Mycetoma by *Monosporium Apiospermum* in St. Croix, Virgin Islands[1]

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In view of the generally limited experience of the medical profession in the subject of mycetoma, a few introductory remarks about the nature and classification of this disease are considered important for the purpose of orientation. Mycetoma is a good descriptive name which means fungus tumor, that is, a tumor produced by fungi. From the pathological viewpoint, mycetoma—also known as Madura foot—is a chronic, granulomatous new growth, usually involving one of the feet. Clinically, it is characterized by enlargement and deformity of the affected region, by abscess and sinus formation, by the presence of characteristic "grains" in the pathologic tissues and exudates and, finally, by the usual absence of general systemic disturbances, notwithstanding the frequent penetration of the infective process into the deeper tissues and even the bony structures. The characteristic grains are essential to diagnosis. They consist of small bodies of different shape, dimension, and color, each of them representing a colony of the infecting fungus. The color of the grains, which may be black, white to yellowish-white, or red, has led some investigators to distinguish three different varieties of mycetoma—the melanoid, the ochroid, and the red, the latter being rather uncommon.

Recent investigations have revealed that the disease may be caused by a large number of fungus species including Hyphomycetes, Actinomyces, and Ascomycetes. It has been further found that grains of the same color may represent entirely different specific agents in different cases. Chalmers and Archibald[2] have classified the mycetomas in two distinct groups: (a) the actynomycotic mycetomas and (b) the maduromycoses—a classification that has been accepted by many well known investigators. These two types of mycetomas may be readily recognized by the microscopic morphology of their grains. In the actinomycotic mycetomas, which may be caused by different species of Actinomyces, the grains consist chiefly of fine filaments, seldom measuring more than one micron

1. Received for publication March 16, 1944.

2. A. J. Chalmers and R. G. Archibald, A sudanese maduromycosis. Ann.Trop.Med., **10**: 169–222, 1916.

in width. These filaments are hyaline; they have a poorly defined cell membrane, are apparently nonsegmented, and do not produce chlamydospores. In the maduromycoses produced by molds, the grains contain coarse, clearly septate, branching filaments with well defined cell membranes and, usually, chlamydospores. The case presented in this communication falls among the maduromycoses and was caused by a Hyphomycete.

CASE REPORT

History. A. G., a Negress 64 years old, engaged in housework and residing in Estate Grove Place, St. Croix, Virgin Islands, was admitted to the Hospital at Christiansted on July 8 and discharged on July 24, 1937. The patient complained of a chronically swollen and painful right foot.

The pathologic process started in 1929 when the sole of her right foot, which had become locally harder than normal, began to pain and itch. "It came on like a stone bruise," the patient said. However, there was no puncture wound, cut, or abrasion on the skin. The lesion opened and discharged a very small amount of pus and then closed over. Her foot was not swollen at that time and the patient could walk with but very little pain. There were no cracks or infection between the toes or around the nails, neither were there any general symptoms.

A short time later, part of the foot below the instep began to swell and to hurt again. Between May and July 1929, the swelling was incised twice without exuding any pus. Since then there had been a gradual and continuous progression of the swelling, and walking had become painful.

Examination. Examination revealed an elephantiasic swelling of the right foot and lower leg (Plate I). The skin covering these regions was hyperpigmented and there were many scars and several open sinuses on its surface. Some of the sinuses discharged a thin, yellow, serous fluid while others were covered with crusts. The affected tissues were neither hot nor did they appear to be acutely inflamed. Movement of the foot and toes was preserved to a certain extent. On palpation the swelling was firm; there were no points of acute tenderness and no areas of fluctuation. The tissues around the sinuses were very hard; hardly any discharge could be expressed. A crust covering one of the openings was lifted and, upon pressure, discharged a thick exudate containing white grains. The rest of the right lower extremity showed no apparent involvement. The subinguinal glands were somewhat enlarged, discrete, hard, and tender,

and were covered by a mass of fat that produced a visible swelling. The left leg revealed a mild degree of elephantiasis and showed some pitting edema over the crest of the tibia. There were no varicose vessels.

The patient was a very large, somewhat obese woman. Physical examination did not show any fundamental disturbances that might be considered important, with exception of the condition of the lower extremities already described.

Roentgenologic study. The X-ray pictures revealed pathologic changes in all the bones of the right foot, down to the phalanges (Plate II, Figs. 2 and 3). The joints between the tarsal and metarsal bones had been destroyed; the latter were dense but smaller than normal in diameter and there was evidence of new bone formation along the margins of each of them. The joints between the tarsal bones were also destroyed, but the ankle joint was only slightly involved. The tibia and fibula showed periosteal thickening through almost their entire length. An X-ray picture of the left leg did not reveal important changes.

Histopathologic report. A biopsy of the tissues, which included part of a sinus, revealed marked acanthosis of the epithelium in the epidermis, with elongated and irregular rete pegs projecting into the underlying tissues (Plate III, Fig. 4). The corium showed a granulomatous reaction and was densely infiltrated with round cells and polymorphonuclear leukocytes (Plate III, Fig. 5). The ulcerated portion of the tissues was covered superficially by a thick fibrinopurulent exudate in which several lobulated fungus granules were found (Plate IV, Fig. 7).

Therapeutic problem. Since science has not yet provided specific treatment against mycetoma, the only hope for recovery would have been radical surgery. However, considering the age of the patient, her relatively short life-expectancy, the absence of general symptoms and the slow progress of the infective process, together with the fact that she could move about to do simple housework, including ironing and feeding her fowls, it was decided that amputation was not the most convenient measure in this particular case. Moreover, as the patient was a heavily set woman and the unaffected leg and foot were not entirely normal, it seemed doubtful if she could get about well on a peg leg or with the aid of crutches. Local treatment was conducted on general principles and plans had been laid to test the action of sulfa drugs against this infection, but the patient was anxious to leave the Hospital and, after she was released, we were unable to follow up the case.

MYCOLOGIC STUDY

Infecting fungus in lesions. In the pathologic tissues, as well as in the exudate draining from abscesses and sinuses, the parasite could be observed as numerous dull whitish, lobulated, soft granules ranging from 1 to 2 mm. in length (Plate IV, Fig. 7). Each of these granules represented a fungus colony. When examined microscopically, it was found to consist chiefly of a network of coarse, hyaline, septate hyphae, bearing many pyriform, terminal or lateral spore-like cells (Plate IV, Fig. 6). The hyphae measured from 3.3 to 5 microns in width and the spores, 7.8 to 8.8 by 4.3 to 5.2 microns.

Cultural methods and material used. Many of the grains from the exudate were carefully and repeatedly washed in sterile saline solution, divided into smaller particles, and finally inoculated into a series of glucose agar slants. After a week of incubation at room temperature, all the tubes revealed multiple cultures of one and the same fungus. Following the process of isolation, this fungus was inoculated into two groups of Petri plates with Sabouraud's proof medium and 4 percent glucose agar, respectively, to observe the gross morphology of the colonies. In order to study their microscopic morphology, additional slide cultures and chamber cultures (Henrici)[3] were made on the above media, as well as on cornmeal and Czapek agars and in dextrose broth (hanging drops).

In addition to the fungus obtained from the St. Croix case, three other organisms in our fungus collection, belonging to the same species, were studied for comparative purposes. Among these, there was one isolated by Gay and Bigelow from a case of mycetoma occurring in Massachusetts.[4] A second was obtained by Gellman and Gammel from another case in Maryland,[5] and a third was found in Dr. Bailey K. Ashford's collection, apparently obtained from the University of Paris.

Gross cultures of the fungus. Two weeks old cultures on Sabouraud's proof medium were irregularly circular in contour and measured approximately 6 cm. in diameter (Plate V, Fig. 8). Superficially, there was a profuse, irregular growth of waxy white to white aerial hyphae, characteristically arranged in simple or branched, often anastomosed, moist looking tufts.

On 4 percent glucose agar, the rate of growth was approximately

3. A. T. Henrici, Molds, Yeasts, and Actinomycetes (New York: John Wiley and Sons, Inc., 1930).

4. D. M. Gay and J. B. Bigelow, Madura foot due to *Monosporium apiospermum* in a native American. Am.J.Path., 6:325–336, 1930.

5. M. Gellman and J. A. Gammel, Madura foot. A third case of Monosporosis in a native American. Arch.Surg., 26:295–307, 1933.

the same as on proof medium. The tufted character of the hyphae was evident, though not to the same degree; the general appearance of the cultures was not as moist, and the aerial growth was whiter, somewhat cottony and, toward the periphery, even powdery (Plate V, Fig. 9).

Cultures on both media became drier and greyish in color with aging. No sclerotia were produced.

Microscopic morphology. Microscopically, cultures on all the media revealed essentially the same morphologic picture. There was an abundant, aerial and submerged mycelial growth, consisting of septate, branching hyphae, some of which showed local thickenings at different points. The hyphae measured from 1 to 3.5 microns in diameter; their cell membrane was thin; the protoplasm was hyaline and granular and contained many refractile droplets of various sizes (Plate VI, Figs. 10 to 18). In most of the cultures, the hyphae showed a peculiar tendency to stick together forming irregularly anastomosing bundles. This explains the tufted character of the aerial growth already noted in plate cultures. When examined under the microscope, these tufts from plate cultures showed large numbers of closely packed hyphae bearing many lateral spores.

Sexual reproduction was not observed, but conidia were produced abundantly by all the cultures in both the aerial and submerged mycelium. Many of the fertile hyphae showed no difference whatsoever from the vegetative filaments, the conidia being developed both laterally and terminally, either sessile or on short stalks, mostly singly but occasionally in bunches of two to four (Plate VI, Figs. 10 to 12 and 15 to 18). Somewhat differentiated conidiophores were also noted as simple, fairly straight, tapering branches, each of them bearing an individual spore at its tip (Plate VI, Figs. 13 and 14). These conidiophores were of different lengths but usually could be enclosed in their entirety within the high-power field of the microscope.

The conidia were unicellular and oval to pear shape. They measured 6.2 to 10.4 by 3.7 to 8.7 microns and were linked to the conidiophores at a small, flat facet on their narrower pole (Plate VI, Figs. 10 to 18). Their cell membrane was smooth and darker than that of the vegetative mycelium; the protoplasm was granular and hyaline, becoming dull yellow with age. It usually contained a number of variously sized refractile droplets.

The fungus was classified in the species *Monosporium apiospermum*, Saccardo, 1911.[6]

6. P. A. Saccardo, cited by C. W. Dodge, Medical Mycology (St. Louis: The C. V. Mosby Company, 1935).

COMMENT

The case of mycetoma here studied is presented with a triple purpose: (1) to establish beyond doubt the existence of the disease in the Virgin Islands; (2) to stimulate medical interest in this important mycosis and, (3) to help promote a better understanding of its etiology.

Mycetoma is not a common malady, but its geographic distribution is widespread throughout the civilized world, especially in tropical regions. It is a chronic, penetrating, destructive, incapacitating infection which may, though rarely, lead to metastases and even death. The disease is often confused with many other pathologic conditions that may be very similar clinically, such as mossy foot, leprosy, chromoblastomycosis, and syphilis, hence the differential diagnosis should be based on the presence of grains in the infected tissues or exudates and on the identification of the etiologic fungus.

Up to the present time, no method of treatment, except radical surgery, has proved entirely successful against this infection. Of course, the sulfa drugs and penicillin have not been given a fair trial, as yet; it has been stated that the latter may be effective in actinomycosis.[7] If this were confirmed, it is possible that at least many of the actinomycotic mycetomas would respond to the drug. It should be remembered, moreover, that the iodides may be useful coadjutants in the treatment, irrespective of other therapeutic agents employed.

One of the most striking features of mycetoma is the large number of fungus species, of even different genera, that are capable of producing the disease. In 1927 Gammel[8] published an excellent review of the literature on the subject and presented a long list of etiologic fungi that were classed in nine different genera, namely: Actinomyces, Madurella, Indiella, Glenospora, Scedosporium (Monosporium), Allescheria, Aspergillus, Sterigmatocystis, and Penicillium. To these we may add the genus Cephalosporium, species of which have been recently found to be of etiologic importance in Brazil,[9] Mexico,[10] and Puerto Rico.[11]

7. W. E. Herrell, Further observations on the clinical use of Penicillin. Proc.Staff Meet. Mayo Clin., 18:65–76, 1943.

8. J. A. Gammel, The etiology of maduromycosis. Arch.Dermat.& Syph., 15:241–284, 1927.

9. A. E. Arêa Leão and J. Lobo, Mycétome du pied a *Cephalosporium* recifei, N. sp. Mycétome a grains blancs. Compt.rend.Soc.de biol., 117:203–205, 1934.

10. A. González Ochoa, Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F. Personal communication to the author, 1944.

11. A. L. Carrión, Estudio micológico de un caso de Micetoma por *Cephalosporium* en Puerto Rico. Mycopathologia 2:165–170, 1940.

The classification of the etiologic agent in cases of mycetomas has often been a confusing problem. This is due, in part, to incompleteness of the original descriptions for some of the fungi responsible for the infection; in part, to the susceptibility of the individual fungi to undergo mycologic variations and, finally, to the tendency of some investigators to create new species on the basis of such variations. In view of the evergrowing scope and complicated character of the problems in medical mycology, every effort should be made to review imperfect descriptions, to establish definitely the essential morphology of legitimate species, and to eliminate the names of all fungi found to be identical with such species.

The morphology of the fungus cultures from the present case is essentially consistent with Saccardo's original description of the species *Monosporium apiospermum*.[12] Microscopically, it is identical with three other members of this species in our stock fungus collection. It is true that comparable plate cultures of the four fungi show great differences in gross appearance (Plate VII, Figs. 19, 20, 21, and 22), but we believe that such differences should fall within the range of mycologic variations. In support of this view is the behaviour of the St. Croix fungus, which has produced variants entirely different in pattern from their mother culture (Plate VII, Fig. 22).

It is important to point out certain features in which the four specimens of *apiospermum*, studied by us, differ from Saccardo's definition of the species.[13] In his definition the conidiophores are given as "not erect" and no mention is made of the production of lateral or clustered conidia. The specimens in our collection produce erect or ascending conidiophores; a large proportion of the conidia—perhaps the majority—are produced laterally rather than terminally, and the production of conidia in small clusters is always observed in cultures, though only as an occasional phenomenon. These features have been observed by other investigators. In addition, we should like to call attention to the tendency of our local isolate to produce thick tufts of fertile hyphae somewhat resembling coremia. It should be emphasized that these characters do not warrant the creation of new species; they are qualities which should be added to the original description of the species and should be borne in mind for future classifications.

12. P. A. Saccardo, *op. cit.*
13. *Ibid.*

SUMMARY

The first recognized case of maduromycosis in the Island of St. Croix, Virgin Islands, is herein studied and reported. The infection was of the white grain type and its etiologic agent was a Hyphomycete, identified as *Monosporium apiospermum*, Saccardo 1911. Attention is called to certain morphologic peculiarities of this species, which should be taken into consideration for future classifications.

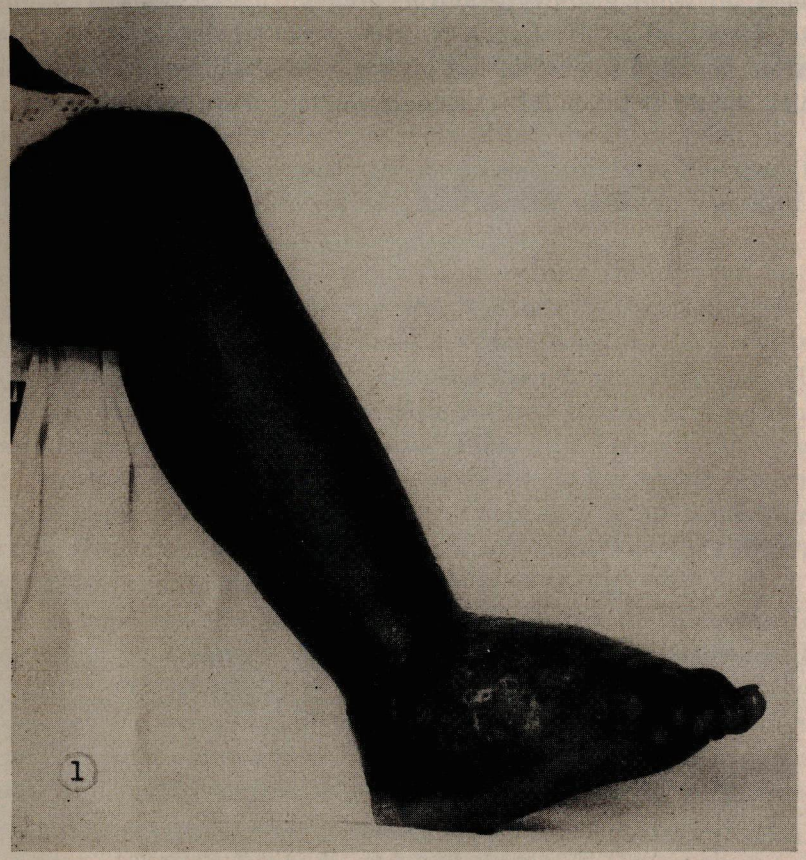


PLATE I

Fig. 1. Maduromycosis: the St. Croix case. Elephantiasic swelling of foot, hyperpigmented skin with many scars and open sinuses.

LÁMINA I

Grabado 1: Maduromicosis: caso procedente de la isla de Santa Cruz. Agrandamiento elefantiásico del pie; piel hiperpigmentada, con muchas cicatrices y bocas de trayectos fistulosos.

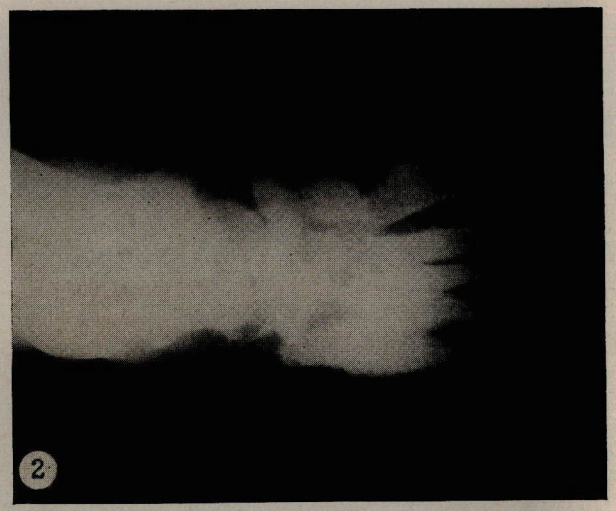


PLATE II

Roentgenologic pictures of infected foot: antero-posterior (Fig. 2) and lateral (Fig. 3) views.

LÁMINA II

Radiografías del pie infectado: vistas antero-posterior (grab. 2) y lateral (grab. 3).

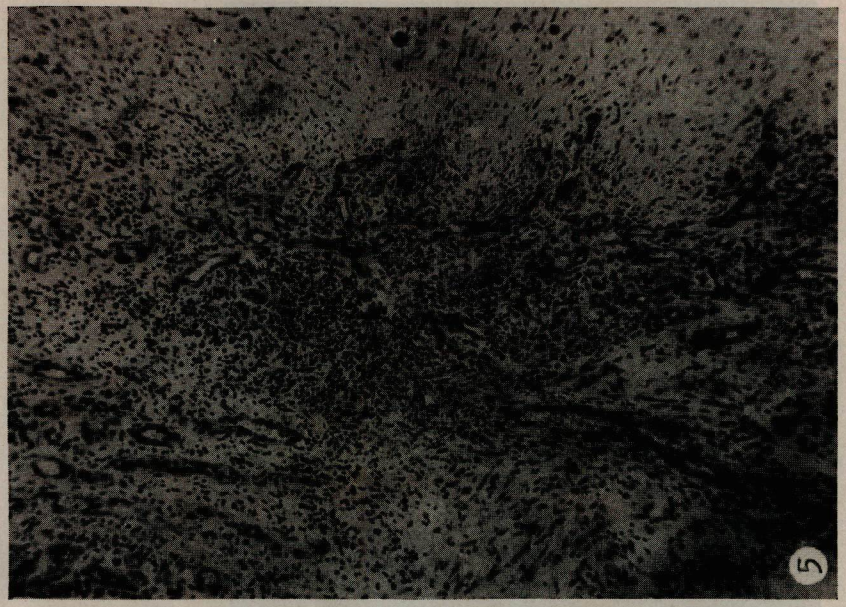


PLATE III

Histopathologic reaction of the infected tissues showing changes in the epidermis (Fig. 4) and in the corium (Fig. 5) (Magnification: x 80).

LÁMINA III

Reacción histopatológica de los tejidos infectados con las alteraciones de la epidermis (grab. 4) y del corion (grab. 5) (x 80).

PLATE IV
LÁMINA IV

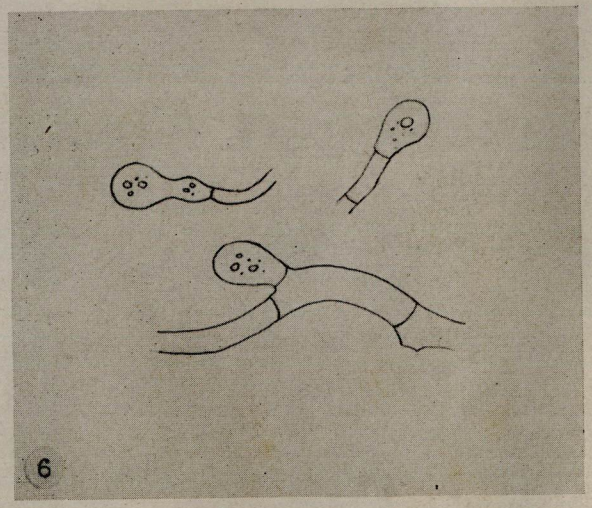


Fig. 6. Drawing from fresh preparation of a grain on microscopic examination. Note septate fungus filaments and spores.

Grabado 6: Dibujo representando una preparación reciente de un gránulo al examen microscópico. Nótanse los esporos y la tabicación de los filamentos micósicos.

Fig. 7. Histopathologic section of infected tissue showing multiple

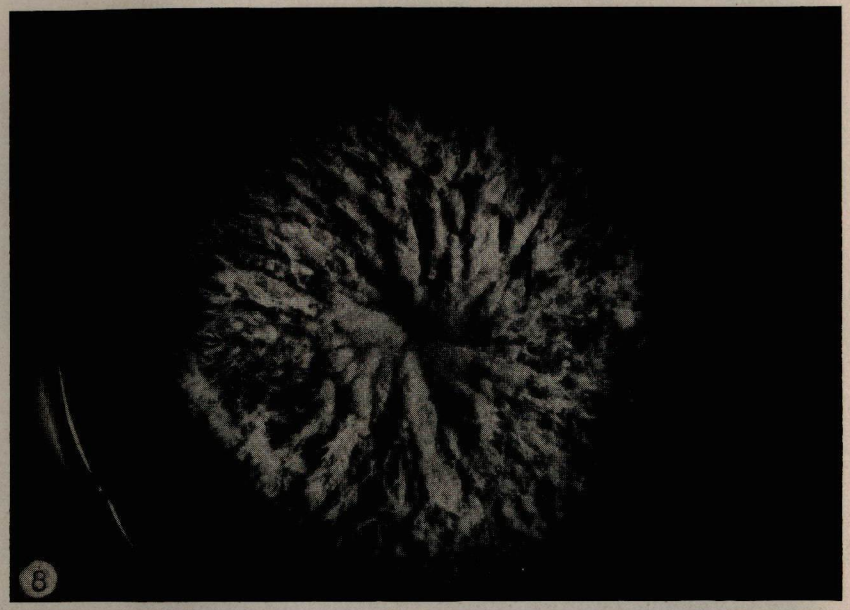


PLATE V

Fig. 8. *Monosporium apiospermum*, St. Croix case: cultures two weeks old on Sabouraud's proof medium.

Fig. 9. Culture of same fungus at same age on 4 per cent glucose agar.

LÁMINA V

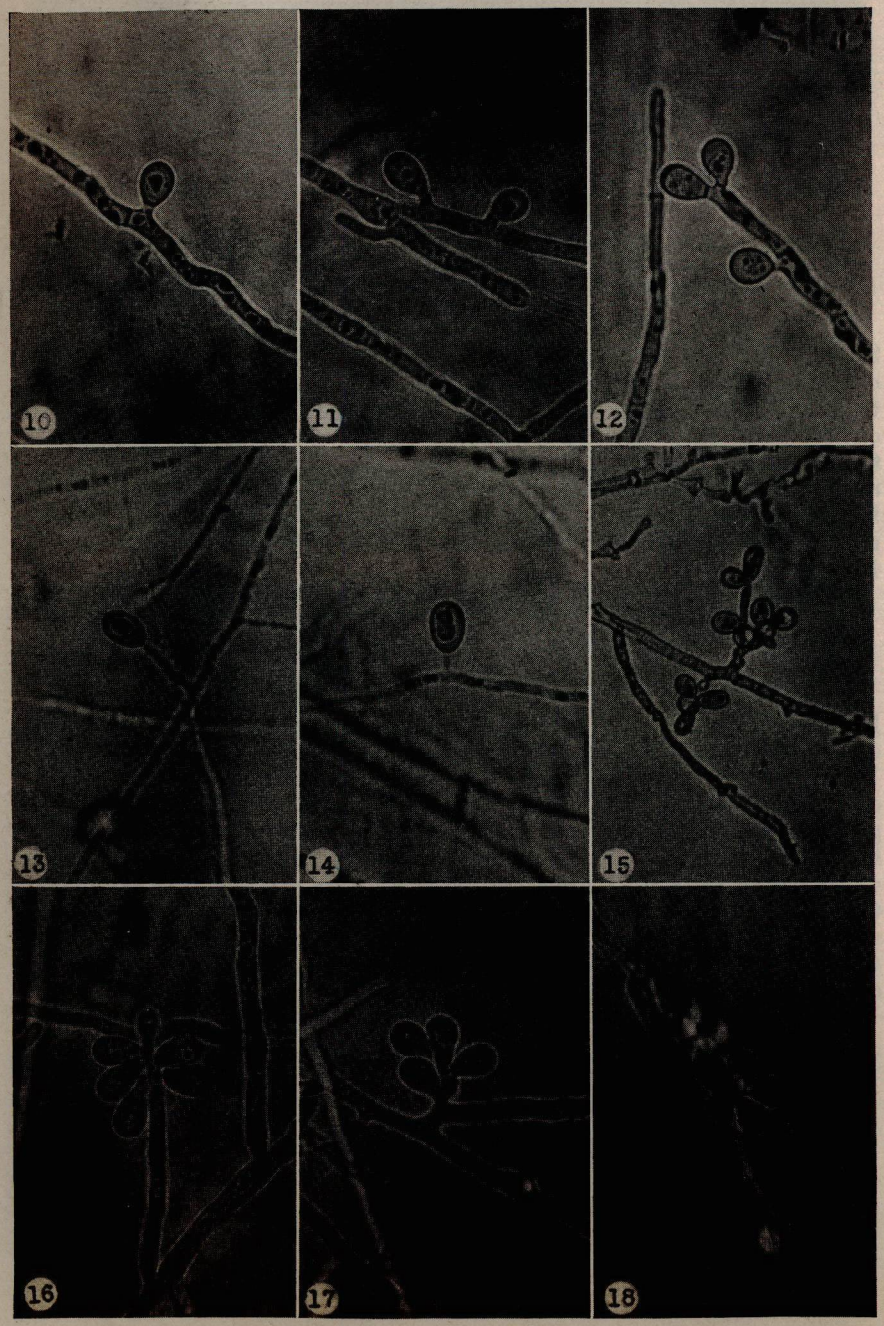
Grabado 8: *Monosporium apiospermum* aislado en el caso de Santa Cruz: cultivos de dos semanas, en medio de prueba de Sabouraud.

PLATE VI

Sporulation in *Monosporium apiospermum*. Note oval or pear-shaped conidia produced either singly (Fig. 10 to 14) or in bunches (Fig. 12 and 15 to 18), both laterally and terminally; single conidia at the tip of differentiated conidiophores in Fig. 13 and 14. Fig. 10 to 16 illustrate the St. Croix isolate. Fig. 17 and 18 illustrate the Baltimore isolate. Fig. 10 to 12 and 16 to 18 are from slide cultures on Czapek's agar. Fig. 13 and 14 are from slide cultures on cornmeal agar. Fig. 15 is from a hanging-drop culture in 4 per cent glucose broth. Magnification: x 1,000 in all figures, excepting 15, which is: x 600.

LÁMINA VI

Esporulación en el *Monosporium apiospermum*. Nótense los conidios ovalados o piriformes brotando aislados (grab. 10 al 14) o en ramilletes (grab. 12, 15 al 18), implantados terminal o lateralmente. Los conidios únicos, en el extremo de conidióforos diferenciados, vense en los grabados 13 y 14. Los grabados 10 al 16 ilustran muy bien el hongo aislado en el caso de Santa Cruz, y los grabados 17 y 18, el aislado en el caso de Baltimore. Los grabados 10 al 12 y 16 al 18 proceden de cultivos en portaobjetos, en agar de Czapek. Los grabados 13 y 14 son de cultivos en portaobjetos en agar-maíz. El grabado 15 es de un cultivo en gota colgante, en caldo glucosado al 4 por ciento (magnificación de 1000 diámetros en todos los grabados, excepto el 15, que es de 600).



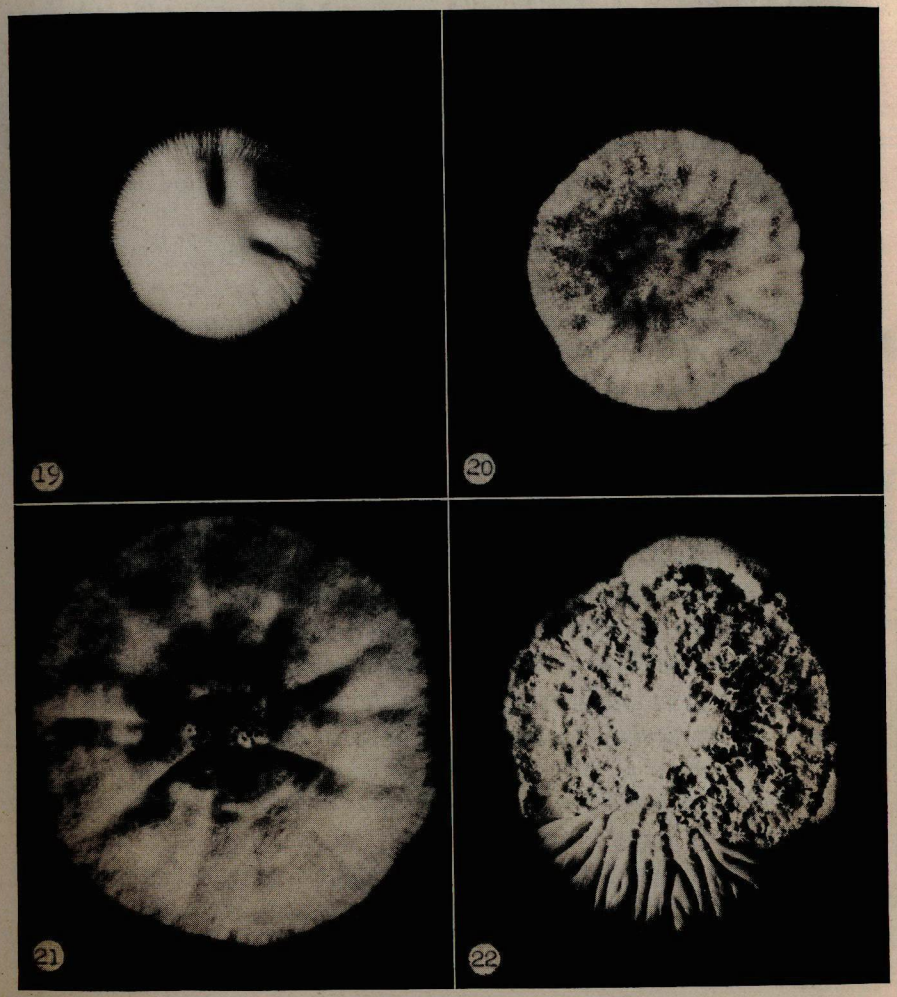


PLATE VII

Different isolates of *Monosporium apiospermum* presented to show various patterns in gross morphology. Colonies developed on Sabouraud's proof medium, same age (2 weeks).

Fig. 19: Baltimore isolate. Fig. 20: isolate from University of Paris (Dr. Ashford's collection). Fig. 21: Massachusetts isolate. Fig. 22: St. Croix isolate. In St. Croix isolate, note three variant sectors, two of them cottony and one membranous. Subcultures from the sectors revealed identical microscopic morphology with mother culture.

LÁMINA VII

Diferentes ejemplares de *Monosporium apiospermum*, en que se pueden apreciar las diferencias de configuración de las colonias. Colonias en medio de Sabouraud de la misma edad (2 semanas).

Grabado 19: Hongo aislado en Baltimore. Grabado 20: Ejemplar obtenido en la Universidad de París (colección de Dr. Ashford). Grabado 21: Hongo