MYCETOMA-LIKE CHROMOBLASTOMYCOSIS AFFECTING THE HAND

Further Findings and Comparative Mycologic Studies*

GEORGE M. LEWIS, M.D., MARY E. HOPPER, M.S., WILBERT SACHS, M.D., FRANK E. CORMIA, M.D. and CLEMENT B. POTELUNAS, M.D.

From the Department of Medicine (Dermatology), New York Hospital, and Cornell University Medical School, New York

While on the staff of Goldwater Memorial Hospital (Welfare Hospital), New York, two of us (G. M. L. and W. S.) presented before the Manhattan Dermatological Society (1) a patient with lesions of the right hand having a resemblance to mycetoma. The patient was again presented by Bechet before the Atlantic Dermatological Society (2). Pathologic studies of the same case were reported subsequently by Symmers (3) and mycologic observations were published by Emmons (4) to whom (and to other mycologists) a cultural growth isolated from the patient had been sent. The identical strain was also submitted to Emmons by Symmers. The present communication is a summary of the cultural findings and experimental animal inoculations from this case, and a comparative study of the causative microörganism with closely related species.

CASE HISTORY

A. N., a white man aged 67, a clerk, was observed first in August, 1939, with an eruption of seven years' duration involving the right hand. He had never left the immediate vicinity of the city of New York. On November 24, 1932, he fell and injured his right hand on a wooden floor. It was thought that several splinters were lodged in his hand and these were reputedly removed on that day. He was admitted to the Neurological Hospital, Welfare Island, with arteriosclerosis, hypertension, cerebral thrombosis and with a mild paralysis of the extremities. Three weeks later the hand became greatly swollen and several fluctuant areas developed. These were incised and pus was evacuated. It was noted that the pus contained dark foreign body-like granules of various sizes. Recurrent tumefactions, some of which opened spontaneously, continued to appear. He later became a patient in the Welfare Hospital.

Examination revealed an irregularly enlarged right hand with several sinus-like openings in the palm over localized swellings. Pus containing granules exuded from some of these openings, while from others only granules could be expressed. The granules were dark and angular and varied in size up to approximately 1 mm. On the dorsum of the hand were many firm, movable, painless tumefactions which were not attached to the skin. Sinus tracts were not present on the back of the hands. There was a partial right hemiplegia with a blood pressure 220/100. Roentgenologic studies of the hand showed no bone involved in the disease process. The Wassermann reaction was negative. A complete blood count and urinalysis were normal.

The patient was observed over a period of three years during which time several courses of potassium iodide were given. Since focal flareup of the disease process was coincidental on two occasions, this form of treatment was abandoned. Thymol was also administered and various types of local therapy were used including wet dressings with potassium per-

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manganate. Surgical drainage was undertaken when the swellings became acutely inflamed. No therapeutic measure had any effect on the slow progress of the disease.

It would seem important to differentiate clinically between mycetoma and the case under discussion in this paper. Although the disease had a superficial resemblance to mycetoma, there are at least three reasons why this diagnosis must be reconsidered. The openings to the superficially located granular masses did not form long tracts so characteristic of actinomycosis and mycetoma. Pus was irregularly present and even after many years, the bones were not invaded. We have been unable to find described any comparable clinical condition. According to Emmons a microörganism similar to the one isolated from our patient, was recovered from a case of mycetoma of a foot presented by Jeanselme, Huet and Lotte (5) but we are unable to agree for reasons to be discussed later.

HISTOPATHOLOGIC FINDINGS

The active disease process was located in the cutis and subcutis, the epidermis showing only mild reactive changes. The inflammatory exudate in the corium did not invade the epidermis except as short sinus-like tracts. Mild acanthosis and hyperkeratosis were present.

The important lesions consisted of dense nests of inflammatory exudate of a somewhat variable character. The central portion of these granulomatous districts was made up of many isolated and conglomerate masses of small, angular black granules. Direct examination of the granules under high power magnification revealed the central portion to be composed of thick-walled elements and the surrounding zones of simple, non-pigmented filaments. The Perl staining reaction was negative. These deeply pigmented masses resembled somewhat in size and configuration the sulfur granules of actinomycosis, although the typical palisade arrangement was not present.

The tissue surrounding the central granule formations reacted to the proliferating fungus in a somewhat variable fashion. The cellular response was pleomorphic, with leucocytes, plasma cells, giant cells and histiocytic proliferation (fig. 2 F). In some districts, the fixed tissue elements were unable to cope with the destructive properties of the fungus and the subsequent tissue degeneration was followed by a profuse invasion of polymorphonuclear cells. In other areas fatty degenerative products were engulfed by epithelioid cells to form considerable numbers of foam cells. These in turn were at times transformed into foreign body giant cells (fig. 2 F). In the areas containing a predominance of plasma cells, small lymphocytes and histiocytes, there was frequently a containing dense connective tissue capsule (fig. 2 E), but this attempt at walling-off the process was imperfect and only partially successful in many areas. However, individual foci were separated ordinarily by bands of fibrous tissue.

The granulomatous areas contained a variable number of blood vessels, but while these were dilated in some sections, the characteristic change was an endarteritis of obliterative type (fig. 2 E). The connective tissue was condensed, thickened, hyalinized and irregularly dispersed by the inflammatory exudate. There was no basophilic degeneration as is seen in actinomycosis; furthermore,

both the histologic appearance of the granules and the presence of foam cells were of value in excluding that disease.

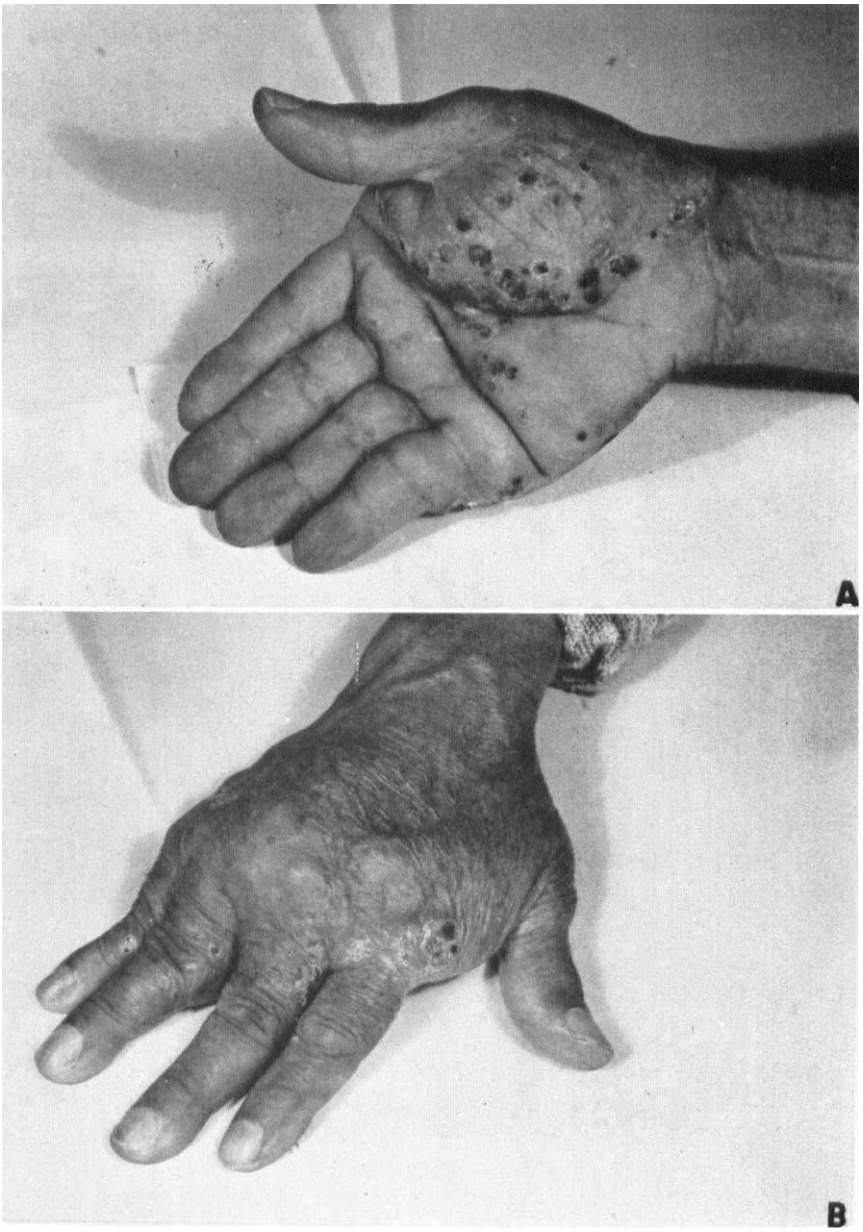

FIG. 1. SUBCUTANEOUS, GRANULOMATOUS LESIONS SHOWING SIMILARITY TO MYCETOMA

In summary, the pathologic findings were those of a deep mycotic infection in different stages of development and of a pleomorphic tissue reaction, often of

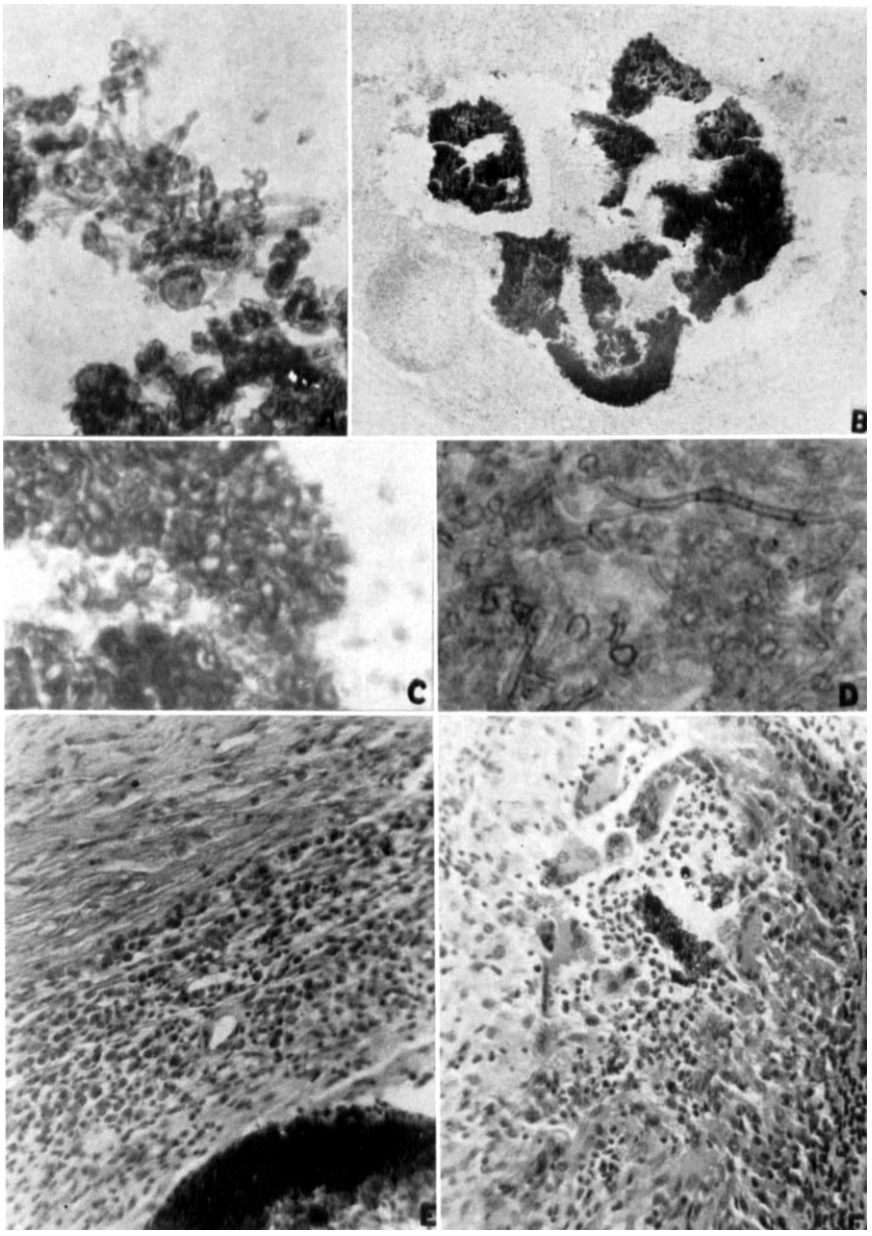

FIG. 2. STRUCTURE OF THE PATHOLOGIC TISSUE

A, the granule has been teased out to show filaments and chlamydospores, ×670; B, histologic section to show position of granule in the tissue, ×100; C, margin of the granule, ×670; D, tangled mass of filaments and chlamydospores in section from experimentally induced lesion in the mouse, ×670; E, cross section of half of granulomatous lesion, showing granular material at base, pleomorphic infiltrate and a thickened blood vessel in mid zone and an upper outer zone of fibrous connective tissue; F, foreign body reaction with central granule, surrounded by a mixture of polymorphonuclear, epithelioid, plasma, foam and giant cells.

foreign body type, to the infection. Despite the imperfect nature of the tissue defense, the infection did not appear to be more than locally invasive.

DIRECT MYCOLOGIC EXAMINATION

The granular material was readily obtained by superficial incision over swollen parts of the hand, followed by pressure at the sides. The material was a black compact mass consisting of granules which were difficult to break apart. After prolonged teasing the granules could be separated and were found to vary in size up to 1 mm. in diameter. The granule was composed of a tangled mass of black, short-segmented filaments and a mixture of thick-walled chlamydospores and arthrospores. In some granules there were thin-walled, hyaline, rapidly-growing filaments at the periphery.

CULTURAL CHARACTERISTICS

A. Dextrose agar

Contrary to the statement of Symmers, no difficulty was encountered in obtaining, repeatedly, cultural growths from granules which had recently been removed as well as from granules stored for several days. Our standard medium containing 4 per cent dextrose agar and 1 per cent Fairchild's peptone was used. The primary isolation required ten days before it could be identified as a fungous growth. At first the growth was black and yeast-like. After approximately 4 weeks, the culture was a compact black mass of filaments with a light thin layer of fluffy surface growth giving a grayish tone to the colony. There was a large central elevation (umbo) but neither sulci nor surface configuration were in evidence. The margin was abrupt, moist and black.

B. Other mediums

When transferred to some other culture mediums, some variation in the size and surface configuration was noted. On maltrose agar, the rate of growth was similar, the color was identical but radial grooves appeared. On corn-meal and on Czapeck mediums, the growth was sparse (fig. 3D and E). This would indicate an obligate parasitism (requiring organic nitrogen not present in these mediums).

FINDINGS ON CULTURE MOUNT

Preparations made from a primary isolate revealed a moniliform growth as illustrated (fig. 4). Later this finding could not be duplicated from the study of subcultures.

In material from subcultures, vegetative structures predominated. These consisted of both thick-walled and thin-walled, simple, branching filaments and thick-walled arthrospores. Spore forms were found only in the fluffy surface growth of colonies at least 4 weeks old. Even then, the number of conidia was sparse. The predominant spores were found as clusters produced at multiple points from the surface of a restricted and thickened tip on the end of short branches and at the junction between vegetative filaments (Acrotheca type of

sporulation). At no time was a Phialophora cup identified although there were some broken filaments which simulate this type of spore producing mechanism. At times, the type of sporulation seen in Hormodendendrum was approached but only as a rudimentary structure (fig. 8 C, D and E).

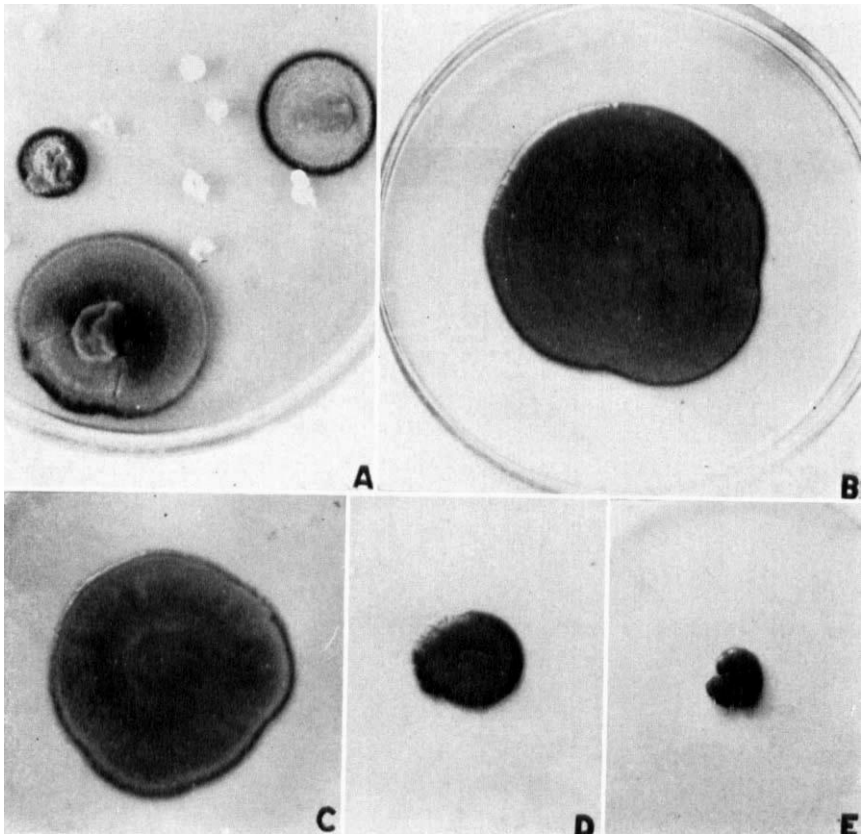

FIG. 3. CULTURAL APPEARANCE OF ISOLATE

A, primary growth; B, subculture after 4 weeks on dextrose agar; C, on maltose agar; D, on Czapek's agar; E, on corn meal agar.

ANIMAL INOCULATION

Both mature guinea pigs and white mice were employed, using a saline suspension of the cultural growth. The following technics were employed: (a) subcutaneous injection; (b) intraperitoneal injection; (c) repeated injections into and below the plantar callosities.

In no case did the animals die spontaneously as a result of the induced infection. The animals were sacrificed from 4 to 10 weeks following inoculation of the material.

RESULTS

In both guinea pigs and white mice, a granulomatous reaction developed in the subcutaneous tissue in the region of the injected material. This could be

detected as a nodule by the palpating finger before removal. On histologic section many non-pigmented and pigmented hyphae were seen diffusely through-

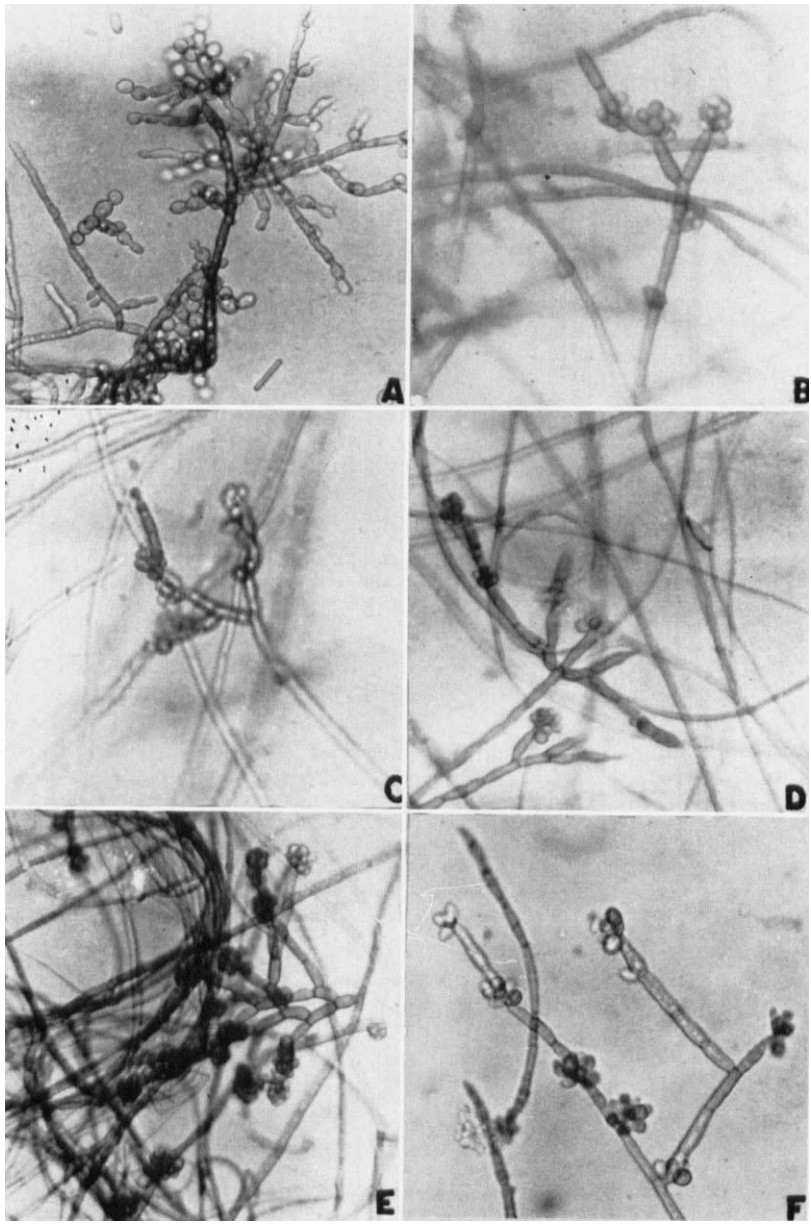

FIG. 4. Culture mounts showing A, simple blastophore sporulation in the first isolate; B, to F, the typical spore clusters are produced at end of branches or between hyphal segments, ×480.

out the cutis, together with many chlamydospores. The tissue reaction was evidenced by the presence of a polymorphous cellular exudate including poly-

morphonuclear leucocytes, lymphocytes, eosinophils and many foam cells. Dilated blood vessels were noted and the entire area was often encapsulated by a

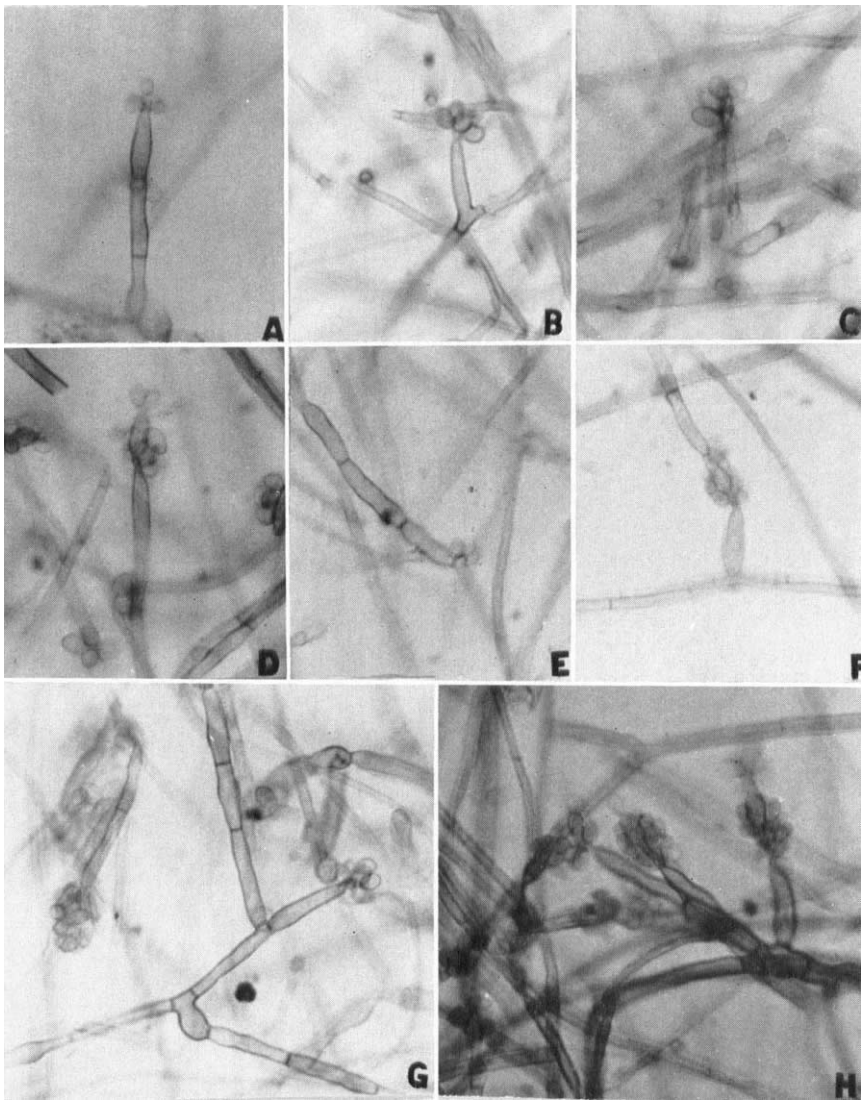

FIG. 5. CULTURE MOUNTS SHOWING ORIGIN, AND RELATION OF SPORES TO SPOROPHORE

In A, B, C, and D, spores are arising from the tip of sporophores from multiple points; in E, three spores may be observed arising from a small lateral apiculum; in F, the continuity of filament is shown with spores attached at point of origin in an intercalary sporophore; in G, the thickened cell suggests a cup except for the attached filament. In H, both terminal and lateral clusters of spores originate from mother cells at multiple points, ×1080.

band of dense fibrous tissue. Experimental infections were not produced by either intraperitoneal injections or when repeated inoculations were made below the plantar callosities.

It is of interest that Symmers was able to produce substantially the same type of response in rabbits (3).

COMPARISON WITH OTHER SPECIES

A. Cultural findings

The black color of this fungus suggested comparison with the fungi which had been isolated from patients with chromoblastomycosis. Cultures of Hormodendrum pedrosoi, Hormodendrum compactum and Phialophora verrucosa (Uruguay) were obtained from Dr. A. L. Carrion, School for Tropical Medicine, San Juan, Porto Rico. Another strain of Phialophora verrucosa was sent to us from Dr. Fred D. Weidman, University of Pennsylvania, Philadelphia. Hormodendrum olivaceum, a common contaminant was also included in the study.

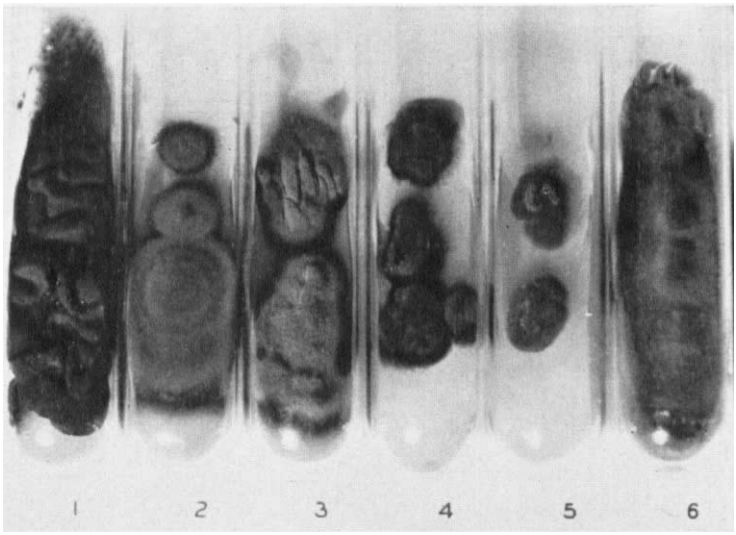

FIG. 6. CULTURAL COMPARISON OF SIX ATRAMENTOUS FUNGI
Reading from left to right are: H. olivaceum, our isolate, H. pedrosoi, H. compactum, P. verrucosa (Philadelphia) and P. verrucosa (Uruguay).

The variation in cultural growth of these fungi is shown in figure 6.

It was found that H. olivaceum produced the most rapid growth, while for H. pedrosoi, our organism and P. verrucosa (Uruguay) the rate of growth was moderate. The remaining two, P. verrucosa (Philadelphia) and H. compactum grew slowly; the Philadelphia fungus grew even less well on a plate than indicated in the picture of the slant (fig. 6). The color in all species was similar and not distinctive, being essentially black with a grayish tone because of the surface filaments. Rapid growth of the colony was accompanied by increased surface fluff. It is of interest that the fluffy surface growth of all species readily collapsed when touched. All species were transferred with difficulty because of the membranous structure of the substrate. The surface pattern was not distinctive but with H. compactum and P. verrucosa (Philadelphia) the growths tended to be irregularly elevated in comparison with the other colonies.

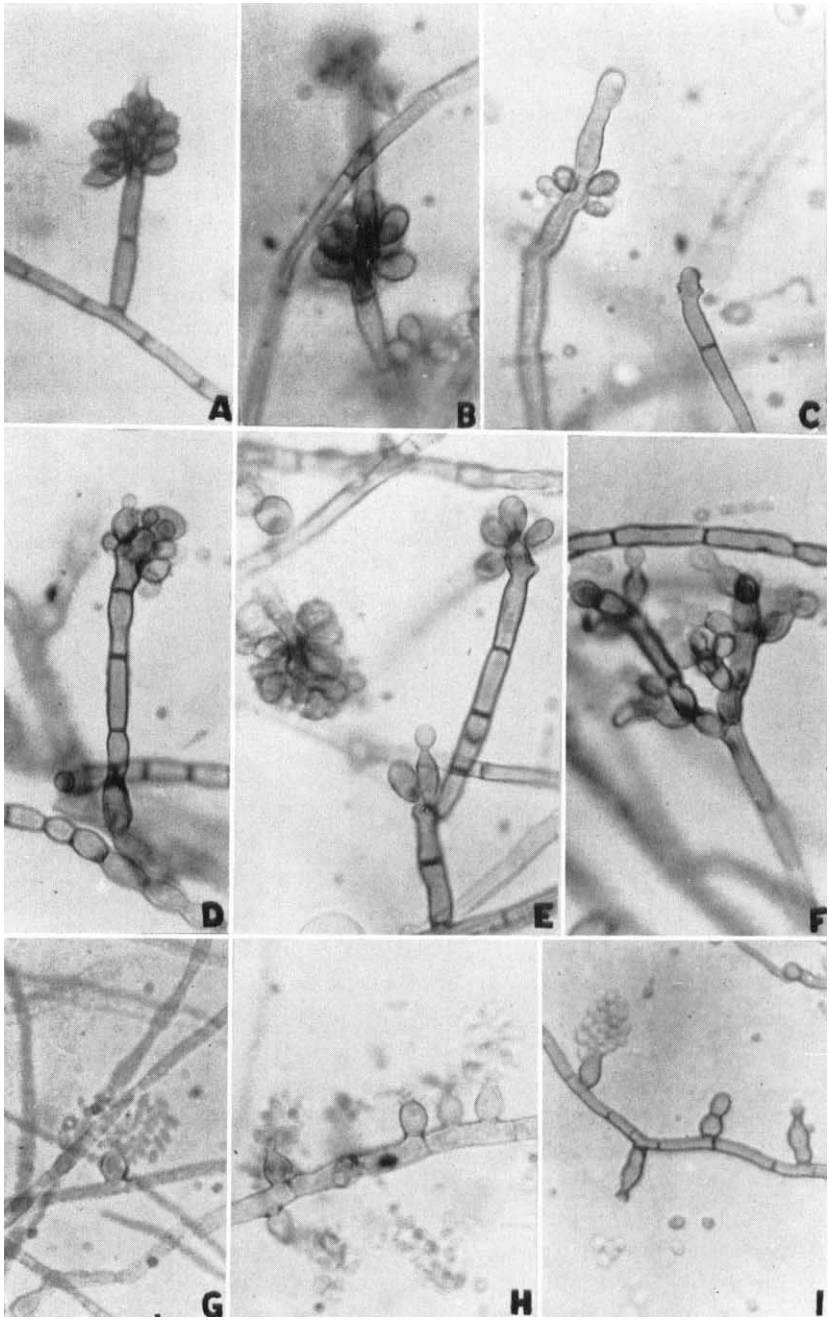

FIG. 7. ACROTHECA SPORE FORMS IN THREE OTHER FULIGINOUS FUNGI

A, B and C, Hormodendrum pedrosoi; D and E, Hormodendrum compactum; G and H, Phialophora verrucosa (Uruguay) in which spores are seen to arise from a filament protruding from cup. Typical Hormodendrum spore organ is seen in F (H. compactum). Phialophora cups are present in H and I (P. verrucosa—Uruguay). Magnification ×1080 except A and H which are ×670.

B. Microscopic findings

It has been demonstrated by Carrión (6) that certain isolates from patients with chromoblastomycosis might have any one or more of three types of sporulation. These types included (1) the Hormodendrum variety—(budding with the youngest spore at the end of a chain), (2) Acrotheca variety (spores produced

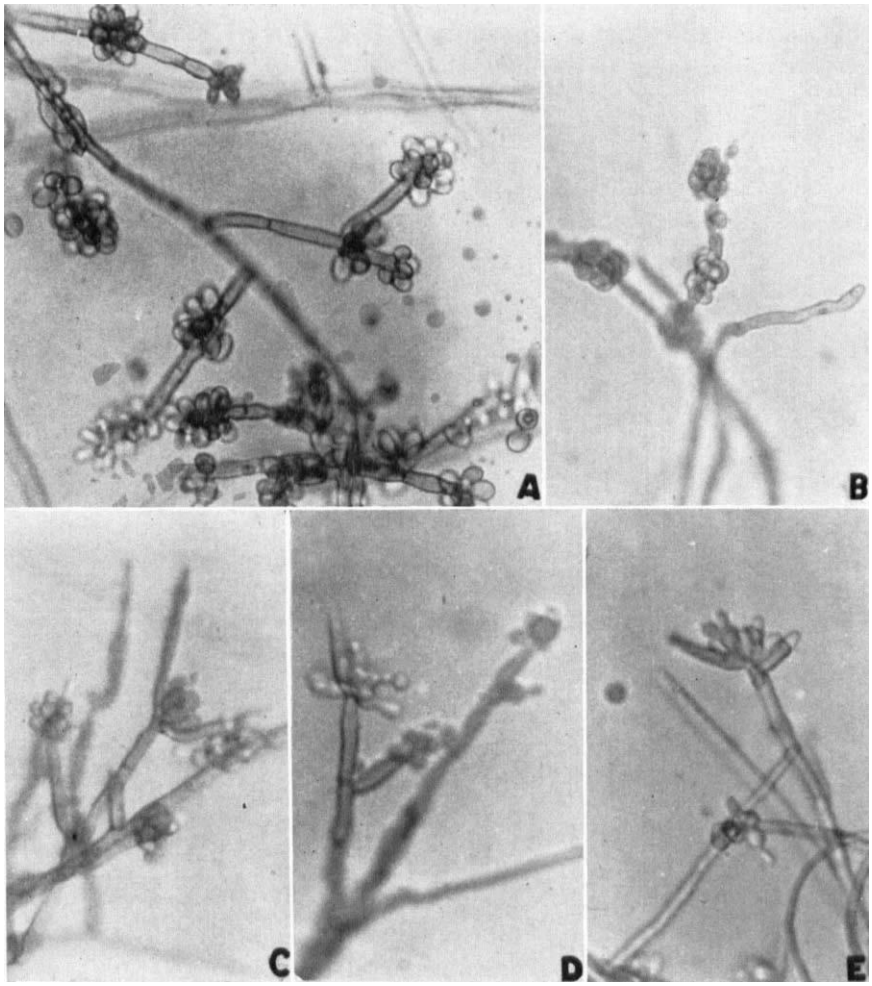

FIG. 8. SPORE FORMS FOUND IN THREE FUNGI SUGGESTING AN INTIMATE RELATIONSHIP
Acrotheca type of spore formation in A,—H. pedrosoi and B,—P. verrucosa (Philadelphia); Hormodendrum type of reproduction in reduced degree in our organism (C, D, and E).

from the surface of a sporophore at multiple points) and (3) Phialophora variety (one spore at a time from a cup, the spores then accumulating in a cluster).

A study of these six strains of fuliginous fungi confirms these findings and is recorded in table I.

It will be noted that for H. olivaceum only the Hormodendrum spore forming mechanism was present. As previously noted, both a rudimentary Hormodendrum structure and an acrotheca formation were demonstrated in mounts from our isolate, the latter being predominant. With both H. pedrosoi and H. compactum all three types of sporulation were found; although the Hormodendrum form was predominant in both, the acrotheca mechanism was frequent and much more common than Phialophora cups. For both strains of Phialophora, the acrotheca mechanism could be found only rarely; the Phialophora cups were numerous in the Uruguay strain and much less in the strain from Philadelphia, corresponding to the lack of vigor of the cultural growth.

TABLE I

Comparison of some cultural and microscopic findings of six atramentous fungi

The surface configuration was not a distinctive feature in any of the species.

	DIAMETER OF CULTURE AFTER 4 WEEKS	UMBO	SIZE OF SPORE	SIZE OF FILAMENT	HORMODENDRUM SPORES	ACROTHECA SPORES	PHIALOPHORA SPORES
	cm.		*μ*	*μ*			
1. Hormodendrum olivaceum	7.5	no			yes	no	no
2. Isolate from our patient	3.	yes	2.5 x 3.5	1 to 2	rudimentary	yes	no
3. Hormodendrum pedrosoi	3.2	yes	3 to 4.2 x 4.5 to 7.	2 to 3	yes	yes	yes
4. Hormodendrum compactum	2.3	yes	3 to 4 x 4 to 6	2 to 3	yes	yes	yes
5. Phialophora verrucosa (Philadelphia)	.7	yes	1.5 x 2.3	1 to 2	no	yes	yes
6. Phialophora verrucosa (Uruguay)	4.4	yes	1.5 to 2. x 2. to 2.5	1.5 to 3	no	yes	yes

DISCUSSION

It has already been pointed out that while the clinical features at first impress one as mycetoma, the relative superficiality of the process, the lack of deep sinuses and the failure to involve the bony structures are against that diagnosis. When granules derived from the lesions were minutely examined, the palisade arrangement of mycelium characteristic of actinomyces was lacking. The presence of foam cells and of a foreign body reaction also distinguished the histologic picture from that of actinomycosis and mycetoma. A study of the isolated fungus points to a relation with fungi which heretofore have been obtained from lesions of chromoblastomycosis. The clinical features of the disease process in our patient, however, are not typical for that disorder. Chromoblastomycosis is usually superficial with papules becoming nodules and finally verrucous and

cauliflower-like in type. Carrión (6) mentions the variations in clinical appearance that may occur with secondary infection from pyogenic microörganisms and from elephantiasis but states that the deeper tissues are not usually involved. Weidman (7) first suggested that the disease process might be best referred to as chromomycosis. It would seem reasonable to postulate that the clinical features observed in our patient, while at variance with the published accounts of other cases of chromoblastomycosis, are due to the combination of traumatic introduction of a fungus of weak pathogenicity into the subcutaneous tissues of an individual in a debilitated state of health. The pathogenicity of the fungus for animals was sufficient to reproduce the disease and further prove its etiologic rôle. According to Emmons, the fungus is identical with a microörganism identified by Langeron (8) as Torula jeanselmei. However, Langeron stated that the granules were soft, that cultures developed readily within 24 hours and had a blackish down with greenish shadows (sheen). On microscopic examination there were many budding spores (blastospores). In contradistinction, the granule in our case was hard, the colony developed slowly and a green tone was not found. Budding spores were observed only in the primary isolate and conidia were always scanty. For these and other reasons we believe these fungi to be different species. In suggesting the name of Philophora jeanselmei for our isolate, Emmons stated that "on all of the types of conidiophores described here the conidia are borne at the tip. They are pushed out serially through an opening at the end of the conidiophore. . . " Our observations indicate that the spores are produced from multiple points on the surface of a constricted tip (acrotheca type). This tip may be terminal or intercalary but the process is the same. In figure 5 F the attachment of the spores to a constricted tip is shown with mycelium in continuity so that an opening would be impossible. Fracture of the filaments and dislodging of conidia were noted to be frequent and might well result in a picture simulating a flaring cup; this can be imagined for the filament in figure 5 G. Furthermore, the youngest spores are often seen at the tip of the conidiophore while older cells are still attached, manifestly impossible if the organ was a cup. The predominant and habitual spore form from our observations is of acrotheca type and this should constitute the chief basis for identification. From prolonged study, structures suggesting the Hormodendrum type of spore formation were very occasionally seen.

From our studies of the fungus and in comparison with other strains isolated from cases of chromoblastomycosis, we believe that Carrión is correct in bringing these fungi together in one genus which he has called Fonsecaea (6).

SUMMARY

A patient with subcutaneous, granulomatous lesions of the right hand was first observed in 1939 and throught to have mycetoma. Further investigations, including a critical review of the clinical features and histopathologic findings, together with a detailed study of the causative microörganism, indicate that the disease might be better classified as chromoblastomycosis. The exact botanical identification of the fungus responsible for the disorder has been difficult. From

prolonged studies and from comparison with morphologically similar species it is concluded that this fungus should be placed in the genus Fonsecaea together with species of Hormodendrum and Phialophora as suggested by Carrión.

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