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GENERALIZED MYCOSIS DUE TO A HITHERTO UNDESCRIBED FUNGUS (GLENOSPORA GAMMELI)

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Case reports of generalized mycosis are infrequent; first, because the condition is probably not very common; secondly, because few clinicians are interested in this type of disease and do not possess the training necessary to study the fungi; and thirdly, since Koch's postulates cannot be fulfilled in dealing with diseases due to fungi, pathologists and bacteriologists hesitate to make clinical reports on incomplete studies.

We report this case because we believe it to be an instance in which an apparently harmless, though hitherto unrecognized mould has invaded the tissues of man and produced a serious disease, which once recognized, could be treated satisfactorily. Furthermore, we think that the detailed description of the method of identifying the strain of this fungus may serve as a guide to others in similar investigations.

CASE HISTORY

The patient, aged forty-five years, walked into the dispensary of Lakeside Hospital in March, 1925, complaining of pain in the chest, cough with bloody expectoration and loss of weight. This he had endured for about four months, thinking that it had started from a cold, and although unable to work he had not come for medical aid until very recently he had been alarmed by seeing blood in his stools. He had had the malaise and weakness common to destructive lung disorders but no night sweats. He looked quite ill and could scarcely speak on account of persistent cough. He could give no definite account of the appearance of certain skin lesions other than that they had been present for not more than four weeks.

He had had no previous illnesses and had worked steadily as a coal miner, but immediately before becoming sick had worked at heavy outside construction, pouring concrete. Examination showed that he had a partial consolidation of

his right upper lung with coarse and fine moist râles suggestive of pulmonary tuberculosis.

At various places within the skin or under the skin there were lesions not compelling interest, until more carefully investigated. They occurred in three forms (figs. 1 and 2). On his back, face and neck they were like furuncles but with much necrosis and little inflammation; they were not tender nor had they been painful. Other lesions of the face, back, neck, scalp, soft palate and tongue

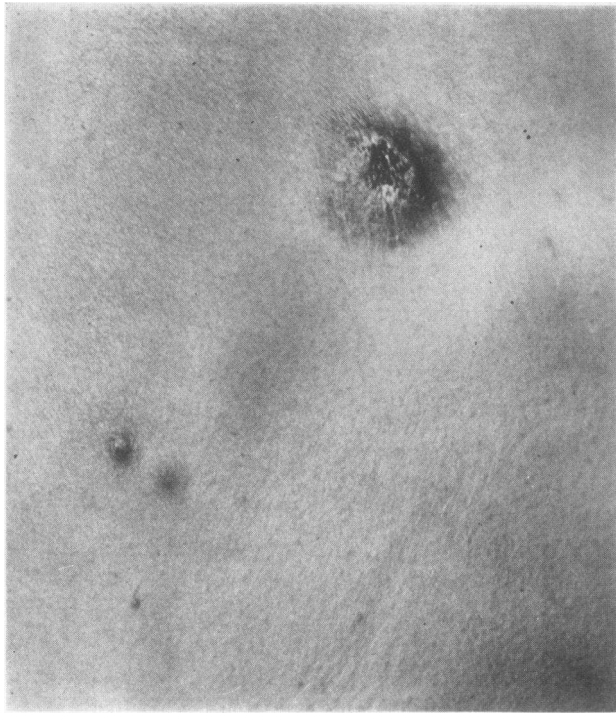


FIG. 1. ULCERATING GRANULOMATOUS LESION OVER RIGHT SCAPULA

Below and to left two small papules

were more tumorous in nature, with no necrosis or inflammation. They seemed to be within the skin or just beneath the mucous membranes, where they could be seen as yellowish-gray, soft bodies, from 3 to 10 mm. in diameter. They were not tender. The third type of lesion was larger and lay deeper. These were soft, movable and semifluctuant, being more like lipomata than any other common condition. One such, 5 or 6 cm. long, was attached to the sheath of the right triceps, another in the right biceps, one over the right deltoid near its lower inser-

tion and another on the anterior aspect of the right thigh. The axillary and supraclavicular lymph nodes of the right side were definitely enlarged and quite hard. All these lesions increased in size so that a few of the cutaneous nodules softened and ulcerated within a few days, and some of those already ulcerated about the face crusted over in a manner to resemble blastomycosis. This diag-

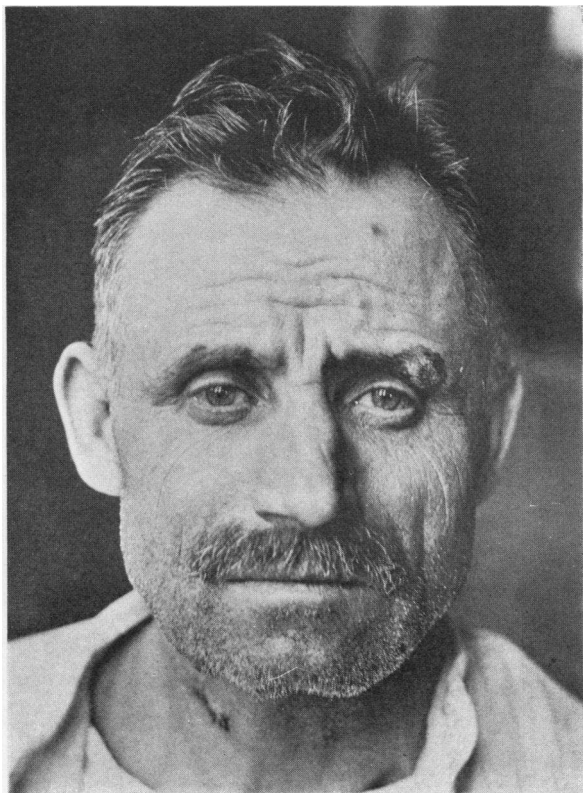


FIG. 2. LESIONS ON FACE. TWO SMALL PAPULES ON FOREHEAD, LARGER CRUSTED LESION ON LEFT EYEBROW, ONE DEEP-SEATED NODULE ON LEFT SIDE OF CHIN

nosis was made by the dermatologist consulted. Careful examination of his heart and central nervous system showed no signs of syphilis.

His temperature averaged 38°C ., going as high as 38.5°C . practically every day. The blood, except for a leukocytosis of 10,000, showed nothing abnormal and the Wassermann reaction was negative. Urine was likewise negative. The sputum was very purulent and bloody and contained a few undetermined organisms, but

no acid fast bacilli or fungi. Material from several lesions showed no forms whatever suggestive of fungi. The stools throughout his stay in the hospital were entirely normal.

Roentgen-ray of the chest was reported as follows (fig. 3): "Stereoscopic films of the chest show a roughly triangularly-shaped shadow of increased density, extending outward from the right hilum. There seems to be some exaggeration of the bronchial markings and some haziness of the right apex. The right dia-

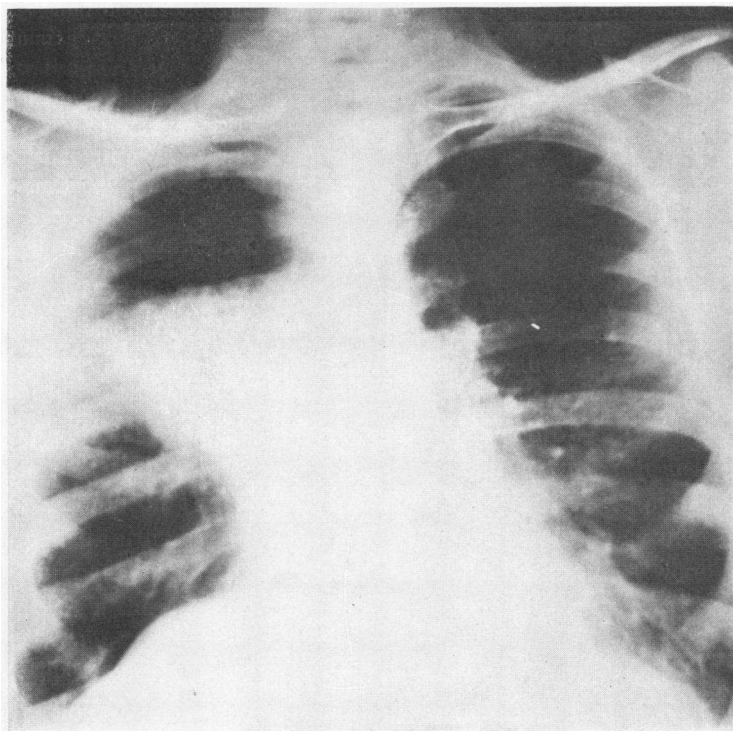


FIG. 3. X-RAY SHOWING TRIANGULAR SHADOW EXTENDING OUTWARD FROM RIGHT HILUS

phragm seems somewhat deformed, probably in part by adhesions. The appearance on the right looks distinctly more like a neoplasm than an infectious process such as tuberculosis. The trachea is seemingly pulled well to the right. There is apparently an anomaly in the form of the first left rib, the entirety of which is not shown on these films.—C. M."

Cultures made from under a large crust on the face showed a white fungus in all media. Cultures taken by aspiration from several deep tumors and cultures

made from tumors removed surgically yielded the same fungus. Blood cultures were negative.

Lesions removed for biopsy were reported once as granuloma and again as chronic inflammatory tissue. The larger lesions removed from the muscles proved to be abscesses, filled with thick, greenish pus in which no organism could be identified.

The patient was given as medication iodide of potash in 5-grain doses three times each day, and neoarsphenamin to a total of 4.65 grams. Protein shock with vaccines of *Bacillus typhosus* caused no reaction either locally or systemically.

Under treatment the skin lesions healed promptly, the fever subsided, as did the cough and expectoration. Physical signs and x-ray showed the condition of the lungs to be much improved.

He was discharged from the hospital after sixty-one days as entirely well, and has been working in the mines ever since.

ESTABLISHMENT OF THE STRAIN

On April 7, 1925, we attempted for the first time to isolate the organism which we believed to be a blastomyces or sporotrichum. As media for all primary cultures we used ordinary glucose agar and Sabouraud's milieu d'épreuve. Pus from the small follicular abscesses in the periphery of the lesion on the left eyebrow was used for inoculation. Other culture tubes were inoculated with the material from a nodule on the right shoulder. This lesion resembled more an unopened furuncle. Pus was aspirated with a sterile syringe after careful preparation of the skin, and cultured. Six tubes were incubated at 37°C., the other 6 were kept at room temperature.

After four to five days some of the tubes at room temperature showed colonies of a fine white mycelial growth spreading slowly toward the periphery. During the next few days the same growth was noticed in 10 tubes. One tube was contaminated with *Staphylococcus albus*, 1 remained sterile. The tubes in the incubator showed a much slower growth.

On April 12, a second series of cultures was made in the same way using this time only the aspirated material from two superficial, fluctuating, closed abscesses. A pure culture of a white mold grew in all tubes.

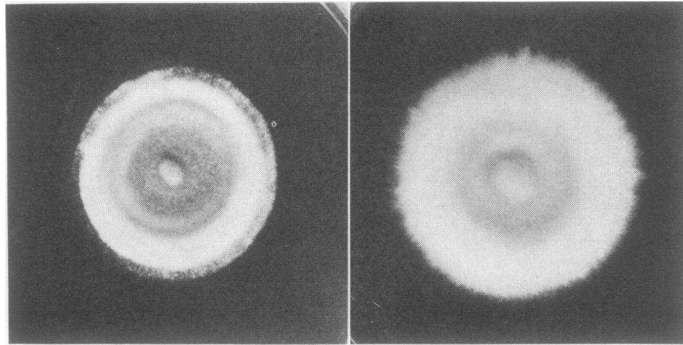
On April 21, a third series of cultures was taken. The material was obtained under most rigid precautions by puncturing an abscess of about a walnut size about 2 cm. below the skin in the biceps of the

right arm. The same growth as in the first and second culture series resulted.

When the first biopsy was performed, cultures were also made by the surgeons on the routine media. The pathological laboratory reported "no growth after forty-eight hours" but when the cultures were reexamined about ten days later and compared with our first cultures, a white mold was noted.

Smears from the pus showed only polymorphonuclear leukocytes, but no organisms of any kind.

During the following weeks cultures of these four series were studied and compared. They were found identical on macroscopic



FIGS. 4 AND 5. CULTURES ON SABOURAUD'S GLUCOSE PEPTON AGAR THREE WEEKS OLD

and microscopic examination; therefore, only the strain obtained on April 21, was used for the further mycological study.

Macroscopic examination. The fungus was successfully cultured on almost all available culture media as plain agar, glucose agar, Sabouraud's milieu d'preuve and de conservation, Grütz Nervina Malz Pepton Knoll agar, Löffler's serum agar, endoagar, potato, human serum, gelatine, whole milk, litmus milk, plain broth, Sabouraud's glucose pepton broth, pepton water, potato water, and several litmus sugar media. The only medium where no growth could be obtained was Raulin's fluid for molds.

The mycelium on Sabouraud's glucose pepton agar consists of a dense, thick, rather undifferentiated feltwork of delicate hyphae with an occasional tendency to concentric arrangement. In Erlenmyer flasks the central portion may become elevated, knoblike, and often fissured. The colony forms usually an almost per-

fect circle, although at times the periphery may assume a rather irregular wavy appearance. The color varies from white to light brown and yellowish-brown. Cultures on Sabouraud's glucose agar three weeks old have a diameter of 26 to 32 mm. (fig. 4).

On Sabouraud's maltose agar the mycelium remains almost white and shows less tendency to spread. The growth is much slower.

On top of old cultures on Sabouraud's proof media, sometimes a white duvet is noted. Pure white duveteuse growth as in the polymorphic form of trichophytons etc. is frequently observed (fig. 5) but experiments have shown that in subcultures the brown growth and the forms observed in our first cultures can be obtained again. Therefore, we believe that we are here dealing only with simple individual variations, id est pleiomorphism of this organism. A true polymorphic form as established so well by Sabouraud for most ringworms was not observed.

The most luxuriant and brilliant growth occurs on Grütz-Malz agar where the color may become brown.

On ordinary glucose agar a heavy, uniform white growth is obtained with a marked tendency to radiate from the center and spread peripherally far beyond the margins of the nutrient substratum. The hyphae frequently cover the walls of the culture tube with a hazy network.

On Löffler's serum agar, endoagar, egg agar, Sabouraud's milieu de conservation, the mycelium is white and of varying density. The concentric and radial arrangement is less pronounced or absent. The hyphae as a rule do not project beyond the medium. On Sabouraud's pepton agar the color is grayish-white.

The growth on agar is limited, the colonies remain white, small and button-like.

The upper portions of carbohydrate media darken slightly. The fine rhizoidal hyphae invade the medium only superficially.

In liquid media our fungus forms a powder-puff-like growth on the bottom of the culture tubes or flasks and ascends later to the surface. There it finally forms a thick white membrane that seals the culture fluid. Sometimes, on milk for instance, a growth appears only on the surface. The location of the growth seems to depend chiefly on the specific gravity of the medium.

The aspect of the cultures on Sabouraud's as well as on other solid media, is subject to variations within certain limits even if all cultures are made and kept under exactly the same conditions. No conclusions as to identity can be drawn from the macroscopic findings.

Microscopic examination. Material of cultures on solid media was soaked in 40 per cent KOH, teased and examined. Cultures on glucose agar and Sabouraud's milieu d'épreuve were embedded in paraffin and serial sections stained with hematoxylin eosin. Hanging-drop cultures with Sabouraud's glucose pepton broth or a medium where the French glucose masseé de Chanut was replaced by the American glucose Pfanstiel, showed the process of reproduction in the most satisfactory way. The mycelium climbing up the walls of the culture tubes was also examined.

The hyphae are cylindric tubes with apical growth, transversely septate, rather straight, hyaline or subhyaline, filled sometimes with a few fine granules or also occasionally with droplets. The young mycelial filaments are undivided by partition walls. Old filaments are hyaline and also in these the septa are frequently invisible. The diameter of the hyphae varies from 2 to 5.3 micra, the interseptal segments have a length from less than 15 to 103 micra. The branching occurs laterally and sometimes by dichotomy. Some thick hyphae form a mycelium "en raquette" as known in *Microsporon Audouinii* and other fungi. There is a

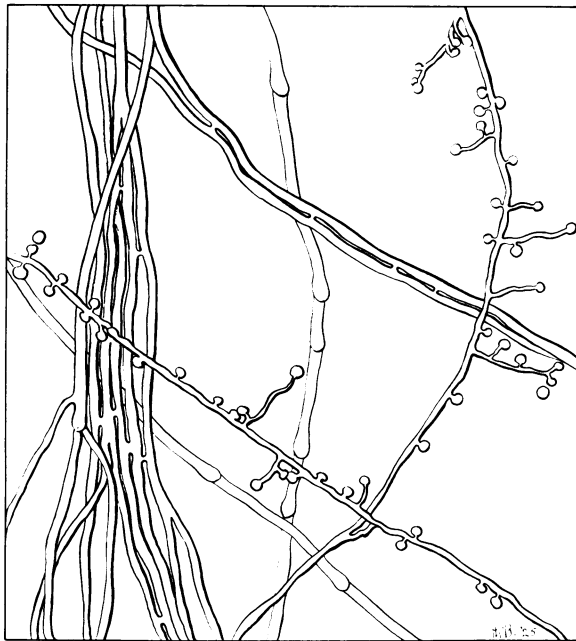


FIG. 6. DRAWING OF HANGING DROP CULTURE. HYPHAE OCCASIONALLY IN COREMIA-LIKE BUNDLES WITH INTERLACING BRIDGES. ALEURIOSPORES EITHER SESSILE OR BORNE ON SPOROPHORES. "MYCELIUM EN RAQUETTE"

marked tendency to form coremia and these hyphae are frequently connected by short bridges (fig. 6). Floating of the protoplasm and Brownian movement of the fine granules in the interior of the fungus could be noted.

The spores are conidia. They are sessile and usually attached directly to the sporiferous hyphae (pleurogamous) or are sometimes borne on simple erect sporophores of 1.6 micron diameter and varying length. These sporophores are unseptate and barely differentiated from the hyphae. They as a rule bear a single terminal spore, but exceptionally one sporophore may support two or three spores,

the second and third one then being lateral to the axis. Short sporophores may suggest phialides.

The size of the conidia varies from 3.3 to 6.6 micra with an average diameter of 4.2 micra (fig. 7). They are in young cultures, pale green, usually one-celled, homogeneous or filled with small granules, spheric or slightly ellipsoidal with smooth walls. In older cultures the shape of the spores becomes more irregular, they may be filled with globules, and two-celled spores are occasionally observed.

One mycelial segment may give rise to several of these spores. Where the walls

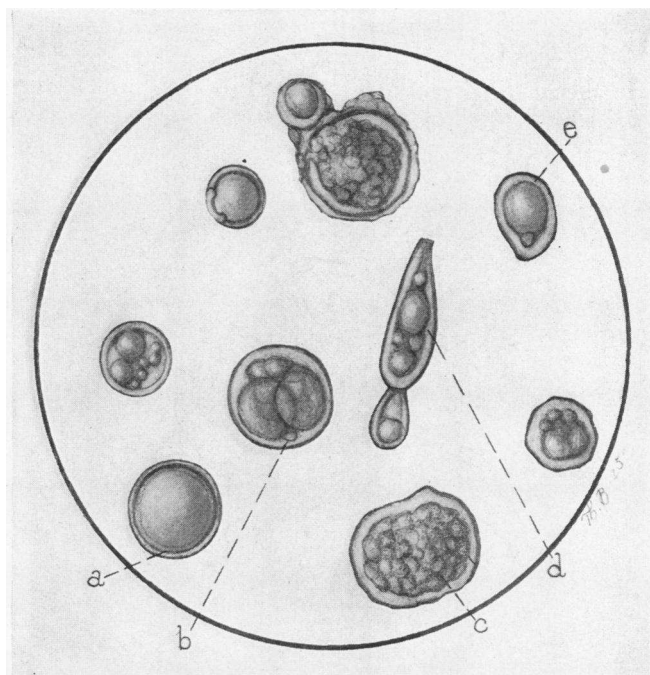


FIG. 7. VARIOUS FORMS OF CHLAMYDOSPORES

of the hyphae bud spores, short chains are sometimes noted. The spores are cut off very late and always remain close to the hyphae from which they originated.

Hyphae and spores may be concolorous, but usually the spores are darker than the mycelium, especially in the old cultures.

Large globose spores of 10 to 12 micra diameter, and darker than the ordinary spores, are found in some old hanging-drop cultures and in teased preparations from old cultures on solid media. They are usually terminal but show occasionally also an intercalary position. The wall may become thickened, double-contoured (fig. 7, a) and a round or oval body may be formed (fig. 7, c). Sometimes

coarse, rather regular granules of 1.6 to 2 micra diameter fill these cells and the color changes gradually to brown. In some of these cells the granules may disappear again while a two or three-celled spore remains (fig. 7, b). Some of these spores resemble somewhat the oospores of *Peronospora viticola*. At no time, however, were organs suggesting sexual reproduction seen. No opening in the wall or its rupture was noted, therefore, we interpret them as chlamydospores.

In parts of cultures where the formation of conidia is sparse or absent, the ends of a few mycelial filaments may form a loop (fig. 8) or become spirally twisted. Neighboring hyphae, single or in bundles, interlace with them and form finally an irregular, round or egg-shaped body of 43 to 85 micra diameter. In cultures on glucose agar they may be stalked. These bodies are very rare, but were found in old hanging-drop cultures as well as on the walls of tubes containing cultures

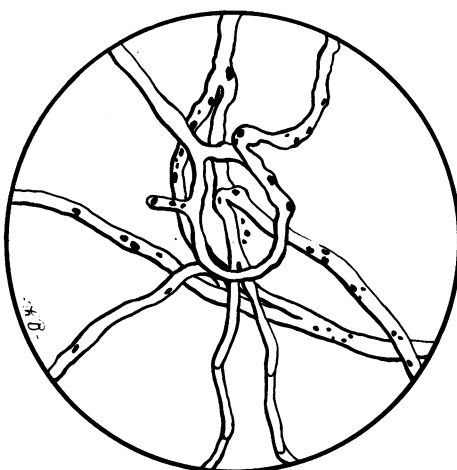


FIG. 8. MYCELIUM WITH BEGINNING LOOP FORMATION

on solid media. They suggest somewhat loose rudimentary perithecia analogue the "organes nodulaires" or minute sclerotes. In this connection, of course, we do not think of sclerotes as of the highly organized resting bodies of hyphae in the strict definition of Anton de Bary, but we employ the term here in the wider sense as it is used in the French and American medical mycological literature. When studied under high power (fig. 9) they appear as gray or light brown bodies consisting of closely packed mycelium. An opening could not be observed, their significance could not be made out.

All attempts to study perithecia in sections failed. Sections of cultures on solid media fixed in formalin and embedded in paraffin give a rather uniform appearance; small threads (caliber 1.3 to 1.6 micron) and abundant globose bodies

of a diameter of 11 to 12.5 micra with a thick wall. Some have a slightly granular appearance (chlamydospores).

Since Nannizzi (1) in Pollacci's (2) Institute succeeded in recent years in revealing the ascospore stage of certain hyphomycetes as *Trichophyton*, *Microsporon*, *Achorion*; etc., by culturing these organisms on bird feathers, skin, hair and bones, we attempted to grow the fungus isolated by us on chicken feathers and human hair, however, without result.

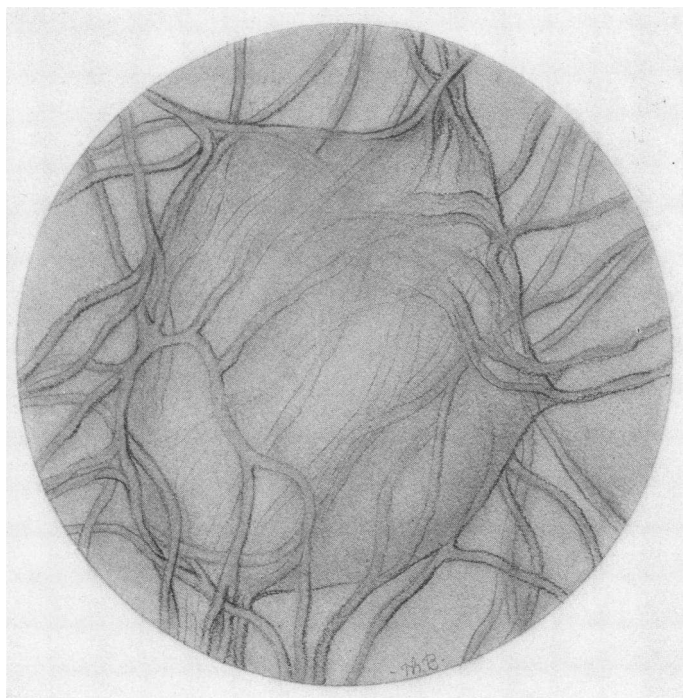


FIG. 9. SCLEROTIA-LIKE STRUCTURE FORMED BY DENSELY INTERLACED MYCELIUM

Chemical action. The fungus produces in the media a marked alkaline reaction. The blue color in litmus milk and litmus broth is deepened considerably. Litmus milk is cleared and assumes a port-wine tint. In old liquid cultures the envelope crystals of calcium oxalate and other crystals suggesting monocalcium phosphate may be found.

Ferment production. Litmus broth with dextrin, maltose, mannite and saccharose, does not change its color. Lactose litmus broth assumes a slight port-wine tint. Litmus broth with galactose, glucose and levulose, is very slowly decolorized

after the fourth week. No gas formation in any of the litmus sugar media was noted. The fringe of the otherwise white mycelium that is in contact with the wall of the culture tube assumes a deep blue color.

Gelatin is liquefied after three to four weeks; a dense yellow mycelial growth covers the surface. Brown pigment diffuses from it down into the translucent medium and darkens it slowly. The bottom of the culture tube is covered with a fine sediment.

Coagulated human serum exhibits surface growth.

Whole milk does not show any change during the first two weeks except for a heavy growth on the surface, no coagulation takes place. After four weeks the milk has a light yellow color and is turbid. After eight weeks the fermentation is completed. The fluid is clear and dark yellow with a brown tinge. A light brown growth fills the upper third of the medium, while a coarse, heavy flocculent precipitate covers the bottom of the tube.

The optimal temperature is between 18° and 22°C. Development in the incubator at 37°C. is slow and very poor, this did not change the subcultures.

The fungus is strictly aërobic. The development of young cultures in broth was immediately inhibited when the fluid was sealed with a thick layer of vaseline.

Growth in bouillon over chloroform was slightly restrained. Old cultures have a slight fecal odor.

The organism is Gram-negative. Some parts, however, may take the stain occasionally. It tinges readily with the usual laboratory dyes, Safran blue, eosin, etc. The most satisfactory preparations probably were obtained with highly diluted fuchsin.

Agglutination and similar tests were not carried out.

Animal experiments in a monkey, 2 guinea pigs and 7 rabbits, with pus from the lesions and suspensions from cultures, failed to reproduce the disease.

CONCLUSIONS

It is obvious that as the fungus in question is a parasite with septate filaments, and reproducing by spores it belongs to the *Eumycetes* of Schroeter 1892. Until the formation of asci and perithecia is proved, it is equally manifest that it must be placed in Fuckel's class (1869), *Fungi imperfecti*, and in Vuillemin's subclass *Hyphales*. According to the modus of spore formation it belongs to order IV *Conidiosporales* Vuillemin 1910.

When we reported our clinical and mycologic observations before the Association of American Physicians in May, 1926, we suggested that the fungus should be placed in the suborder *Aleuriosporineae*. Since the necessary mycologic literature for further classification was not at our disposal, we sent the organism together with our report

to the director of the Institut of Botany of the Royal University of Siena (Italy) for identification which Professor Gino Pollacci and Professor Arturo Nannizzi very kindly performed. These mycologists verified our findings and agreed with our tentative classification. Since they considered it as a hitherto undescribed species they proposed for it the name of *Glenospora Gammeli*, which they define as follows:

"*Glenospora Gammeli* sp. n. Pollacci et Nannizzi.—Hyphis sterilibus hyalinis vel subhyalinis, rectis, cylindricis, saepe guttulatis, junioribus continuis, adultis plus minusve distincte transverse septatis, 2 to 5.3μ diam., segmentibus 15 to 100μ long, monopodice ramosis, nonnumquam dichotomis, majoribus crebre septatis, articulis clavatis ut in '*Microsporo Audouinii*' et aliis micetis specierum, haud raro fasciculatis, hinc inde anastomosis brevibus inter se connexis; hyphis fertilibus concoloribus decumbentibus, paullo tenuioribus, varie breve ramulosis, superne aleuriis acro-pleurogenis gerentibus. Aleuriis sessilibus vel aleuriophoris longitudine varia suffultis, sphaericis, levibus, initio 3.3 to 6μ diam., hyalino-chlorinis, postremo majoribus, 10 to 12μ diam., crassiuscule tunicatis, granuloso farctis, ramulis diu haerentibus, dilute luteo-fuscis.

Hab. in cute hominis, Cleveland, Ohio, America borealis (Gammel)-Coloniis (in agaroglucosato Pollacci) initio rotundis, applanatis, candidis, byssineis, concentricis zonulatis, dein confluentibus, interdum luteolis vel brunneolis, in senectute crustam applanatam matrice arctiuscule adnatam efformantibus."

In an additional note Professor Pollacci and Professor Nannizzi state that according to their investigations *Glenospora Gammeli* produces one single kind of reproductive elements, i.e., aleuria. These become separated from the mycelium very late, and at maturation they become enveloped by a membrane of their own. The membrane belonging to the portion of hypha in which each of them was formed becomes lacerated and drops off. Outlines of perithecia or sclerotia such as we described were not observed by the Italian mycologists.

The genus *Glenospora* belongs to the suborder *Aleuriosporineae* of the order *Conidiales*. It was originally published by Berkeley and Curtis (3) with a very few words: "Flocci fastigiati fasciculati parce articulati, hic illic sporangia globosa sessilia vel pedicellata ferentibus." Saccardo's (4) description is also brief: "Hyphae biogenae, in crustam atram intextae, varie ramosae, septatae. Conidia ramulis diu haerentia, globosa, majuscula, levia."

Several species of this genus have been found already as pathogenic for man (5). *Glenospora graphii* was observed in otomycosis by Hassenstein, Bezold, Hallier, Stendener and Siebenmann. Morax and Pinoy isolated it in 1910 from a case of keratomycosis. *Glenospora Semoni* and *Glenospora khartoumensis* were found producing maduromycosis of the black grain type. Henseval in Ghent isolated an organism from the sputum of a fetid bronchitis, which was studied by Vuillemin and termed *Glenospora gandavensis*.

Other species of the genus *Glenospora* as *G. Curtisii*, *G. ramorum*, *G. sacchari*, *G. microspora* are known as plant parasites (6). During the last year we attempted to culture our fungus on small pieces of bark and wood of certain trees (beech, cherry, ash, hickory, soft maple), however, without result.

The way the culture material was obtained and the course of the disease entitle us to pronounce *Glenospora Gammeli* in the case described as pathogenic. We do not think that all requirements of Koch's law can be fulfilled in diseases caused by fungi.

We desire to express our gratitude to Professor Gino Pollacci and Professor Arturo Nannizzi of the Institute of Botany of the Royal University of Siena (Italy) for the classification of this organism.

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