower parts of the thigh and in the knee, which were seemingly the regions most recently affected, there were numerous, well limited, dull red or violaceous patches of dermatitis showing a fairly marked degree of infiltration. These patches were of different sizes, the largest measuring about 3 inches in diameter. The borders were usually well marked and irregularly rounded. In certain places where several adjacent lesions had become confluent the affected portions naturally extended over much larger areas, the borders taking a more irregular and polycyclic character. The inflammatory areas rose about one or two millimeters above the normal epidermis and showed marked exaggeration of the lines of cleavage to the skin.

In most of the plaques the general evenness of the surface was interrupted by the presence of markedly elevated, variously sized, dull red or grayish nodular growths, often fungating in character, which gave to the lesions a characteristic appearance. These nodules were sometimes confluent, forming large, irregular, prominent masses. They were usually hard to the touch and by pressure a certain amount of grayish-white cheesy material could be extracted from some of them. Certain nodules had softened and contained an opalescent fluid sometimes stained with blood. Ulceration had occurred in some of the lesions, but the eruption as a whole had a rather dry appearance. Slight external injuries readily produced bleeding.

In many places the skin showed areas of hyperpigmentation within which were seen fading inflammatory foci quite similar in appearance to those already described. It is evident, therefore, that the spontaneous healing of some of the lesions is possible in this disease.

II. Lesions on the leg: Farther down, in the middle of the leg, the lesions were evidently older. In this region the nodular growths described above had attained a much larger size and their number was increased to such an extent that coalescence had taken place and most of the patches were now transformed into irregular, prominent, fungating areas, having a dull red color and being covered here and there by more or less adherent, grayish squamae or crusts.

III. Lesions on the foot: In the lower part of the leg and over the dorsum and sides of the foot, where the pathologic process had persisted longest, the eruption offered a most striking picture. Here the skin appeared edematous, rather elephantiasic, covered with numerous, markedly elevated, cauliflower-like masses which were extremely characteristic. The size of these masses varied roughly from that of a large pea to that of a hen's egg. Some had a hemispherical or oval form; others were more irregular in morphology due to coalescence of two or more lesions. In some places the fungating masses overgrew the base of the lesions, giving to them a somewhat pedunculated appearance. The surface was dry, irregular, granular, and often covered by dirty, grayish crusts. Toward the border of the foot, where the skin is normally more corneous, the lesions had taken a very typical warty appearance.

The pathological reaction was not limited to the skin proper, since in various points of the affected limb there were lesions deeply situated in the subcutaneous tissue. Some of these lesions were adherent to the dermis producing a certain degree of bulging, and when this was the case the skin usually participated in the reaction, taking a reddish or violaceous color. In other instances the nodules were more or less movable under the integument, the tissues covering them re-

maining normal. Subcutaneous nodules not infrequently softened and broke out through the skin.

Itching had always been a prominent feature of this eruption, and during the last few years there had been more or less pain on the lower part of the leg and foot on walking. It was very remarkable that the general health of the patient was not seriously disturbed, notwithstanding the relatively enormous extent of the infected areas.

Metastatic lesions: A careful examination of the patient revealed a number of interesting lesions far removed from the original site of the infection. In the lower third of the ulnar side of the left forearm there was a hard, lumpy mass, about 3 inches long by 1.5 inches wide, deeply situated in the subcutaneous tissue and seemingly adherent to the bony structures. It was of four months' duration. Though the region was considerably deformed by the presence of this growth, the skin was not affected in the least and could be easily displaced over the hard underlying mass. There was a slight pain on pressure and the condition as a whole was somewhat suggestive of a viciously healed fracture. However, the history was negative, and an X-ray picture failed to show any deformity in the bone.

Shortly after the patient was admitted to the hospital, a certain degree of fluctuation was noted in the lesion, and upon incision about 2 cc. of a whitish opalescent fluid came out. From this fluid the causative organism was isolated in pure culture and sections from the abscess wall revealed the same pathological changes observed in the leg lesions (see "Pathology"). The cavity was left to drain for several days and the wound gradually healed, but the infiltration did not disappear entirely.

In the anterior aspect of the middle third of the right thigh another deeply seated nodule was discovered.* This was about the size of a large bean, and it lay within a pocket in the muscle just beneath the fibrous sheath of the *rectus femoris*. The lesion was excised and examined histologically and showed characteristic changes with typical fungus cells.

On the chin there was a superficial, moderately infiltrated, dull red patch about ¾ of an inch in diameter and somewhat irregular in contour. It resembled very closely some of the less infiltrated lesions noted on the leg. About 2 cm. below this efflorescence, in the submental triangle, a small subcutaneous pea-sized nodule was discovered. The mycotic origin of the latter was readily disclosed upon pathological examination.

It is presumable that the superficial patch observed on the chin might have resulted from the direct inoculation of the skin by the contaminated hands of the patient. From this location infection was probably propagated downward by way of the lymphatics producing the submental nodule. But the development of deep foci such as those described in the left forearm and right thigh, respectively, can hardly be ascribed to transcutaneous inoculation. It is difficult to conceive of the fungus as traversing the skin and corium without leaving traces of its passage, to then become implanted in the deeper

* Note that the original eruption was located on the opposite side.

tissues. So that these lesions are best explained as the result of a true metastasis of the fungus cells, most probably by way of the lymphatics of the leg to the thoracic duct and thence to the left subclavian vein and general circulation. In the light of these observations the occurrence of secondary foci in some of the internal organs should be considered as an important possibility in this disease. The clinical characters and the significance of such a complication would, of course, vary greatly in accordance with the organ involved.

LABORATORY DATA: A feces examination for parasites was negative and the urine showed nothing abnormal. The blood revealed a reduction of the red cells to 3,100,000, and of the hemoglobin to 60 per cent (Dare). The leukocytes, on the other hand, were increased to 21,300. The differential count was as follows:

Small lymphocytes	39 per cent
Large lymphocytes	1 per cent
Mononuclears	2 per cent
Polynuclear neutrophiles	57 per cent
Eosinophils	1 per cent

These blood changes were most probably due to complicating bacterial infections which were evident at the time the patient was admitted to the hospital. The blood chemistry showed a slight reduction in the cholesterol (112.4 per 100 cc.) and calcium (8.4 per 100 cc.) contents. One blood culture was negative.

PATHOLOGICAL ANATOMY

Histopathologic studies were undertaken with three main objectives in mind. First, to help establish a definite diagnosis. Second, to determine the inter-relationship of the various types of skin lesions, tracing, if possible, the evolution of the process by analyzing the histologic changes in the lesions at different levels of the affected leg. For it seemed evident, both from the history of the case and from the appearance of the dermatologic changes, that the disease had started in the foot and thence ascended towards the knee. The third main objective was to decide whether the deep seated nodules in the thighs and submental triangle, and a fluctuating swelling in the left forearm, were related or not to the affection of the leg.

MATERIAL AND METHODS: The material studied consisted of (*a*) patches of induration of the skin of the lower third of the left thigh; (*b*) sessile nodules from the skin of the left leg; (*c*) cauliflower-like lesions from the skin of the dorsum of the left foot; (*d*) a small pedunculated nodule from the left leg; (*e*) a nodule from the subcutaneous fatty layer of the left thigh, above the

highest level of skin involvement; (*f*) a nodule attached to the inner aspect of the deep fascia of the right thigh; (*g*) a nodule situated in the subcutaneous tissues of the submental triangle, and (*h*) of tissue scraped from the wall of an abscess-like swelling in the left forearm.

Zenker's fluid was used for the fixation of all the pieces of tissue but three, for which 10 per cent formalin solution was utilized. Paraffin sections were stained with hematoxylin and eosin. The special stains employed were Weigert's for elastic tissue with safranin as a counterstain, Gram's for bacteria and Ziehl-Neelson's carbolfuchsin method for acid fast bacilli. The Prussian blue reaction with potassium ferrocyanid and hydrochloric acid was used for identification of iron-containing pigment.

DESCRIPTION OF LESIONS: (*a*) The material representing this type of lesion was removed from the distal third of the thigh and consisted of two small strips of skin. The epidermal surface was finely roughened in both, and on section the epidermis was slightly thickened and formed short papillary projections, while the corium was not appreciably broadened. In one of the portions the roughening was limited to small areas that were sharply outlined and slightly elevated above the surrounding smooth skin.

The microscopic changes were limited to the epidermis and superficial portions of the corium (Plate III:Fig. 1). The epidermis was moderately and unevenly thickened, and showed acanthosis and elongation of the rete pegs. The horny layer was broadened and completely keratinized. Epithelial pearls were frequently encountered in the malpighian and horny layers. The lengthening of the dermal papillae resulted in the formation of blunt and pointed elevations at the surface. In the papillary portion of the derma were fairly numerous pseudo-tubercles usually found in close proximity to the epidermis. These structures were composed of epithelioid cells and frequently contained a giant cell of either the Langhans or foreign-body type in the centre. The superficial portions of the corium were infiltrated with plasma cells, lymphocytes and large mononuclear cells. The infiltration was rather compact and for the most part diffuse, though at times the cells showed a tendency to form focal accumulations. Small collections of polymorphonuclear leukocytes were seen here and there, particularly at the tip of the dermal papillae; a few leukocytes had wandered into the epidermis. Among the cells of the infiltrate in the corium were occasional eosinophils and a rare Russell's fuchsin body. The reticular layer of the corium was unaltered except for the presence of a few small foci of perivascular infiltration with lymphocytes and plasma cells. The hair follicles and sweat glands were atrophic.

In the center of the pseudo-tubercles, usually within the giant cells, were brown, spherical (Plate VII: Fig. 1) or cup-shaped (Plate VIII: Figs. 1 and 2) bodies with a refractive double membrane and a fairly homogeneous inner substance. They were occasionally found free in the dermis and within epithelial pearls in the malphigian and horny layers of the epidermis. These fungus cells averaged 7 microns in diameter, but varied from 5.7 to 11.4. A few were found divided in two (Plate VIII: Fig. 1) and three chambers by delicate, brown

septa, and a short, brown, septate hypha was seen growing from some of the fungus cells in the horny layer. Not infrequently, the above bodies were pale, with ill-defined limiting membrane, and obviously in process of degeneration (Plate VIII:Fig. 2).

(*b*) The sessile nodules from the skin of the left leg averaged 1 to 2 cm. in main diameter and approximately 0.5 cm. in height. The surface was quite even in all. On section the bulk of the nodules was composed of dense semitranslucent tissue over which the epidermis formed occasional small depressions.

As compared with the lesion first described, acanthosis of the epidermis was only slightly more pronounced, while the interpapillary rete pegs were much longer, and the depressions at the surface much deeper (Plate IV:Fig. 1). The bulk of the nodule consisted of young, loose, edematous fibrous tissue that had replaced the papillary layer of the derma and resembled recently organized granulation tissue. The reticular layer was broad and very dense. The pseudo-tubercles were, as before, superficially situated, but most of the giant cells in them were of the foreign-body type. The cell infiltration differed in being distinctly focal (Plate III. Fig. 2), and in that eosinophils, Russell's fuchsin bodies and polymorphonuclear leukocytes were more numerous. The eosinophils were most frequently found in the immediate neighborhood of pseudo-tubercles, and the polymorphonuclears in minute abscess-like collections beneath the epidermis, or in recesses in the epithelial pegs, or in small groups in the center of pseudo-tubercles. Hemosiderin pigment was present in finely divided form throughout the loose connective tissue and along the collagen bundles of the reticular layer. Weigert's stain showed the elastic fibers to be totally absent from the layer of loose connective tissue, while in the deeper, denser portion they were short and fragmented. The fungus cells were found in the same situations as previously.

(*c*) The cauliflower-like lesions from the dorsum of the left foot measured 1.5 to 2.5 cm. in main diameter, and 1 to 1.5 cm. in height. The surface was uneven, crustaceous and coarsely papillary. On section, the papillae were found to vary from 0.1 to 0.4 cm. in height. In the depressions between them was soft, pale matter. Beneath the epidermis the bulk of the nodule was constituted by dense, whitish tissue.

Microscopically, this type of nodule differed from the sessile ones mainly in the much greater density of the fibrous connective tissue of which they were composed. The interpapillary rete pegs were, in general, thicker and longer, but acanthosis, although more advanced in some areas, had in others given place to atrophy. The pseudo-tubercles were quite superficially situated and had attained a larger size than in previously described lesions (Plate IV:Fig. 2). The giant cells in them were most frequently of the Langhans variety. Eosinophils and Russell's fuchsin bodies occurred in greater number than in any other type of skin lesion, while polymorphonuclear leukocytes were but rarely seen and

never formed abscess-like collections. The fungus cells were located, as before, in pseudo-tubercles, in epithelial pearls of the horny layer and in the depressions of the epidermis. In the last situation a fairly dense mycelial growth (Plate VI: Fig. 2) was occasionally found, and each fungus cell apparently gave rise to but one delicate, septate, brown hypha.

(*d*) A pedunculated nodule was removed from the posterior aspect of the upper half of the left leg. The histologic alterations were essentially similar to those found in the cauliflower-like masses.

(*e*) This nodule was removed from the subcutaneous tissues of the anteromedial aspect of the left thigh, at a level above the highest limit of skin involvement. It was ovoid and measured 2.5 cm. in length and 1 cm. in diameter. The nodule was surrounded by a delicate capsule, and on section presented a large central yellowish area of softening surrounded by whitish semi-translucent tissue.

Histologically, the corium and epidermis overlying the nodule were normal, except for the obliteration of the dermal papillae in some places. The capsule of the nodule was composed of a layer of fibrous tissue in which only a few short elastic fibers could be demonstrated with special stains. Within the capsule was granulation tissue densely overrun by plasma cells, polymorphonuclear leukocytes and lymphocytes. Eosinophils were moderately abundant and Russell's fuchsin bodies formed a prominent feature of the infiltrate. Throughout the granulation tissue were very numerous pseudo-tubercles composed of large epithelioid cells. Giant cells were also numerous and of both the Langhans and foreign-body types, in about equal proportion; they occurred in the pseudo-tubercles and outside of them. The polymorphonuclear leukocytes were for the most part diffusely scattered, but not infrequently formed small abscesses in the granulation tissue, and at times were found in minute groups in the pseudo-tubercles. In the center of the nodule was a large cavity packed with degenerate and necrotic cells that appeared to be large mononuclears in their majority, but amongst which dense collections of polymorphonuclear leukocytes could be recognized. (Plate VI:Fig. 1). Slit-like spaces from which cholesterol crystals had been dissolved occurred here and there in the granulation tissue surrounding this area.

The fungus cells were found in large numbers in the center of pseudo-tubercles, in the large central area of necrosis, and in the granulation tissue. They occurred singly and in groups of not more than four cells. They had not always been enclosed in a giant cell, and when in the center of pseudo-tubercles were often accompanied by small numbers of polymorphonuclear leukocytes. One of the fungus cells was unevenly divided in two parts by a transversely placed septum, and the larger of the sub-divisions presented a small knob-like projection.

(*f*) This nodule was deeply located in the right thigh and attached to the inner aspect of the deep fascia. It measured 1.5 by 0.6 by 0.5 cm. and was enclosed in a delicate capsule.

Microscopically, the structure of the nodule (Plate V:Figs. 1 and 2) was

very similar to that of the preceding one, except that there were no areas of necrosis. Small groups of polymorphonuclear leukocytes were often found in the center of pseudo-tubercles (Plates V:Fig. 2, and VII:Fig. 2).

(*g*) This nodule was removed from the subcutaneous fatty layer of the submental triangle and measured 0.5 cm. in diameter. It was similar, both grossly and microscopically, to the one previously described under "*f*".

(*h*) The tissue scraped off the wall of the abscess-like swelling in the left forearm was composed of loose edematous granulation tissue throughout portions of which a delicate reticulum of fibrin had been deposited. In it were small areas of hemorrhage, and diffuse, not very dense, infiltrations with polymorphonuclear leukocytes, lymphocytes, plasma cells, large mononuclear cells and Russell's fuchsin bodies. Occasional small pseudo-tubercles were found, in some of which were fungus cells. Most of these cells seemed to be in process of degeneration, being rather large and pale, and showing a very thin limiting membrane that was not sharp in outline. No bacteria were found in sections stained by the Gram and Ziehl-Neelson methods.

SUMMARY AND DISCUSSION OF HISTOPATHOLOGIC FINDINGS

The reaction to the presence of the fungus was granulomatous in nature, with formation of pseudo-tubercles even in the earliest of the lesions studied. The essential skin changes were limited to the superficial portions of the corium, especially to the papillary layer.

In one set of skin lesions— the slightly elevated reddish patches—the principal histologic change consisted in pseudo-tubercle formation and diffuse round cell infiltration in the papillary layer of the derma. In the second set— that of the medium-sized sessile nodules—the papillary layer and part of the reticular had undergone replacement by a broad zone of loose and edematous connective tissue in which were pseudo-tubercles and focal accumulations of round cells. In the third set, comprising the large cauliflower-like masses, the changes differed from those in the medium-sized nodules mainly in the much more advanced degree of fibrosis, and in the greater size of the lesion.

The material studied clearly showed the gradation of changes between the medium-sized sessile nodules and the

large cauliflower-like masses, but did not include any lesions presenting the intermediate stages between the slighly elevated patches of induration and roughening of the skin, and the medium-sized nodules. It seems plausible to suppose that such lesions, if studied, would have shown formation of granulation tissue in the papillary layer of the derma. It is apparently through organization of this tissue and progressive thickening and fibrosis of the reticular layer, that the lesions slowly develop until they attain the structure and proportions of the cauliflower-like masses.

The cell reaction, diffuse at first, later became distinctly focal in distribution. Polymorphonuclear leukocytes were most numerous in the patches of early skin involvement and in the medium-sized nodules, while they were very rare in the older cauliflower-like masses. Eosinophils and Russell's fuchsin bodies became more abundant as the lesions grew older, being most numerous in the cauliflower-like masses. The type of giant cell reaction seemed to show a tendency to become preponderantly of the Langhans variety as the lesions aged, but this was not distinct. Hyperkeratosis and lengthening of the interpapillary rete pegs advanced as the lesions progressed in their evolution. Acanthosis of the epidermis seemed to reach its height in the intermediate stage of development of the nodules, but after this, although it progressed in places, atrophy began in others.

An interesting feature was the finding of fungus cells enclosed in epithelial pearls in the malpighian and horny layers of the skin. This may represent a mechanical defense reaction by means of which some of the fungus cells are extruded from the tissues.

The mycotic lesions found in the subcutaneous tissues and deep fascia, in places far removed from the original site of the disease, were all of relatively recent development and showed formation of granulation tissue and pseudo-tubercles, but no organized connective tissue, excepting that in the capsule.

In some of the metastatic lesions the fungus apparently was capable of inducing widespread necrosis, softening, and mild suppurative changes. There is reason to believe that these changes were not due to secondary infection, since no bacteria were isolated upon culturing the pyoid material found in the abscess-like swelling of the forearm, and no

organisms other than the fungus were discovered in sections stained by the Gram and Ziehl-Neelson methods in any of the two lesions showing the above changes. The pyoid material, however, was not cultured anaerobically.

On the method of primary infection with the fungus, the material studied affords no direct information. There is no reason to suppose, from the evidence at our disposal, that the fungus first entered in any way other than directly through the skin. It would only necessitate a relatively slight trauma to the epidermis for implantation in the loose, superficial layer of the derma, where it seems to thrive best. From this situation, slow extension along the superficial lymphatic network of the corium probably occurs. This would account for the upward extension towards the knee and for the superficiality of the lesions. Reproduction within the tissues and occasional extrusion to the surface within epithelial pearls or otherwise may account for the presence of the fungus in the horny layer at all levels of the affected limb. The fungus seems capable of surviving for some time in this situation, where it was not infrequently seen to be producing a mycelium. It is also conceivable that from the skin surface it could be transported, perhaps by scratching, to the skin in other parts of the body, and it may be in such manner that the patch of dermitis in the chin of our patient originated.

MYCOLOGY

Cultural Methods: The organism as described was isolated not only from the various types of lesions on the affected limb, but also from all the metastatic foci. Its cultural characters were determined by growing the parasite immediately after isolation on glucose agar containing 1 per cent Chassaing peptone (Cogit & Co., Paris) and 4 per cent Pfanstiehl's dextrose which has a specific rotation of +52.5°. The resulting pH was 5.4. This medium was poured into petri plates to a depth of slightly over ¼ of an inch. The plates were inoculated on the following day and were kept at room temperature, great precautions being taken to avoid contaminations.

The microscopical characters were determined by the hanging drop culture method using Sabouraud's broth to which, in some instances, ascitic fluid was added; also by the direct examination of plate cultures under the microscope.

MACROSCOPICAL STUDY OF CULTURES: The organism grows slowly. At the end of three weeks each culture had developed a prominent, black, irregularly hemispherical mass the surface of which was covered by tiny grey hairs. This mass was about 8 mm. in diameter and 4 or 5 mm. high.

During the next few days a shallow peripheral growth was noted to emerge from the base of the dome-shaped projection above described. This growth soon formed a band encircling the more elevated central portion (Plate X:A) and subsequently the peripheral extension of the colony was comparatively more rapid.

At the end of forty days the diameter had increased to approximately 35 mm. From the central portion, which was still markedly prominent and convex, the culture sloped down gradually toward a more shallow marginal zone limited externally by a regularly circular border. Superficially it was remarkably smooth and upon close examination it revealed a delicate hairy structure at the periphery with a somewhat powdery center. The color was grayish-black with a slight but definite touch of green and the surface appearance in general was not unlike that of old black velvet.

After fifty days the cultures had grown to about 45 mm. in diameter. The central umbo and the sloping zone surrounding it showed a more greenish tone and had become definitely powdery in structure. The marginal area was still shallow and hairy (Plate X:B). After this period there was nothing of importance to be noted.

MICROSCOPICAL CHARACTERS: Young colonies showed numerous germinating spores giving birth to bud-like cells which continued to divide in a single plane to form mycelial elements. The first cells produced would retain the spherical character of the mother spore, and consequently the initial portion of each filament exhibited festooned borders; but, as a rule, this feature was gradually lost upon subsequent growth (Plates XI: A and XV: A). In some of the cultures the tendency to produce rounded cells became quite pronounced, many of the filaments showing a definite beaded appearance (Plate XII: A). Occasionally a striking yeast-like growth was observed and then the cells would resemble rather closely the spherical elements found in the tissues (Plate XI: B).

In fully developed drop cultures the thallus generally

From these characters we were forced to suspect that we were dealing with a species of Hormodendrum, and this was confirmed by C. W. Emmons, who kindly examined this fungus with us at Columbia University.

CLASSIFICATION: At least two types of fungi have been isolated from cases of chromoblastomycosis. The first fungus carefully studied from this disease was reported by Medlar [4] and Lane [5] in 1915, and was classified by Thaxter as *Phialophora verrucosa,* new species. This fungus develops a distinctive conidiophore—an oval or barrel-shaped cell with a terminal cup in which the conidia are developed. This same parasite was recovered from a second case recently studied in Texas [6].

The fungus of South American chromoblastomycosis was first isolated by A. Pedroso [2b], of Brazil, in 1911; but a careful study of its behavior in cultures with the purpose of classification was not undertaken until ten years later when Brumpt became interested in the subject. Thus, in the third edition of his Précis de Parasitologie, published in 1922, this author gave a description of the parasite under the name *Hormodendrum pedrosoi* Brumpt, 1921 [7]. As will be seen below, Brumpt subsequently accepted a different classification [8]. However, his observations established beyond doubt that the etiologic agent in the Brazilian cases was different, at least morphologically, from *Phialophora verrucosa.*

In 1922 da Fonseca and Area Leao, who made an intensive study of the Brazilian strains, called attention to a certain type of conidiophore observed in their cultures which led them to place the fungus in the genus Acrotheca, Fuckel [3]. This conidiophore is described as a hypha, the terminal cell of which becomes elongated and knotted in appearance due to the development of a certain number of small protuberances, each of them carrying a single, ovoid, sessile, unicellular spore. Knotted structures were not observed in cultures of our organism *. After the work of da Fonseca

* In a more recent study of the fungus grown on thin layers of cornmeal agar we found certain conidiophores that do show tiny protuberances at their distal portion as described by da Fonseca (Plates XVI and XVII). When each of these protuberances bears a single spore, the fructification group resembles very closely the Acrotheca type of sporulation (Plate XVII:B). However, the general tendency of the spores thus developed is to form chains of the Hormodendrum type (Plates XVI and XVII). This feature evidently excludes our organism from the genus Acrotheca, where the conidia are not catenate. It would seem, therefore, that the Acrotheca-like structures noted in our cultures represent only an early stage of development of the more complicated spore groups produced by this organism.

and Area Leao was published, the term *Acrotheca pedrosoi* became rather popular, particularly among German authors.

More recently Ota [9] and Langeron [10], after a thorough study carried on independently of each other, arrived at the conclusion that the Brazilian organism should be included in the genus Trichosporium, Fries, 1829, a view which was later accepted by Brumpt [7]. Members of this genus are characterized by a well developed mycelium forming a thick growth over the surface of the medium; the spore-bearing hyphae appear more or less branched, and the dark conidia are borne singly or in clusters at the extremities or along the sides of undifferentiated conidiophores. The essential difference from Hormodendrum is the absence of chains of spores and the presence of pleurogenous sporulation.

According to Ota and Langeron, the elements which form the branching chains described by Brumpt in 1922 (*Hormodendrum pedrosoi*) are not to be considered as actual spores, but rather as ovoid cellular forms of a vegetative nature. In other words, these branching structures would represent a more highly developed type of conidiophore, true spores being borne only by the terminal articles of the chains. Langeron also stresses the absence of disjunctors between the cells of these chains. It is chiefly on these grounds that he excludes the Brazilian organism from the genus Hormodendrum, though such disjunctors are not mentioned in the definition of this genus. We might say that in cultures of the Puerto Rican strain (which is quite similar to the Brazilian as described by Langeron), it was often impossible to discriminate morphologically between the cells constituting the sporogenous chain which would be a vegetative structure according to the French author, and those produced by the terminal element of this chain, which are the only cells regarded by him as true conidia.

The possible relation of the fungus to the genus Acrotheca is also discarded by this same author on the grounds that, in the latter, the mycelium is practically obsolete while the organ of fructification is a well specialized, simple, erect, pluroseptate conidiophore, the spores being attached to small knot-like prominences which occur at the terminal portion of the organ. These characters, it is claimed, are not consistent with those obtaining in the fungus studied.

The morphology of the organism here described as a Hor-

parasite to which were added a certain amount of sterile sand and 15 cc. of sterile saline. The mixture was set aside for 20 minutes and the supernatant fluid was employed for inoculating the animals. To be sure that the suspension contained the organism and was active, a drop of the fluid was examined under the microscope and inoculations were made on slants of Sabouraud's agar. The microscope revealed the presence of spores and fragments of mycelium, and a pure culture of the fungus was recovered on every slant except one where a colony of aspergillus developed together with the parasite.

The skin of each animal was next shaved over an area of approximately 10 sq. in. and then scratched roughly with the razor. Immediately after this, 2 cc. of the above suspension was injected intravenously into each of them. The whole group was carefully observed over a period of four months. Skin lesions were never developed on the scratched areas or elsewhere.

In a second series, 2 monkeys and 4 rabbits were used. After a careful shaving on the back, the left scapular and interscapular regions were scratched with the razor and then smeared with a live culture of the organism. On the right side three small subcutaneous pockets were opened in each case, to be subsequently inoculated with culture pieces of the fungus. The two monkeys died of tuberculosis two and seven months, respectively, after inoculation, none of them showing signs of chromoblastomycosis at autopsy. The rest of the animals were kept under observation over approximately the same period as the above series. None of them showed evidence of infection.

The last series included 8 white rats which were treated with the same suspension used in the first group. Three of these animals received a subcutaneous injection of 0.75 cc. each, while the other five were inoculated intraperitoneally with a dose of 1.5 cc. One of the latter rodents, which had been starved in vitamin A during the period preceding the experiment, died on the day following inoculation and was discarded. The others were observed over a period of from 7 to 10 months. Those injected subcutaneously were all negative. However, in the group treated intraperitoneally there was one which died 4½ months after inoculation showing definite signs of infection upon post mortem examination.

At autopsy, no gross pathologic findings were in evidence in any of the organs except the lungs, which presented extensive pneumonia. The peritoneum was unaltered and the abdominal lymph glands were not enlarged. Paraffin sections of the Zenker-fixed liver revealed several rounded microscopic nodules that were sharply outlined against the hepatic parenchyma and were located in different portions of the liver lobule (Plate IX: Fig. 1). Some of these nodules consisted only of dense, hyalinized fibrous tissue. In others, a small portion in the center was less dense and presented a few shrunken epithelioid cells and lymphocytes. In still others, this central area was larger, and showed an occasional giant cell in addition to epithelioid cells. Rounded or oval, yellowish or faintly brown fungus cells were found in the giant cells, and outside of them (Plate IX: Fig. 2). They often occurred in short chains of two or three. Some of the fungus cells were very pale and shrunken and apparently in process of degeneration.

TREATMENT

Chromoblastomycosis has been generally considered as an incurable disease. Many drugs have been tried to combat the infection, but no favorable results are reported with any of them. However, the fact that the iodides have been so extensively used in many of the mycoses, particularly in blastomycosis, led us to give a thorough trial to the sodium salt in this particular case. Treatment was initiated, accordingly, on February 27, 1931, with a daily dose of 1 gram by the intravenous route. Since then the injections were continued with a few short interruptions, the doses being gradually increased. Fortunately, the patient showed such a remarkable tolerance for the drug that at the time of discharge, in January 17, 1933, he had been receiving for several months a daily dose of nine grams.

On June the 29th, 1931, some of the large foot lesions were excised with a view to determine any possible influence of surgical treatment on the evolution of the lesions.

Secondary infections of minor importance, which occasionally developed in different parts of the eruptive areas, were treated with mild antiseptic applications.

No other indications, either local or general, were made. Of course, the patient was kept in good hygienic surround-

ings, his diet being plain and nourishing throughout the period he remained in the hospital.

When treatment was suspended in January, 1933, the therapeutic influence of the iodide salt was perfectly apparent. Most of the lesions showed extensive healed areas where the skin appeared somewhat atrophic and hyperpigmented, while the large prominent masses noted at first on the leg and foot were reduced to one-fourth or one-fifth of their original size. Indeed, there was a time when a complete cure was seriously contemplated. It should be noted, however, that after the improvement effected during the first year, the eruption took on a more or less stationary character. Moreover, an interruption of the treatment for a few weeks shortly before the patient left the hospital was followed by the development on the upper part of the thigh of a number of deep, subcutaneous, hard, lumpy masses, most probably metastatic lesions, which were very much improved upon the resumption of iodide therapy. Last of all, a biopsy taken on November 30, 1932, revealed the same pathological picture observed when the case was first studied with the presence of typical fungus cells in the lesions. The parasite was also recovered in cultures.

According to the above observations, it is concluded that the intravenous administration of large doses of the iodides over extensive periods of time may be a good method to keep the disease under control, although it is doubtful that a complete sterilization of the infected body could ever be accomplished by this means, at least, in advanced infections. Intolerance to the drug would be another serious difficulty in certain cases.

With reference to the lesions removed surgically, it should be stated that in their place were left a number of simple large ulcerations. These ulcerations were treated on ordinary principles, and complete healing took place after some time.

It would be premature to judge of the results of surgical methods in other cases not receiving iodide treatment. In early infections, it is possible that the elimination of all pathological foci by surgical or electrotherapeutic methods might result in a permanent cure. However, in cases where the infection is as extensive as in our patient, where the lesions have penetrated deeply into the tissues and partic-

ularly where the infecting organism has invaded the circulatory system producing metastatic processes at distant locations, surgical measures would be not only unpracticable but evidently insufficient to absolutely sterilize the body against the infecting fungus.

Obviously, the treatment of this mycosis still remains an open problem. It may be possible to find a solution of this problem in the field of chemotherapy or perhaps, through immunological methods. Further research along these lines should be warmly encouraged.

SUMMARY

A case of chromoblastomycosis is recognized, studied and reported for the first time in Puerto Rico. This adds a new focus of this very rare disease to those already known to exist (Brazil, Boston, Texas).

The eruption had existed for 15 years at the time of admission to the hospital (1931); it affected the left lower extremity and, clinically, it was of a rather polymorphous nature. Some foot lesions were hard, irregular and definitely warty in character. On the leg, and partially on the foot, the eruption consisted of large, prominent, irregular, papillomatous masses, many of them having a cauliflower appearance. Farther up (knee and lower thigh) the lesions were less infiltrated, less elevated and more patchy in appearance. Itching was a troublesome subjective symptom and there was pain on locomotion.

The occurrence of metastasis is considered very remarkable as this has never been reported before in chromoblastomycosis, and may be of extreme importance from the viewpoint of prognosis.

The essential pathologic changes were characterized by a granulomatous reaction with the formation of pseudo-tubercles and giant cells, and a focal round cell infiltration in the superficial portions of the corium, leading first to the development of slightly elevated skin lesions which through progressive fibrosis were later changed into nodules of gradually increasing size. In the metastatic lesions the tissue reaction was as in the skin, except that in the stages studied the tendency was to softening and suppuration rather than to fibrosis. The fungus occurred in the form of brown, yeast-

like cells. In the skin lesions it was situated in the epidermis and in the superficial portions of the corium, both within pseudo-tubercles and outside of them. Septate forms were occasionally encountered in the corium, while in the horny layer of the epidermis mycelial elements were at times observed to be growing from the yeast-like cells.

The etiologic parasite evidently falls in the genus Hormodendrum, although the species is not definitely established as yet. Upon Sabouraud's medium it produced thick, dark, velvety, circular colonies reaching at the end of the seventh week a diameter of approximately 4.5 cm. At this time the cultures showed a smooth dome-shaped, central prominence around which there was a gradual sloping down toward a more shallow marginal zone. Hanging drop cultures revealed a tangled growth of septate, straight or moderately undulating mycelial elements branching mostly at acute angles. Asexual spores were produced abundantly, but their morphological arrangement and their relations to the fertile hyphae showed variation. Certain spores were attached to the distal portion of short conidiophores forming clusters not unlike those often observed in cultures of sporotrichum. Others appeared singly both laterally and at the tips of fertile hyphae. Finally, there were certain cellular forms, morphologically indistinguishable from the above spores, that were developed in short chains, either simple or branched. In a general way, the characters just described correspond fairly well with those given for the fungus of South American chromoblastomycosis.

Of 4 monkeys, 8 white rats and 10 rabbits, experimentally inoculated by various methods, only one of the white rats showed signs of chromoblastomycosis at autopsy. This was inoculated intraperitoneally and the resulting lesions were essentially similar to those of the human tissues, there being microscopic pseudo-tubercles that underwent partial replacement by fibrous tissue but that were limited, however, in their location to the liver.

The daily administration of high doses of sodium iodide intravenously over a period of approximately two years resulted in the complete disappearance of many lesions and a considerable reduction in the size of those remaining. The improvement was most marked during the first year of treatment. After this period the reaction to the drug was not

nearly as effective as it was at first. In fact, there was a time when the disease would seem to have become stationary. It is doubtful that a complete sterilization of the patient could ever be effected through iodide therapy.

REFERENCES

(1) Strong, R. P., Shattuck, G. C., Bequaert, J. C. and Wheeler, R. E.: Dermal Granulomatous Spirochaetosis. Medical Report of the Hamilton Rice Seventh Expedition to the Amazon, in Conjunction with the Department of Tropical Medicine of Harvard University, 1924–25. Page 36. Harvard University Press. 1926.

(2 *a*) Pedroso, A.: Quoted by da Fonseca, O. and de Area Leao, A. E.; Las Cromoblastomicosis. Quinta Reunión de la Sociedad Argentina de Patología Regional del Norte. 1: 329. Imprenta de la Universidad, Buenos Aires. 1911. (1930).

(2 *b*) Da Fonseca, O. and De Area Leao, A. E.: Quinta Reunión de la Sociedad Argentina de Patología Regional del Norte. 1: 329. Imprenta de la Universidad, Buenos Aires. 1930.

(3) Terra, F., Torres, M., Da Fonseca, O. e Area Leao, A. E. De: Novo typo de dermatite verrucosa, mycose por Acrotheca, com associacao de leishmaniose. Brasil Medico, 2: 365. 1922.

(4) Medlar, E. M.: A Cutaneous Infection caused by a New Fungus, *Phialophora verrucosa*, with a Study of the Fungus. Jour. Med. Research. 32: 507. 1915.

(5) Lane, C. G.: A Cutaneous Disease Caused by a New Fungus (*Phialophora verrucosa*). Jour. Cut. Dis., 33: 840. 1915.

(6) Wilson, S. J., Hulsey, S. and Weidman, F. D.: Chromoblastomycosis in Texas. Arch. Derm. Syph. 27: 107. 1933.

(7) Brumpt, E.: Précis de Parasitologie. 3e édition, Paris, Masson et Cie. Page 1105. 1921.

(8) Brumpt, E.: Précis de Parasitologie. 4e édition, Paris, Masson et Cie. Page 1333. 1927.

(9) Ota, M.: Quoted by Langeron. Parasitic Fungi of Man (Written in Japanese). Jap. Jour. Derm. & Urol., Vol. 28, No. 4. 1928.

(10) Langeron, M.: Le *trichosporium pedrosoi* (Brumpt, 1921) agent de la dermatite verruqueuse Brésilienne. Ann. Parasit., 7: 145. 1929.

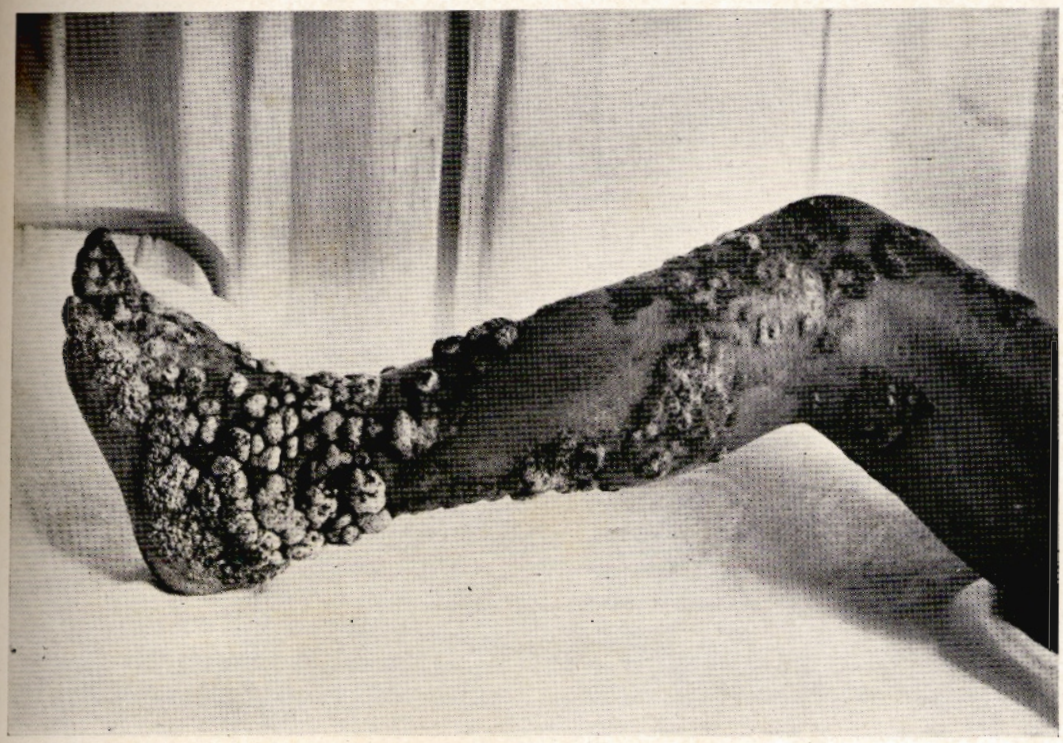


PLATE I

Outer aspect of infected limb showing various types of skin lesions.

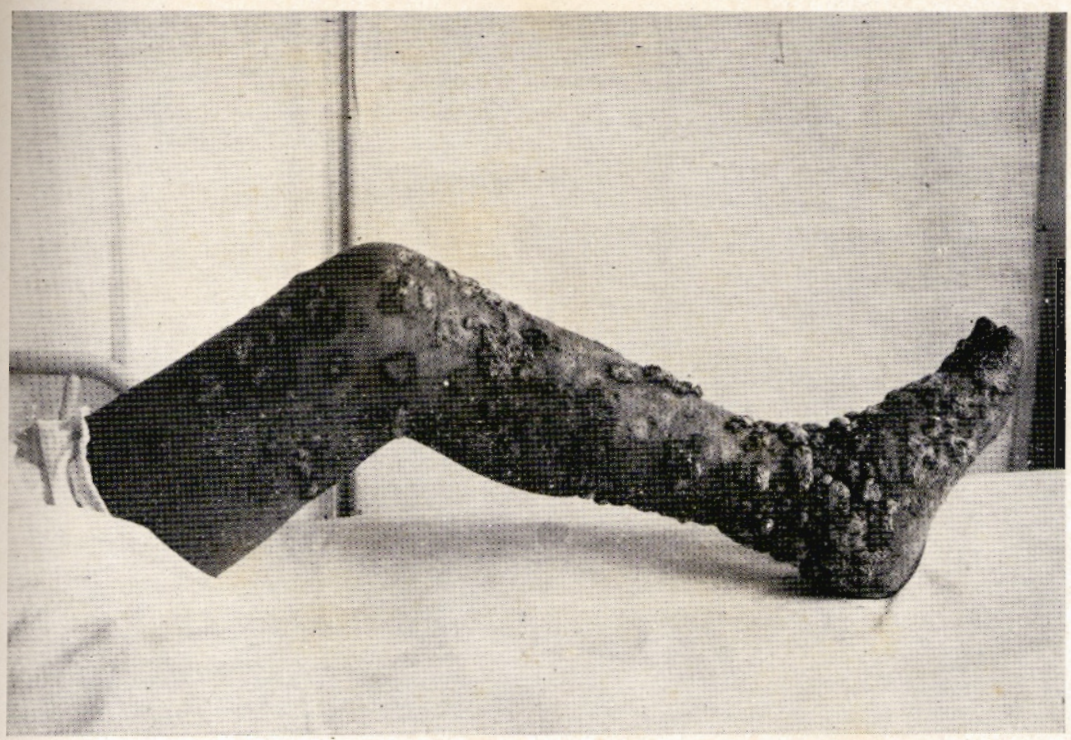


PLATE II

Inner aspect of same limb (See Plate I).

EXPLANATION OF PLATES

PLATE III

Fig. 1. Early skin lesion showing epidermal changes, pseudo-tubercle formation and diffuse cell infiltration in superficial parts of corium.

Fig. 2. Skin nodules, intermediate stage of development, with replacement of corium by recently organized granulation tissue presenting focal cell infiltrations.

PLATE IV

Fig. 1. Skin nodule, intermediate stage of development, demonstrating epidermal changes; also, pseudo-tubercle formation and focal infiltrations of corium. Note miliary accumulations of polymorphonuclear leukocytes in rete peg.

Fig. 2. Cauliflower-like nodule: acanthosis, hyperkeratosis, pseudo-tubercles, infiltrations of round cells and fibrosis of corium.

PLATE III

Fig. 1

Fig. 2

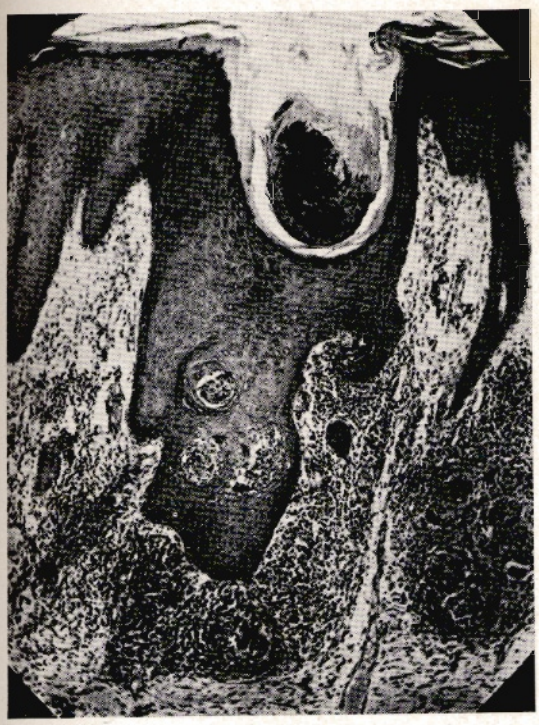


PLATE IV

Fig. 1

Fig. 2

PLATE V

Figs. 1 and 2. Metastatic nodule (right thigh) showing pseudo-tubercles in granulation tissue. In Fig. 2, miliary abscess. Note presence of fungus cells in pseudo-tubercles and giant cell in Fig. 1.

PLATE VI

Fig. 1. Metastatic nodule (left thigh). Focus of softening and suppuration surrounded by granulation tissue.

Fig. 2. Cauliflower-like nodule (left foot). Fungus cells, mycelial elements and keratinized débris in superficial fold of epidermis

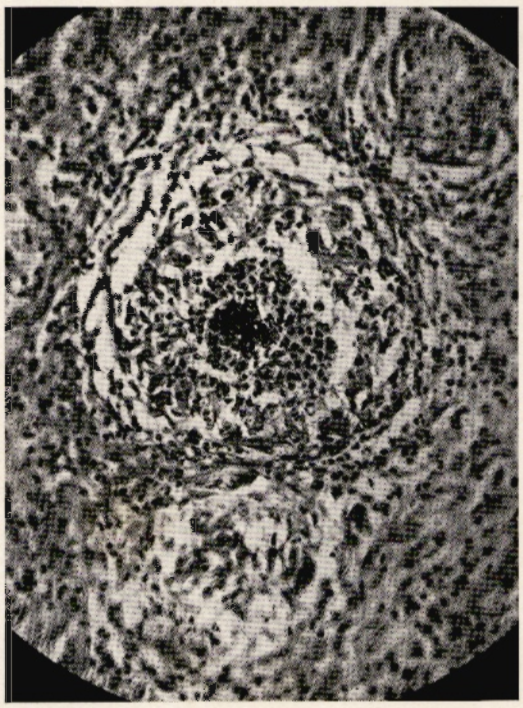
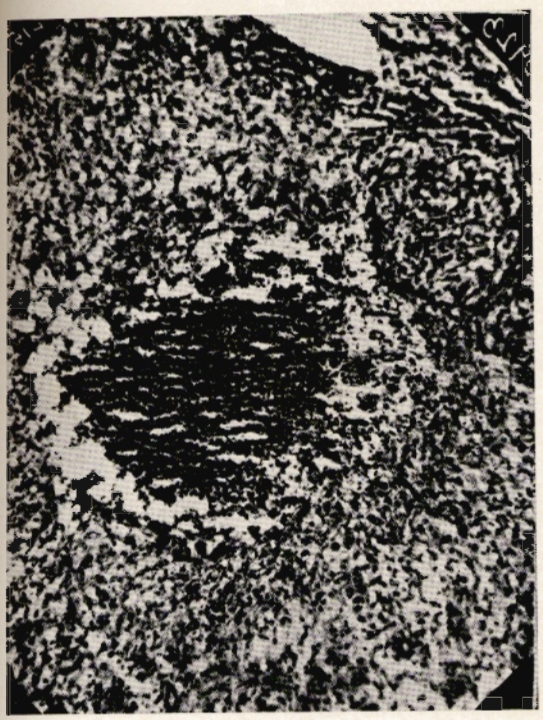


PLATE V

Fig. 1

Fig. 2



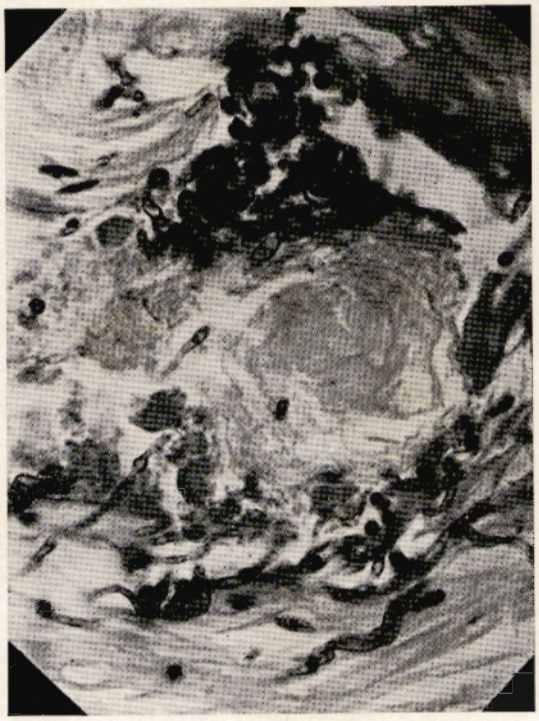


PLATE VI

Fig. 1

Fig. 2

PLATE VII

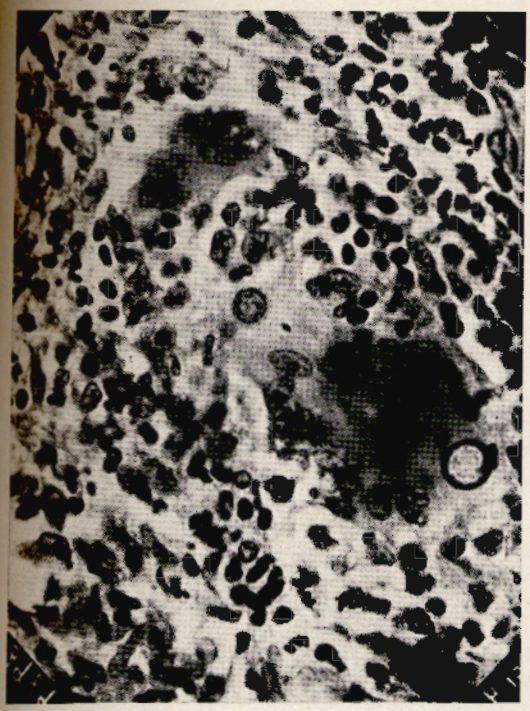
Fig. 1. Skin nodule, intermediate stage of development. Foreign-body giant cell containing two spherical fungus cells.

Fig. 2. Metastatic nodule (right thigh). Leukocytic infiltration about fungus cells in granulation tissue.

PLATE VIII

Fig. 1. Early skin lesion. Granulomatous changes in corium. Cup-shaped and septate forms of fungus in giant cell.

Fig. 2. Early skin lesion showing tip of dermal papilla surrounded by epidermis. Note cup-shaped forms of organism; also degenerating fungus element in larger giant cell.



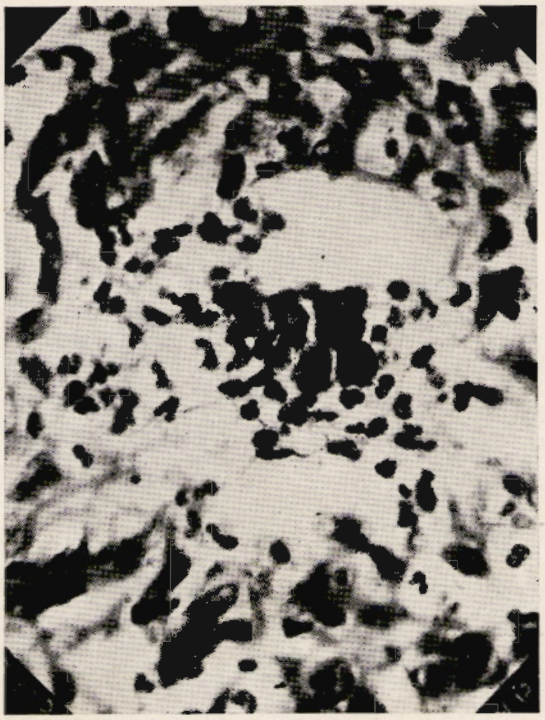


PLATE VII

Fig. 1 Fig. 2

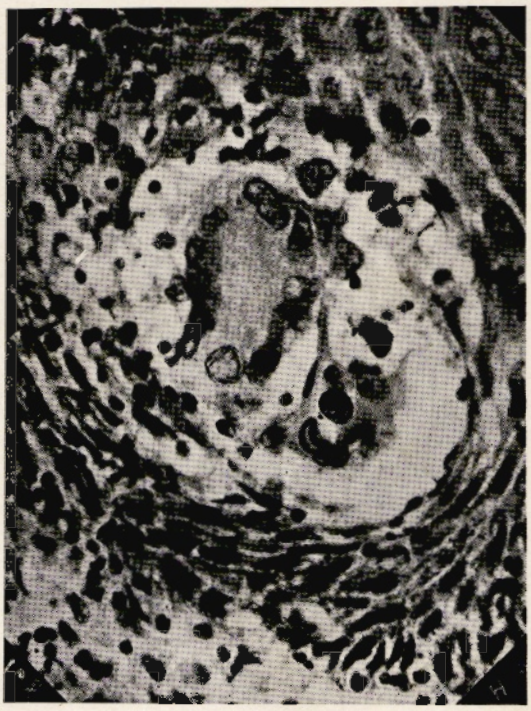


PLATE VIII

Fig. 1 Fig. 2

PLATE IX

Nodule in liver of experimental rat: Fig. 1. Low-power magnification showing small giant cell and abundant fibrous tissue.

Fig. 2. High-power view of pseudo-tubercle with chain of fungus elements in center.

PLATE IX

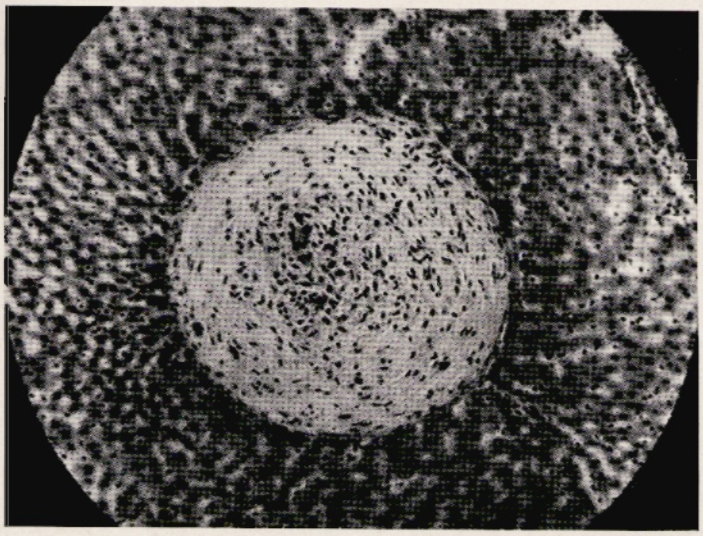


Fig. 1

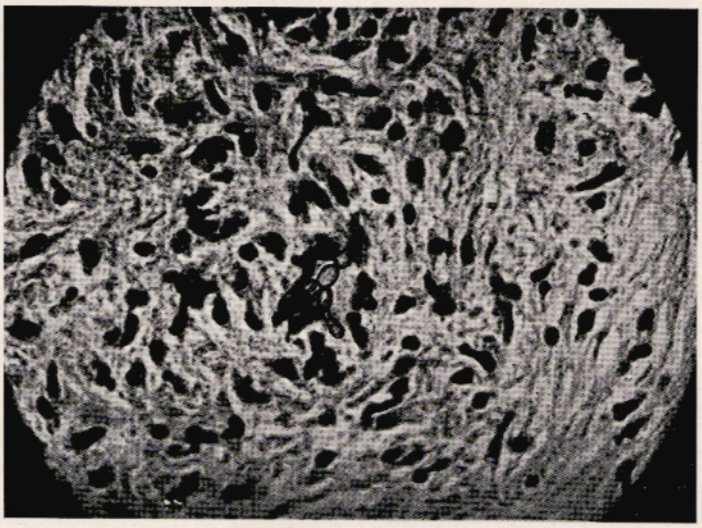


Fig. 2

PLATE X

Cultures of fungus causing the Puerto Rican case; A and B, colonies 21 and 50 days old, respectively, upon Sabouraud's medium; C, drawings from a hanging drop culture showing bunches of conidia, some of which form branching chains.

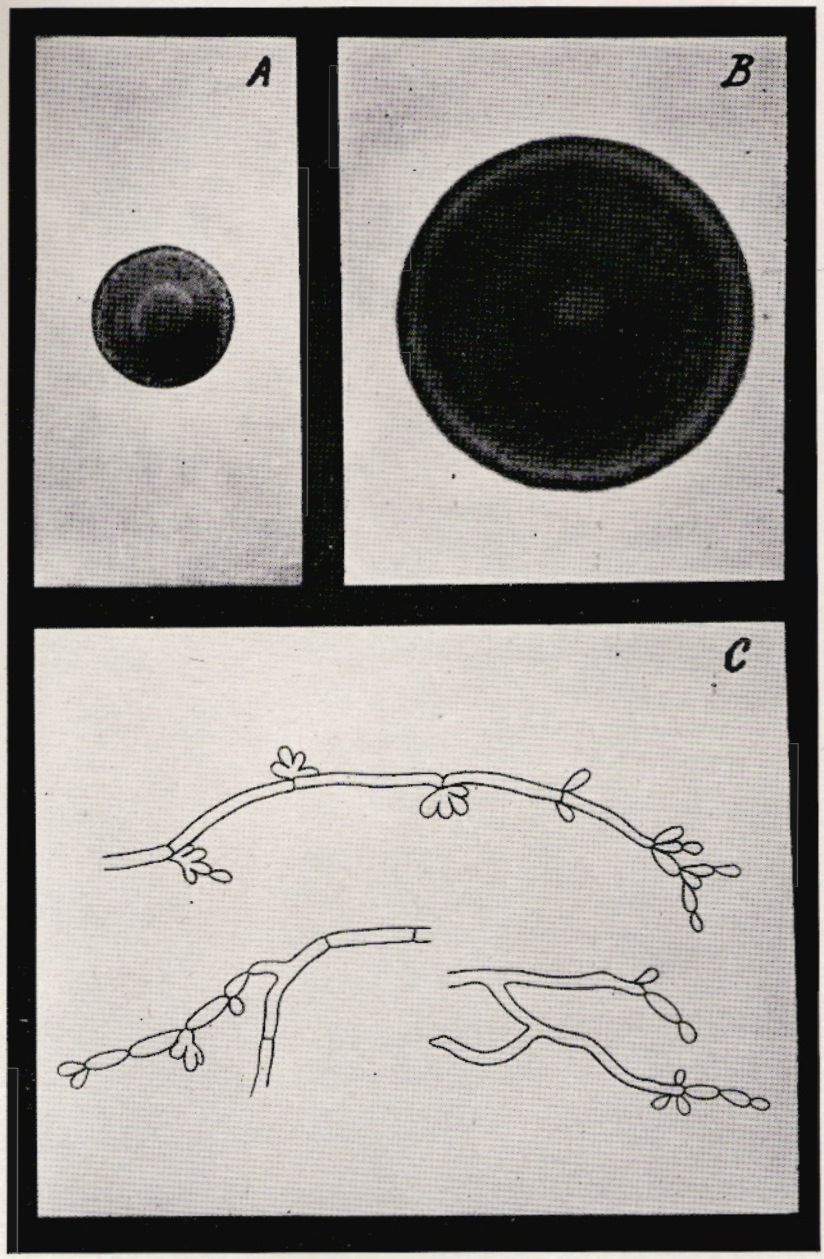


PLATE X

PLATE XI

Hanging drop cultures showing early growths—yeast-like structures in B.

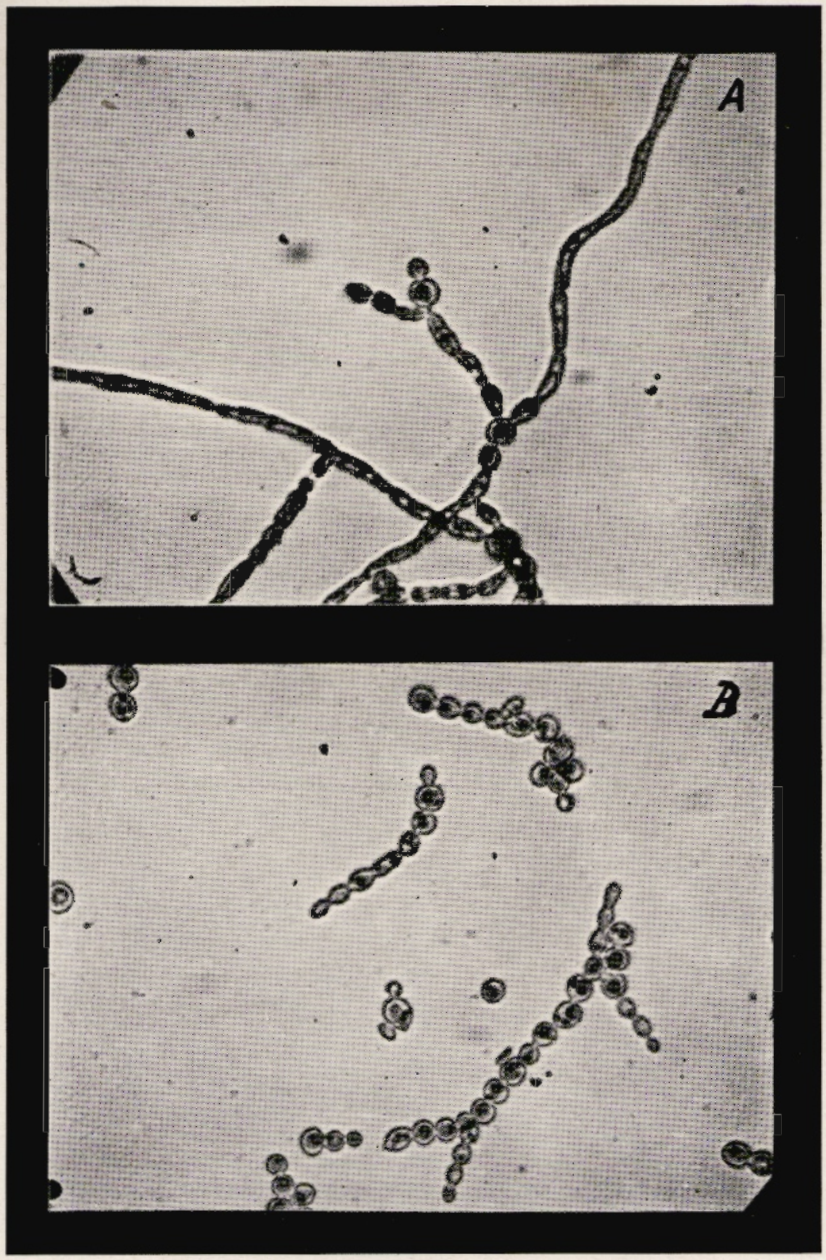


PLATE XI

PLATE XII

Hanging drop cultures showing in: A, beaded growth of the mycelium; B and E, single lateral conidia and terminal bunches; C and D, lateral clusters; F and G, terminal groups.

PLATE XII

PLATE XIII

Hanging drop cultures showing in: A, B, and C, both lateral and terminal bunches with a single lateral conidium in A; D, conidiophore (see arrow) giving rise at its tip to a pair of conidia-like structures arranged in chain formation (right) and a secondary conidiophore bearing a thick cluster of conidia (left).

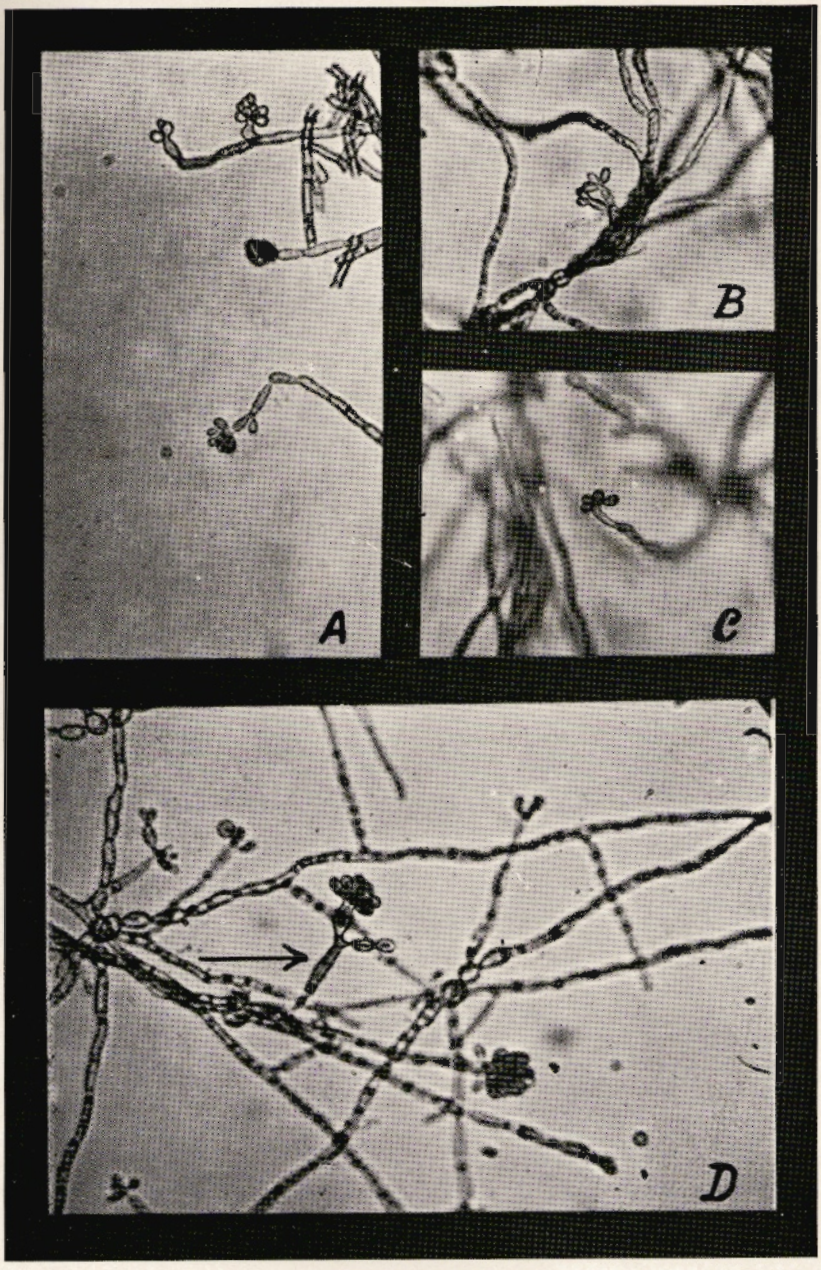


PLATE XIII

PLATE XIV

Hanging drop cultures showing the tendency of certain conidia to form short chains.

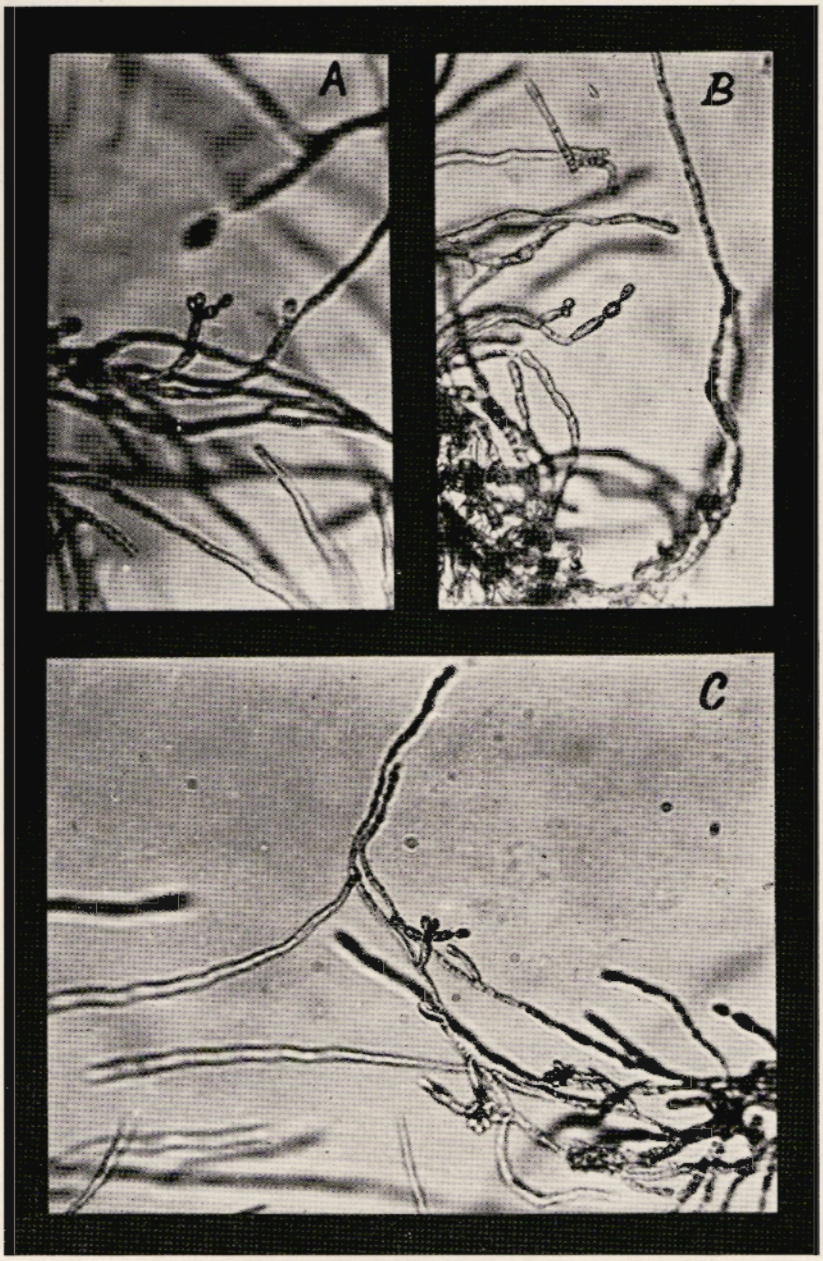


PLATE XIV

PLATE XV

Hanging drop cultures showing in: A, clusters of conidia borne terminally and laterally by fertile hypha. Also accessory group of conidia developed from one of the elements of a terminal cluster (see arrow). B, an element from a spore head giving rise to a secondary group, while another element develops a secondary spore showing tendency to chain formation (see arrow).

PLATE XV

PLATE XVI

Slide cultures on cornmeal agar (oil immersion lens) showing stages of development of spore heads (See well-developed head in Plate XVII). Note conidiophores with knotted tips and tendency of conidia to form chains; disjunctors visible in B.

PLATE XVI

PLATE XVII

Slide cultures on cornmeal agar (oil immersion lens) showing in: A, well developed spore head with chain formation; B, spore head where the various conidia are still single; C, sporophores with knotted tips and chain of conidia with disjunctors.

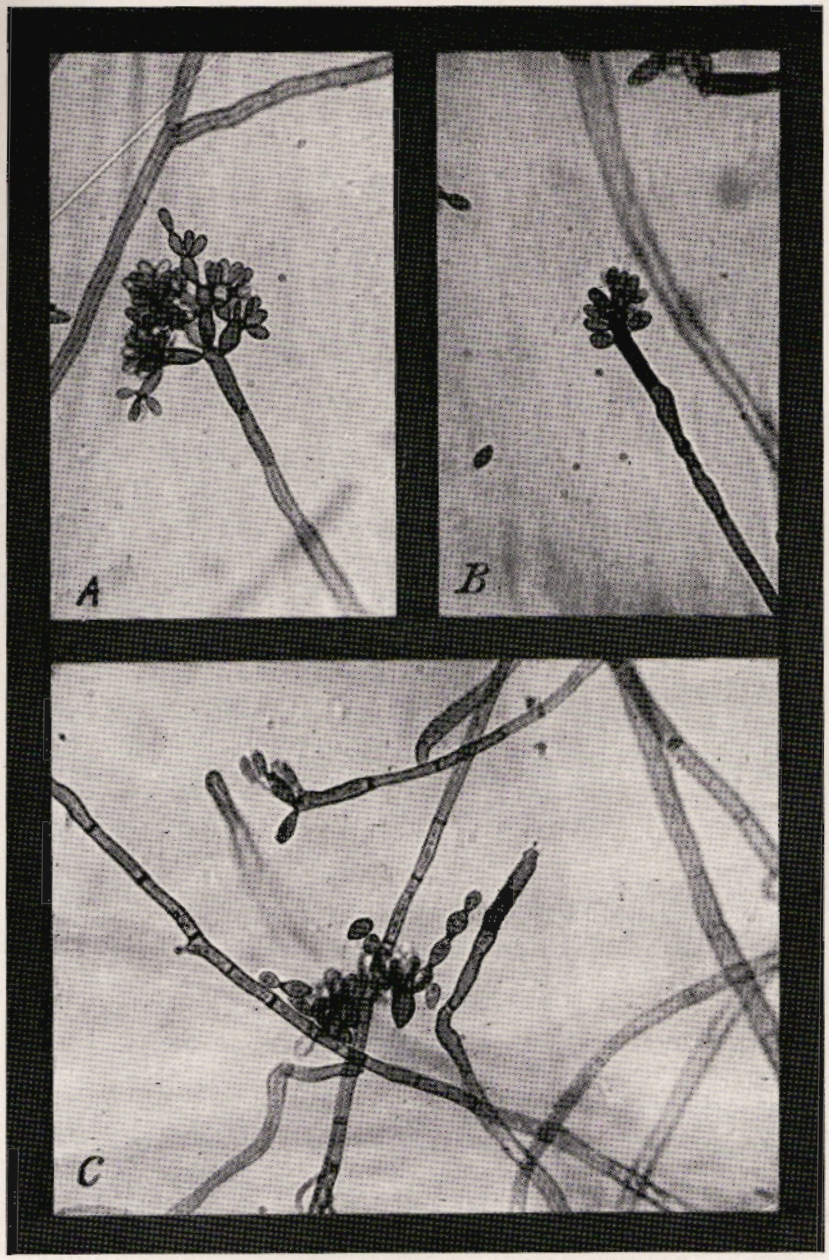


PLATE XVII

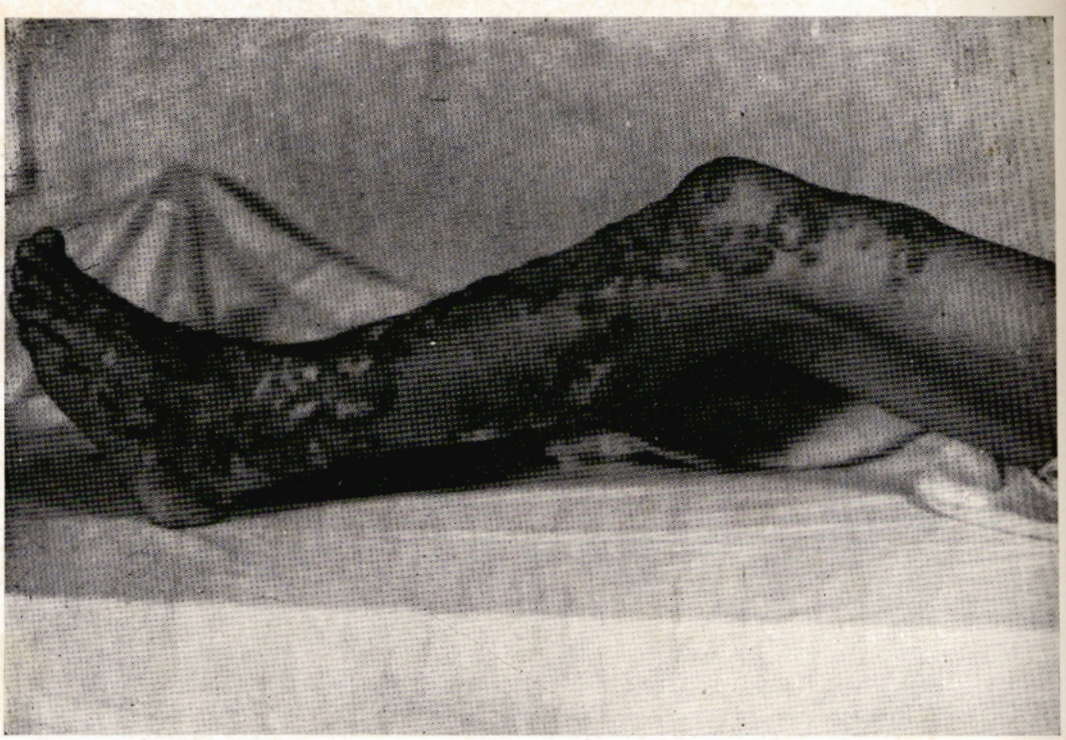


PLATE XVIII

Outer aspect of affected limb after 2 years of treatment with sodium iodide.