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SVMMARIVM — Due describuntur organisma mucoida, quorum maxime variae sunt formae: coccoides, bacilloides, cryptococcoides, amoeboides, etc. Cum autem nulla ratione potuerint diversae formae separari, videtur non esse symbiosis. Mycologi et bacteriologi, quibus eiusmodi culturae missae sunt, putarunt nullum eas locum proprium habere in bacteriorum mycetorumque systemate, quo harum rerum periti nostra aetate uti solent: utilitatis causa Auctor alteri binomium proposuit *Myxomicromium multiplex*, alteri autem *Myxogeotrichum filarioides*.

Eorum microorganismorum culturae haberi possunt ex ipsius Auctoris Instituto (Instituto de Medicina Tropical, Junqueira, Lisboa) et ex American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland 20852, USA).

MYXOMICROBIUM MULTIPLEX

Isolated by me from two cases of a chronic ulcerative dermatosis of the leg with gumma-like lesions and open sores which are practically neither painful nor tender. The clinical

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picture somewhat resembled sporotrichosis but no sporotrichum fungus was found in either the open or closed lesions. Instead, from both types of lesions a peculiar pleomorphic slime-producing organism was grown.

Cultural characters. — On primary isolation in glucose agar slants six to eight days old, kept at room temperature, the surface is covered with an abundant translucid film of slime in which opaque areas are here and there visible, especially when the culture is viewed in reverse. The colour of the slime is often pinkish.

In older cultures isolated, heaped, hardish colonies (withish, yellowish, reddish or red) may at times appear, and these may have some similarity to the so-called fruiting bodies of certain Myxobacteriales. The organism does not grow, or only very badly, on potato, rather scantily on CZAPEK's agar, not very abundantly on blood agar and usually not at all or very poorly on coagulated serum. It produces neither acid nor gas in any of the usual carbohydrates. In the first three weeks gelatine is not liquified, but partial liquefaction may then develop.

Liquid Media. — In SABOURAUD's maltose broth and in peptone water growth is fairly abundant at the bottom of the tube, the column of liquid above remaining clear. No pellicle, no ring. In old cultures strings of mucus may often be seen on shaking.

Pathogenicity. — Intraperitoneal injection of 2 ml. of a ten days' peptone-water culture (well shaken) kills a young guinea-pig in ten to twenty-one days. The peritoneal liquid is very scanty; it is mucoid and contains no leucocytes.

Microscopy. — In wet and stained preparations made from glucose-agar cultures, especially if these are made from the sediment, numbers of variously shaped bodies are seen.

1. « *Cryptococcoid bodies* ». — Numerous roundish or irregularly rounded cells occasionally budding and approxima-

tely 2-5 micron in diameter. In preparations stained and undiluted carbol-fuchsin (twenty-five seconds) a few of these are diffusely stained while some stain deeply at the periphery and very little or not at all in the centre, producing ring forms with one or two deeply staining granules. They are gram-negative constantly; only exceptionally an occasional cell may appear slightly doubtful. As a rule they do not stain with ZIEHL-NEELSEN, but occasionally minute portions appear to retain the fuchsin stain.

Microscopical examination of the cryptococcoid cells shows that they usually contain one or two (or four or more) ovaloid or roundish bodies which for convenience I shall call « endospores ». Some cryptococcoid cells may show one or more buds, and endospores will frequently be seen in these buds also.

2. « *Cystis* ». — Scanty and irregularly shaped among the cryptococcoid cells are larger roundish or ovaloid cells, 6-12 micron in diameter, with a thickish deeply staining wall and filled with numerous endospores. These structures may be referred to for the time being as « cysts » or « sporangia ». At certain stages of development, apparently the wall breaks and the spores come out.

Quite often one or more buds are noted on the wall, which originate strings of similar cells by repetition.

4. *Cryptococcus-like cells.* — These generally appear in glucose-agar cultures a few months after isolation. They are about 3-5 micron in diameter, perfectly round, occasionally sub-round the central portion of which is strongly gram-positive while the peripheral portion is gram-negative. They are capsulated. They may become the prevalent type and in fact almost the only type present. After a time, however, they may again become rare.

5. *Coccoid bodies.* — Some months after isolation often extremely numerous small coccoid cells are present which to the non initiated may appear as common cocci. They are in reality endospores which have been freed from the dissolution of the « cysts ».

6. *Elongated forms.* — Thick bacillary fusiform and somewhat crescentic cells may be seen.

7. *Transition forms.* — These are frequent and of many diverse shapes.

8. *Motile forms.* — These are not very rarely observed. They may be of different size and shape. Some are minute globular bodies, possibly freed endospores which have become motile. Occasionally a cell is seen about the size of the smaller cryptococcoid cells presenting amoeboid changes in its contour (no true pseudopoda emitted) and also peculiare turning over movements with or without translation movements. Electron microscopy, not yet available to me, will probably throw light on the nature and structure of these motile cells and their means of locomotion. The usual methods of staining for flagella and cilia have given unsatisfactory and conflicting results owing probably to the large amount of slime present.

9. *Filamentous and branching forms.* — These I believe are probably artefacts due to inspissated mucus. Some of them, however, closely simulate true mycelial hyphae and pedicellate and non-pedicellate conidia.

Classification. — This is extremely difficult. One of the expert taxonomic mycologists to whom I sent cultures replied that the he could not give the organism any taxonomic position in the generally accepted classifications of fungi and bacteria, and ended his letter: « I confess I have never seen anything

like it before. » I have received practically the same reply from the other five well known mycologists and bacteriologists who were supplied with cultures.

Is it a *symbiosis*? Repeated attempts to separate the various forms by plating and replating and other procedures have so far failed completely in my hands and in the hands of all other worker. It is therefore probable that we are dealing with one single, most pleomorphic organism which may pass through different stages of development. I venture to express again the opinion that the organism presents certain features found among the myxomycetes (Slime Fungi) such as the abundant production of slime, the variously shaped forms, the presence of motile cells and possibly motile spores. As, however, true plasmodia *sensu botanico,* that is to say multinucleated, motile, protoplasmatic masses with no distinct wall have never been found, it cannot be placed among the myxomycetes. If Fungi (Mycota) are given divisional status as done by AINSWORTH and others with two subdivisions, Myxomycotina and Eumycotina, it may be found necessary perhaps to create a third subdivision. Taxonomic work, however, is better left to the experts on taxonomy. For practical purposes, however, and especially for purposes of reference, it is useful to give it a name, and the organism might be continued to be referred to as *Myxomicrobium multiplex.* — the first term of the binomial indicating the production of slime and the second its extreme pleomorphism, almost suggesting a symbiosis.

MYXOGEOTRICHUM FILARIOIDES

This organism was isolated by me 1964 from an adult Portuguese affected with psoriasis. The condition was fairly typical on the body but on the scalp several crusty somewhat rupoid-looking lesions were present. On removing the crust

from one of these a scanty amount of mucoid exudate was seen which was immediately inoculated into three glucose-agar tubes and one glucose-agar plate. It was probably a case of what the old dermatologists called Psoriasis empyodes although in my case the exudate was mucoid and not at all purulent.

Within a few days a number of colonies appeared in the inoculated media, all of the same type — rather large, roundish, mucoid, translucid, non-pigmented. No colonies of Staphylococci or other common skin bacteria developed, while they did abundantly on the media inoculated with the crust. Six of the colonies on the glucose-agar plate were replated and once more all the colonies which developed appeared to be the same type macroscopically and microscopically. The further investigation of the organism was carried out on similar lines as those followed in the investigation of *Myxomicrobium multiplex*.

Cultural Characters. — Ahundant mucoid growth occurs on glucose-agar, plain agar and many other media and substrata including sterile glass, but in contrast to *Myxomicrobium multiplex* no pinkish or red or other pigmentation is ever noticed. When a strain is subcultured daily or every other day for a time the amount of mucus produced may be truly enormous. Old cultures which have not been transplanted frequently usually lose their mucoid appearance. They are white, never pigmented.

The organism grows well on blood agar and coagulated serum, the latter being rapidly liquefied within a week. Gelatine is also rapidly liquefied. The rapid liquefaction of coagulated serum and gelatine is in contrast to what one notes in *Myxomicrobium multiplex* which does not liquefy serum and liquefies gelatine only very slowly, seldom if ever before three weeks. Litmus milk becomes partially decolourised and peptonised with often some small whitish coagula at the bottom of the tube, reaction alkaline. None of the usual sugars, viz. glucose, maltose, galactose, saccharose, lactose are fermented with prod-

uction of gas, but fairly strong acidity is generally produced in glucose, maltose, saccharose and lactose. Work on pathogenicity is not completed. The organism seems to be non-pathogenic to guinea-pigs and rabbits.

Microscopy. — In wet and stained preparations from a glucose-agar culture a number of different forms may usually be seen which for convenience can be classified as follows: 1) geotrichoid and bacillary; 2) globular and subglobular; 3) elongated (ampulliform, fusiform etc.); 4) long filiform or filarioid; 5) other forms and transitional; 6) motile.

Geotrichoid and Bacillary forms. — These are always present in large numbers; they are straight or somewhat curved cells of 4-8 micron in length and 1.5 to 2.5 micron in breadth (dimensions may vary a great deal) with fairly often square ends as seen in the segments of true *Geotrichum* fungi. Dark ground observation shows that many of these cells are encased in a capsule, and that externally to the capsule there may be a translucid reticular, almost invisible involucrum. Electron microscopy, not available to me, will throw light on the subject.

At times short chains of two or three or four or a few more geotrichoid cells are seen and occasionally ofter complete disarticulation these cells become arranged in a zig-zag pattern as seen not rarely in the arthrospores of true *Geotrichum*.

A number of geotrichoid cells contain vacuoles and also some refringent microspherules often arranged in two or three parallel lines. These refringent spherules are not fat, nor would it appear granules of common reserve materials. They may be considered, I am inclined to believe, endospores (microsendospore) using the term *sensu lato,* and each cell containing them might be considered in reality a microsporangium. Not very rarely one comes across a ghost cell in which the cytoplasm has disappeared but the microspherules remain intact and very visible and in those cases when even the outline of the ghost

cell has disappeared they remain intact, situated in two or three rows, later to become scattered.

In addition to the geotrichoid cells, much thinner bacillary cells are at times seen which stain diffusely and do not show any spore-like bodies.

Gram staining gives variable results, this being due probably to the large amount of mucus in which the cells are embedded.

Globose and Subglobose Forms. — Tyo types may be distinguished. Some are clearly geotrichoid cells which after complete disarticulation have become round or roundish like the free arthrospores of true *Geotrichum* fungi. The other type is represented by round or roundish bodies with deeply staining wall, they resemble the cysts or sporangia of *Myxomicrobium multiplex* but are of much smaller dimensions. They are filled with microendospores which hardly take the stain.

Elongated Forms. — Ampulliform, fusiform, barrel-like forms may be seen. A fairly frequent one is the « screw worm » form which but for the enormous difference in dimensions reminds one on first inspection of a fly larva.

Thread-like Forms. — Interspersed among the geotrichoid cells a few scanty unbranched filaments are seen, 100-200 micron and much more in length, having an angulliform or serpentine aspect. These long filaments with very few or no Septa are generally tapering at one or both extremities, and at the tapering end often show a minute spicule, or a minute bacillus-like, or a minute globular formation which does not take the stain or takes it very badly; it probably represents the initial stage of a new filament.

Other Forms. — Large, somewhat amoeboid, vaculated or not vaculated immobile forms are at times seen and also, in stained preparations, some bizare, multi-angular or asteroid forms. They are in reality masses of condensed slime embedding one or two free cells or a close aggregation of cells. It must

be kepts in mind also that slime, especially in films fixed at the flame, may give rise to most diverse and most bizare artefacts.

Rarely, *branching forms* are seen which may possible be true branching mycelial hyphae surrounded by sline and generally non-septate. It must be kept in mind that filaments of mucus may closely simulate true mycelium in preparation fired at the flame and stained.

Motile forms are similar to those noted in *Myxomicrobium multiplex*. Some are extremely minute, one micron or less, and move about in a peculiar jerky way. Others are of somewhat larger dimensions, 2-3 micron, and a few even larger, 4-6 micron in diameter, and may show amoeboid changes in their outline but do not emit true pseudopoda. The possibility of some of the minute forms being motile free endospores comparable to zoospores or swarm spores, cannot be ignored, but much further investigation is required including electron microscopy which has not yet been available to me.

Classification. — The remarks made on the classification of *Myxomicrobium multiplex* apply in a general way to the new organism which also appears to have certain features found among the Myxomycetes (Slime Fungi). Its most distinctive characteristic is the large number of free geotrichoid cells, mostly capsulated, with here and there a few free long, usually unbranched, flexuous, anguilliform or serpentine filaments which may perhaps bear some resemblance to the elatria of certain Trichiales. The constant absence of true plasmodia (*sensu botanico*) does not permit, however, placing it in that order or in any other category of the Myxomycetes. In fact, it would appear that this organism, similarly to what is the case with *Myxomicrobium multiplex*, can hardly be given a taxonomic position in the present-day generally accepted classifications of fungi and bacteria. The possibility of a *symbiosis* cannot be discarded with absolute certainty but the

constant failure to separate the various forms by all the procedures tried makes the hypothesis unlikely.

The organism, although possessing some features in common with *Myxomicrobium multiplex,* differs profoundly from it in its morphological and physiological characters; suffices to mention again the geotrichoid appearance of its cells and its strong proteolytic action. There can be little doubt that in any attempt to classify the two organisms they will have to be given separate generic and specific positions.

Notwithstanding all the taxonomic difficulties and the impossibility of applying strictly the code of botanical/ nomenclature, I believe it might be useful in practice, and especially for purposes of reference, to give it a name, and I would suggest as an apt one *Myxogeotrichum filarioides* — the first term of the binomial indicating its production of slime and microscopic morphology, and the second indicating the presence of the long free sinuous hyphae with a filiform appearance.

I shall be glad to send cultures of *Myxomicrobium multiplex* and the new organism to laboratory investigators desiring them.

FIG. 1 — First case from which *mycomicrobium multiplex* was isolated.

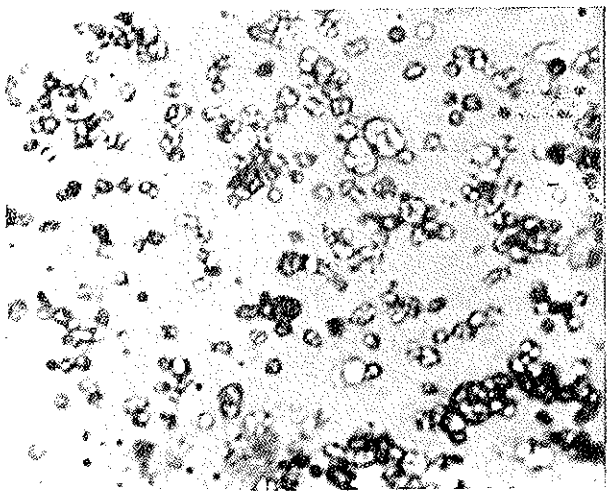

FIG. 2 — *Myscomicrobium multiplex*. Preparation from glucose-agar culture obtained with undiluted carbol-fuchsin. Many of the white spots which look like vescicular endspores which harea hardly taken the stain.

FIG. 3 — Preparation from a glucose-agar culture stained with undiluted carbol-fuchsin. Note variously shaped cells including amoeboid, cryptoccoid and bacillary ones.

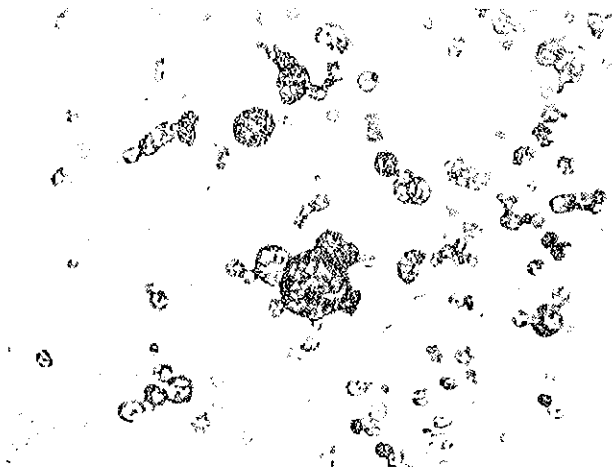

FIG. 4 — Preparation from glucose-agar culture stained with undiluted carbol-fuchsin. Note in the center a large cyst with several buds.

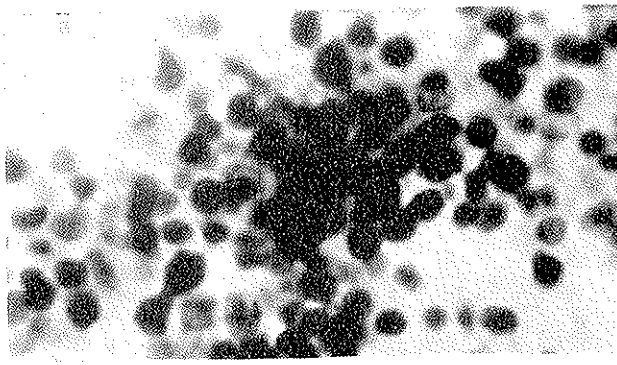

FIG. 5 — *Myxomicrobium multiplex*. Preparation from glucose agar culture stained by Gram's method, note large aggregation of cryptococcus-like cells kept together by slime. Approx. 1.200.

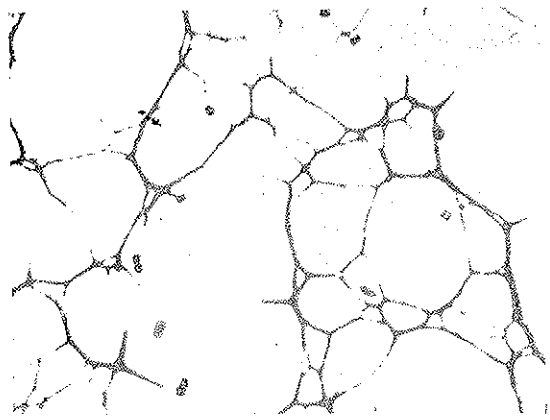

FIG. 6 — Mucoid reticulum simulating mycellium. A few cryptococcoid celles present. Approx. × 100.

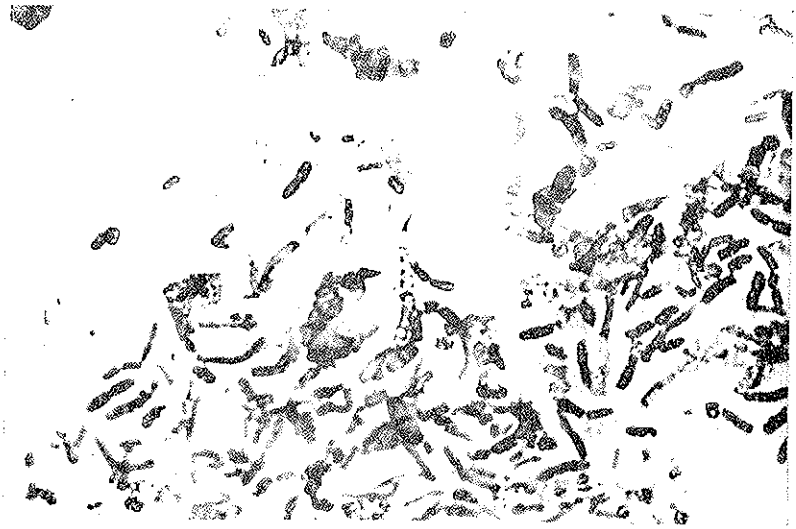

FIG. 7 — *Myxogeotrichum filarioides*. Preparation from glucose agar culture stained with undiluted carbol-fuchsin. Geotrichoid and other cells. Approx. 1.000.

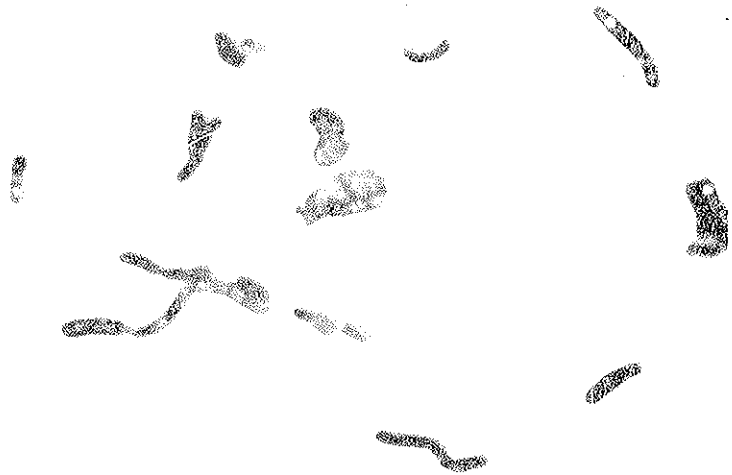

FIG. 8 — *Myxogeotrichum filarioides*. Preparation from glucose agar culture stained by undiluted carbol-fuchsin. Geotrichoid and other cells. Approx. 1.200.

FIG. 9 — *Myxogeotrichum filarioides*. Portion of a filarioid hypha and Various cells. Approx. 1,000.

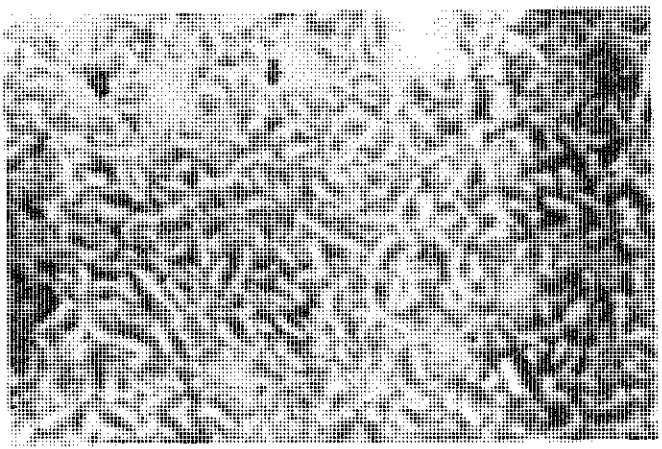

FIG. 10 — Myxogeotrichum filarioides. Note capsulated cells. Approx 1,000.

FIG. 11 — Detail of Microphotograph under dark ground illumination showing well capsulated cells.

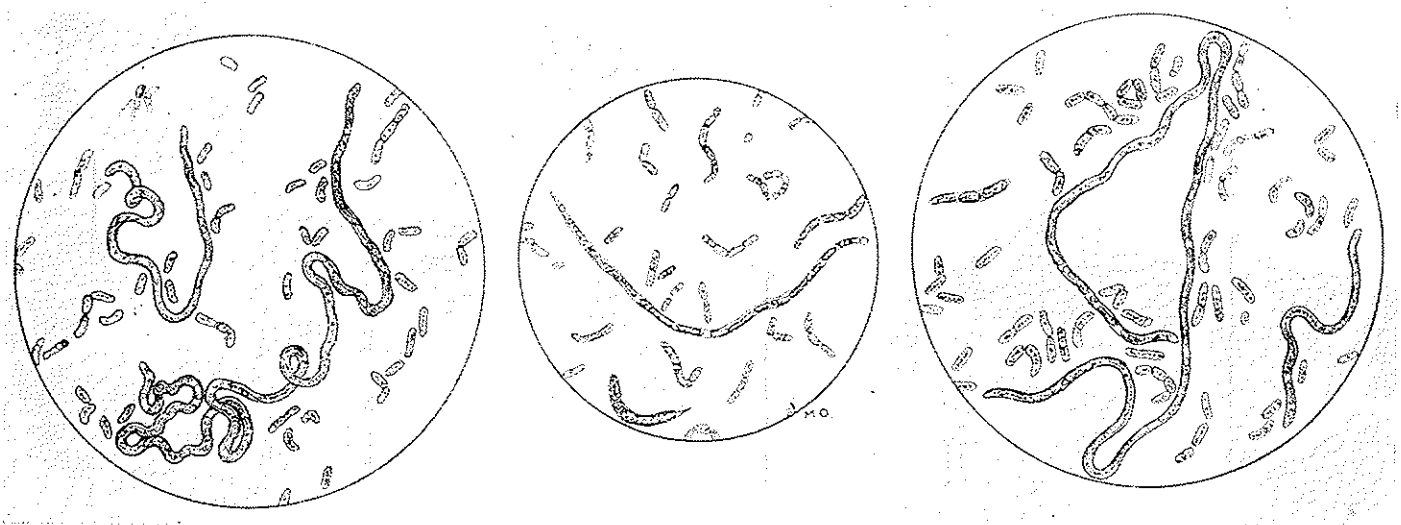

FIG. 11 — *Myxogeotrichum filarioides*. Photocopy of three composite drawings by Miss M. Ottolini. Note variety of forms including filarioid hyphae.

REFERENCE

CASTELLANI A. (1964), *A peculiar pleomorphic slime organism associated with a gummatous-ulcerative dermatosis*. Lancet, July 11, 1964, pp. 72-73.