A CUTANEOUS INFECTION CAUSED BY A NEW FUNGUS, PHIALOPHORA VERRUCOSA, WITH A STUDY OF THE FUNGUS.*

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INTRODUCTION. — The literature on the higher fungi pathogenic for man is large, but for the most part is of relatively little value for three reasons: 1, a fairly large proportion of the cases reported were diagnosed, without cultural or histological study, simply by the examination of pus or by the clinical appearance of the lesion; 2, in a number of the cases the lesion was not recognized until it was impossible to obtain cultures, and diagnosis was based entirely upon the appearance of the fungus in the tissue; and 3, in only a part of those cases in which cultural and histological examinations were made was a careful morphological and cultural study of the fungus attempted. These conditions make it difficult to determine whether a fungus from any given case has been observed before, and also increases the difficulty of properly classifying it. Moreover, the classification of pathogenic fungi is, on the whole, very unsatisfactory, because those that have been observed are only imperfectly known.

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The large majority of fungus infections of man, other than hair and superficial skin infections, are placed under two groups: (a) sporotrichosis and (b) blastomycosis. Under the latter, oidiomycosis and coccidioidal granuloma may be included. The first group has been fairly well systematized through the work of de Beurmann and Gougeroti. The latter class of cases has been studied by a number of authors, but at present constitutes a very ill-defined series of cases. Obviously this condition can be overcome only by the careful cultural and morphological study of the fungi causing the lesions.

The present case is reported because of the opportunity it offered for study of the fungus in tissue and in culture. It shows very clearly that laboratory diagnosis should be resorted to in certain types of chronic skin lesions, and also that all fungi which appear as yeast cells in tissue are not necessarily of the same variety of fungus.

GROSS DESCRIPTION. — The specimen received for histological examination consisted of two small, elliptical pieces of tissue. The smaller piece measured 2 x 1.5 centimeters x .5 centimeter in depth and consisted of epidermis and subcutaneous tissue. In the central portion of the epidermal surface there was an irregular, raised, finely nodular, grayish-brown area which measured one centimeter in greatest diameter. The cut surface showed considerable elevation in this region and was opaque and of a bluish-gray color. The rest of the cut surface was of a uniform grayish color. There did not appear to be any marked increase of epidermis.

The larger specimen resembled the smaller except that it was two centimeters thick and the nodular area on the epidermal surface was not so prominent. On section soft, gelatinous, bluish-red tissue eight to ten millimeters in diameter was seen to extend downward from the lesion on the surface to a depth of .5 to one centimeter into the subcutaneous tissue. The diameter of this gelatinous tissue corresponded roughly to the nodular area seen on the

surface. The epidermis did not seem to be much thickened at the edge of the lesion.

The gelatinous tissue seen in the larger specimen was, in all probability, young granulation tissue formed largely after cauterization of the sinus.

HISTOLOGICAL PATHOLOGY. — The cellular reaction toward the fungus resembles very much a typical, so-called blastomycetic lesion. There is a moderate increase of connective tissue in which the inflammatory reaction varies from acute to chronic in type. This reaction is found for the most part in the corium, but is also found to a slight extent intra-epidermally.

In the regions where the acute inflammatory reaction predominates, the exudate consists chiefly of polymorphonuclear leucocytes and a deposit of fibrin with an occasional endothelial leucocyte and eosinophile. As a general rule, in some portion of these miliary abscesses one or more organisms are present.

The more marked chronic inflammatory reaction consists of collections of endothelial leucocytes and foreign-body giant cells with occasional eosinophiles and lymphocytes. In these regions the fungus occurs singly or in clumps, in giant cells, endothelial leucocytes or free in the tissue. The milder chronic inflammatory reaction consists mainly of lymphocytes, plasma cells and eosinophiles with an occasional endothelial leucocyte or polymorphonuclear leucocyte present. In these areas degenerating forms of plasma cells and of eosinophiles containing basophilic or acidophilic hyaline-like droplets are fairly common. Single organisms or small groups of the fungus are commonly found free in these areas of mild inflammatory reaction.

Sections of the tissue stained for tubercle bacilli give negative results.

CULTURES. — Before the larger piece of tissue was surgically removed cultures were taken with aseptic precautions

from the depths of the sinus. The material obtained was planted upon hydrocele agar, Loeffler's blood serum, dextrose agar and potato. The cultures were incubated at 37° C. with the following results:

Hydrocele agar. — At the end of forty-eight hours a few isolated colonies of Staphylococcus aureus and albus had developed. Nothing further was noticed until the sixth day, when three pin-point, grayish black colonies were seen. Upon microscopical examination these were found to be composed of radiating septate hyphæ which had a distinctly brownish wall. These colonies gradually increased in size until at the end of three weeks the largest colony measured four millimeters in diameter. They were, at this time, round and of a brownish-black color. The surface of the colonies was moist, slightly convex, slightly raised above the surface of the medium, and appeared to be finely striated radially. The growth extended into the medium from one to two millimeters. The colonies were very tenacious. No aerial hyphæ were produced. The medium was colored diffusely, being chocolate brown near the colonies, and gradually changing to a light sepia brown.

Loeffler's blood serum. — The cultural results were very similar to those of hydrocele agar — five pin-point, black colonies appeared on the sixth day. The fungus growth was not so profuse as on hydrocele agar, the largest colony measuring only two millimeters in diameter. Except in size they appeared exactly like the colonies on hydrocele agar. The colonies were firmly incorporated into the medium, which was colored a brownish black around the growth. The serum was not liquefied. No aerial hyphæ were produced.

Dextrose agar. — A single pin-point, black colony was seen on the fifth day. At the end of three weeks the colony measured five millimeters in diameter and was brownish black in color. The surface of the colony was at first convex and later umbonate, slightly cerebrated, and rather dry in appearance. By the end of the second week there was

an abundant production of grayish aerial hyphæ which extended for a distance of about one millimeter above the mycelium. Later the aerial hyphæ became mouse-colored, or purple-tinted brown. The colony grew a slight distance into the medium and was very tenacious. The medium was colored a deep sepia brown for a considerable distance around the colony.

Potato. — Three colonies were first noticed on the sixth day and by the end of the third week measured four millimeters in diameter. At this time they very much resembled small, round pieces of a gray mouse's fur. The production of aerial hyphæ was much more abundant than on dextrose agar. By the end of the fifth week they had become a purple-tinted brown color. This change in color of the aerial hyphæ, as in the case of dextrose agar, probably takes place with the maturing of the hyphæ and conidial formation. The colonies were firmly incorporated into the surface of the medium, which was slightly discolored in the adjoining area.

Transplants were made from these media to various other culture media. While the fungus grows slowly at best, it grows equally well on all ordinary media. The most abundant aerial hyphæ production is found on six per cent glycerine agar, wort agar, carrot and potato, although aerial hyphæ production takes place to some extent on all the solid media used except hydrocele agar and Loeffler's blood serum. Where conidia are planted, the individual conidia produce isolated colonies which after a time may become fused and form a thick, black, felt-like mycelium.

In litmus milk the fungus grows well in isolated colonies and after a time forms a thick, brownish-black mass of mycelium. No pellicle is formed. No aerial hyphæ are produced. The milk is not coagulated or peptonized and gradually is changed to a strongly alkaline reaction.

In Dunham's peptone solution the fungus grows as isolated brownish-black colonies which resemble chestnut burrs in appearance. No indol is produced. No pellicle is formed

and no aerial hyphæ are observed. The colonies have a tendency to cling to the sides of the tube and to remain separate. The medium is colored a light brown to a chocolate brown color in old cultures.

In plain and sugar bouillon the growth appears like that in peptone solution. Aerial hyphæ are not produced and no pellicile is formed.

The fungus does not ferment lactose, maltose, saccharose, mannite, inulin or levulose. Slight acid production occurs in dextrose and dextrin. Titration for the percentage of acid production was made difficult because of the diffuse coloration of the bouillon. The titrations were made with two-week old bouillon cultures. In inulin, mannite, levulose, and maltose the fungus grows poorly.

MORPHOLOGY. — Unfortunately there is at present a certain laxness in the use of botanical terminology in the morphological description of pathogenic fungi. For this reason it seems advisable to define what is meant by some of the botanical terms used in this paper. The term conidium is used to designate an asexual exogenous spore. The term sclerotic cell is used to designate the atypical enlarged cell produced under unfavorable or abnormal conditions, as, for instance, in tissue. The term sclerotium is used to designate a group of sclerotic cells which have been produced by the septation of one or more sclerotic cells in more than one plane. This septation has, in some instances at least, probably been mistaken for endogenous spore formation.

Tissue. — In the tissue the fungus appears in two forms: (1) sclerotic cell; (2) budding form or conidium. The sclerotic cells vary considerably in size, measuring from eight to fifteen μ in diameter. When a sclerotic cell has undergone septation the sclerotium thus formed may measure twenty to twenty-five μ in diameter. The individual sclerotic cell has a heavy brownish-black wall which is thicker in the larger cells. The wall varies somewhat in thickness in individual cells.

The protoplasmic content of the cell varies, but is usually finely granular in appearance with an occasional irregular, coarsely granular area. At times there are a few small, oval vacuoles present. These are probably fat droplets. There is no distinct nucleus discernible. A clear space, probably an artefact due to shrinkage in the process of fixation, is usually present between the capsule and its contents. The sclerotic cell may remain single, or by a process of septation in more than one place, be divided into two, three, four, and perhaps more cells. The sclerotium thus formed is probably analogous to such formations of various saprophytic and parasitic fungi when living under unfavorable conditions. It should not be considered asexual endogenous spore (conidia) formation.

Conidia may arise either from the non-septate sclerotic cells, or the individual cells of the sclerotium. At the point where the conidium is to be formed the wall ruptures and a blunt protoplasmic process is protruded. This enlarges and after a time is separated from the parent cell by the production of a definite septum. Another conidium is then started, and so on until a chain of conidia composed of several units (five in one instance) may be formed. On the other hand, only a single conidium may be formed. These conidia are considerably smaller than the parent cell at first, but gradually enlarge until they are approximately the same size or even exceed it. They may then pass through the sclerotic cell stage and complete the life cycle of the fungus in tissue as far as can at present be determined.

Hydrocele agar and Loeffler's blood serum. — The morphology of the fungus on these two media is described separately because it is on these media that sclerotic cells similar to those observed in tissue are found. The growth of the fungus here consists of a mycelium which is made up of a tangled mass of septate branching hyphæ. The hyphæ are, for the most part, fairly straight and cylindrical and are composed of cells which range from eight to twenty-five μ in length and from two to six μ in diameter. At the periphery

of the growth the lateral branches of the hyphæ are more numerous and are composed of from one to four or five cells. It is in this region, and for the most part in the depths of the medium, that the sclerotic cell formation is found.

The morphological appearance of a single cell is characteristic of the mycelium as a whole. Each cell has a structureless, thick, brownish wall which encloses the protoplasmic content. In fresh mounts the internal structure is composed of a finely granular protoplasm in which refractile droplets of various sizes are embedded. These droplets show considerable Brownian movement, but no true movement is seen. By staining fresh preparations with Scharlach R. or weak carbol-fuchsin the droplets take a bright red stain, showing that they are probably of a fatty nature. By staining fixed specimens with hematoxylin a definite nuclear structure is brought out in practically every cell.

The sclerotic cell formation takes place entirely in the younger portion of the culture, that is at the periphery. It is produced, for the most part, from the end cell of the short lateral branches of the hyphæ, but may be formed by any cell in these branches or near the tip of the main hyphæ. To form the sclerotic cell, a gradual enlargement into a round or ovoid cell from eight to fifteen μ in diameter takes place. In case the sclerotic cell is formed from a non-terminal cell the enlargement is usually toward one side. At first the wall of the sclerotic cell is very thin but as the cell ages it becomes thick and of a brownish black color. As a rule, the sclerotic cells are divided into two, three, four or even more cells by a process of septation in more than one place before the marked thickening of the wall takes place. In this way small sclerotia are formed. The structure of these cells is practically the same as the structure of the sclerotic cells found in tissue.

From the individual cells of the sclerotium or more rarely from a non-septate sclerotic cell, chains of conidia may be produced. The conidial formation here resembles a process

similar to that observed in tissue. On the other hand, the sclerotic cells can produce typical hyphæ and aerial conidial formation if placed under favorable conditions.

The production of sclerotic cells is begun in cultures eight to ten days old and is most abundant in cultures three to six weeks old. The conidial formation is found to a slight degree in cultures three weeks old, but much more commonly in those six weeks old. No sclerotic cell formation was observed in any of the other media used. The cause for sclerotic cell formation evidently does not depend upon anaërobiosis, but rather on the presence of some substance, such as serum, in the medium.

Other culture media. — The only important differences in the morphology of the fungus on hydrocele agar and blood serum and on other culture media are the absence of sclerotic cells and the presence of aerial conidial formation on solid media. As far as can be determined no conidial formation takes place in the various liquid media used.

Conidial formation takes place most commonly on one or two cell lateral branches of the aerial hyphæ, but it may occasionally occur at the end of the hypha itself. The cells of these lateral branches are shorter, broader, and more ovoid than the cells of the aerial hyphæ.

The beginning of conidial formation is shown by the presence of a fine, blunt protoplasmic projection from the distal end of the sporogenous cell. This bud gradually enlarges until it reaches the dimensions of a mature conidium, when a septum is formed between it and the germinating cell and another bud is pushed out. During the maturing of the first conidium the wall of the germinating cell is extended outwards for a short distance and forms a shallow cup. Into this cup the successive conidial buds are projected. In this manner a semi-endogenous conidial formation is produced.

The conidia, instead of falling onto the medium when separated from the parent cell, remain until a spheroid mass containing a varying number (at least sixteen in some instances) has accumulated. The conidia are probably held

together by a mucinous substance, as no capsule can be seen and the conidia tend to remain in clumps or adherent to the hyphæ when separated from the cup. At first this globular mass of conidia was looked upon as a true sporangium filled with endogenous spores, but further investigation failed to substantiate this view.

The individual conidium is ovoid, has a distinct wall which is yellowish at first and brownish later, and contains numerous small, glistening globules embedded in a finely granular protoplasm. The glistening globules are of a fatty nature as shown by a Scharlach R. stain. With a hematoxylin, or eosin and methylene blue stain, each conidium is seen to possess a definite nucleus. The conidia range from four to six μ in length and average about two μ in diameter.

Technic. — Smears of the fungus made in the ordinary way stain well with all routine bacteriological stains, although too diffusely in most instances to bring out cellular detail. Smears stained in dilute methylene blue for ten minutes or longer and then thoroughly washed in water gave fairly good cellular detail. Scharlach R. and dilute carbol-fuchsin stain the glistening globules seen in fresh mounts a bright red. As a rule, the young cells retain the Gram stain while the old cells vary in their staining properties.

The best results for morphological study of the fungus were obtained by embedding cultures in celloidin or paraffin and then staining cut sections. The cultures may be fixed either in Zenker's fluid or in alcohol. In this way the normal relation of the sclerotic cells and the conidia to the hyphæ can be obtained, if the cultures are carefully handled.

The best stains for cultures fixed in Zenker's fluid are eosin and methylene blue, phosphotungstic acid hematoxylin and Mallory's iron hematoxylin. The phosphotungstic acid hematoxylin is of especial value, as it decolorizes the brownish wall and renders cellular detail more distinct. For cultures fixed in alcohol, Mallory's iron hematoxylin and alum hematoxylin are both equally good. For staining the

fungus in tissue the routine eosin and methylene blue stain is very satisfactory.

ANIMAL INOCULATIONS. — Guinea-pigs, mice, and rats have been inoculated with cultures of the fungus. The fungus is not pathogenic for guinea-pigs. The reaction produced is no more than any foreign body would cause, and the lesion is not progressive.

In mice and rats subcutaneous and intraperitoneal inoculation of cultures of the fungus causes progressive lesions. Intraperitoneal inoculation causes the formation of tubercles on the omentum and peritoneum. These tubercles attain the size of a small grain of sand in four to six weeks. The fungus can be recovered from the tubercles. The tubercles are made up of connective tissue, endothelial leucocytes, foreign body giant cells, and occasional lymphocytes. The hyphæ and sclerotic cells are found in various parts of the tubercles. In some instances the sclerotic cells resemble very closely those seen in the human lesion. These sclerotic cells were not inoculated, for the young bouillon culture used showed no such forms.

Subcutaneous inoculation causes the formation of a small localized abscess. In the pus the fungus is readily found and cultures of the pus give an abundant growth. The lesion is progressive but very slow in forming, taking from six to ten weeks to cause a discharge of pus.

From these inoculation experiments it is seen that the fungus is capable of producing a local, chronic, progressive infection as in man.

CLASSIFICATION. — The proper classification of a fungus is very difficult unless the sexual as well as the asexual mode of reproduction is known. However, for the sake of reference, fungi which are at present only partly known, as far as their perfect forms are concerned, should be classified as accurately as possible. From a botanical view-point the points to be considered in classification are: (1) type or

types of spore formation; (2) nature of mycelium produced; (3) color.

In looking over the description of the various pathogenic fungi that have been reported, the only one which resembled the fungus here described was Sporotrichum Gougeroti. The cultural characteristics are practically identical. The morphology corresponds in all respects except the mode of conidial formation. It is very doubtful if Matruchot would confuse the conidial formation of sporotrichum with the semi-endogenous conidial formation which is so characteristic of this fungus.

The fungi grouped by various authors under the head of "blastomycetes," "saccharomycetes," "oidiomycetes" or "coccidioides" resemble this fungus to a certain extent, as far as the forms in tissue and the formation of septate, branching hyphæ are concerned. But the conidial formation is distinctly different from that reported for the various fungi in these groups, and none of these groups of fungi has a distinctly brown-black color, a characteristic which is constant for this fungus.

As far as can be determined there has never been described a fungus, either saprophytic or parasitic, which corresponds closely to this fungus.

In classifying the fungus the Saccardo classification is followed. The fungus can best be placed in the family Dematiaceæ because of its thickly walled, branched, septate, brownish hyphæ, and its general type of conidial formation. The types of conidial formation correspond to those of the subdivision Chalareæ. However, there is no genus or species in this subdivision which is characterized by the collecting of the semi-endogenous conidia into a sporangium-like mass. Because of this characteristic, which differs from the other fungi comprising this subdivision, Professor R. Thaxter has suggested that a new genus be created with this species as the type species. For the generic name the term Phialophora (small shallow cup-bearer) is proposed. As the lesion produced clinically resembles verrucous tuberculosis, the specific name of verrucosa is selected.

A diagrammatic representation of the classification follows:

Branch Thallophyta.
- Class A. The Algæ.
- Class B. The Fungi.
  - Order I. Myxomycetes.
  - Order II. Phycomycetes.
  - Order III. Ascomycetes.
  - Order IV. Basidiomycetes.
  - Order V. Hyphomycetes.
    - Family I. Mucedinaceæ.
    - Family II. Dematiaceæ.
      - Division I. Phaeosporæ.
        - Subdivision XIV. Chalareæ.
          - Genus 1. Conioscypha.
          - Genus 2. Chalara.
          - Genus 3. Thielviopsis.
          - Genus 4. Cirromyces.
          - *Genus 5.* Phialophora, *nov. gen.*
            - *Species 1.* Verrucosa, *nov. sp.*

CONCLUSIONS.

The study of this case has led to the following conclusions:

1. Chronic cutaneous lesions cannot always be properly diagnosed clinically.

2. For this reason, cultural studies and histological examination of refractory skin lesions should be made. Such examinations will, at times, aid in the proper diagnosis and prognosis of the case and will give a clue to rational treatment.

3. Yeast-like cells in tissue probably represent a variety of pathogenic fungi.

4. Careful morphological and cultural study of pathogenic fungi is important before classification of a fungus is undertaken. Had this not been done in this case, the fungus would have been classified as Sporotrichum Gougeroti.

5. This fungus forms conidia in tissue and certain media (hydrocele agar) by a budding process from sclerotic cells. On aerial hyphæ, on other culture media, the conidia are

produced by a semi-endogenous budding process from a special sporogenous cell.

6. A new fungus, Phialophora verrucosa, is added to the list of fungi pathogenic for man.

7. The fungus is slightly pathogenic for rats and mice; non-pathogenic for guinea-pigs.

8. The source of infection is unknown. For prophylaxis the natural habitat of the fungus should be determined.

[In conclusion I wish to express my appreciation for the many helpful suggestions and the generous encouragement given so freely by Dr. F. B. Mallory; for the interest and kindness of Prof. R. Thaxter of Harvard College in aiding properly to classify and name the fungus; and for loans of cultures of sporotrichæ and coccidioides from Dr. S. B. Wolbach and from Dr. D. J. Davis of the University of Illinois, Chicago.

This study was made possible through the kindness of Dr. C. Guy Lane who referred the patient to the laboratory for diagnosis. The case will be reported by Dr. Lane from a clinical view-point.]

EXPLANATION OF PLATES XXIX–XXXIII.

(Figures 1 to 10 inclusive are photomicrographs, made by Dr. F. B. Mallory, of sections of tissue and of cultures.)

PLATE XXIX., Fig. 1, x 200. — Shows general type of reaction. Note miliary abscesses and numerous foreign body giant cells.

Fig. 2, x 500. — Small tubercle in the epidermis. One giant cell with three organisms in it.

PLATE XXX., Fig. 3, x 500. — Miliary abscesses with organism in central portion. Exudate largely polymorphonuclear leucocytes.

Fig. 4, x 1000. — Giant cell with numerous organisms in it. One shows septum across it. Exudate around giant cell consists largely of endothelial leucocytes.

Fig. 5, x 1000. — Giant cell with numerous organisms in it. One organism shows a bud (young conidium). Another organism shows a septum.

Fig. 6, x 1000. — Giant cell in which there are several organisms. There is a chain of five conidia attached to the parent cell present. The end conidium is out of focus.

PLATE XXXI., Fig. 7, x 75. — Section of six weeks old culture on glycerine agar showing the aerial hyphæ, many of which have fructifications. The black part is the surface of the medium.

Fig. 8, x 75. — Section of five weeks' old colony on hydrocele agar showing sclerotic cell formation. These sclerotic cells are found in the depths of the medium at the periphery of the colony.

PLATE XXXII., Fig. 9, x 2000. — Aerial hypha showing numerous typical sporogenous cells. Notice the small cup at the distal end of the cell and the sporangial-like collection of conidia. The photograph also shows the projection of the protoplasm of the cup into the cup at the end. The septa in the hypha also show.

Fig. 10, x 5000. — Hyphæ from a four weeks' old colony on hydrocele agar. The septate hyphæ and sclerotic cell formation is well shown. Some of the sclerotic cells show beginning conidial formation.

PLATE XXXIII., x 1500. — Drawings to illustrate the morphology of the fungus in tissue and in culture. Figs. 1 to 16 from stained preparations. Fig. 17 from unstained preparation.

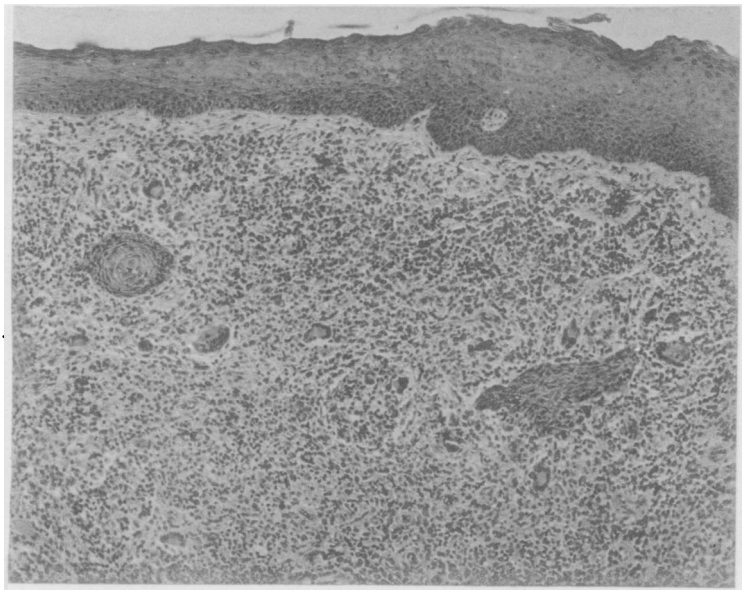
Fig. 1. — Individual conidia adherent to aerial hyphæ — an artefact.

Figs. 2, 3, and 4. — Sclerotic cell formation and septation, and conidial formation as observed in hydrocele agar. Fig. 2 shows chain of conidia arising from the septate sclerotic cells. Note the nuclei and vacuoles due to fat droplets in segments and conidia and not in sclerotic cells.

Figs. 5 to 13 inclusive. — Conidial formation and accumulation on sporogenous cells. Figs. 6 and 9 are optical sections to show the mode of conidial budding. Fig. 10 shows eight, Fig. 12, sixteen conidia. Fig. 11 shows the separation en masse of the conidia from the cup and a bud projecting into the cup.

Figs. 14, 15, and 16 show the morphology as seen in tissue. The clear zone in the organism is an artefact. Fig. 14, a septate sclerotic cell with a chain of conidia from one portion. Fig. 15, a single sclerotic cell in an endothelial leucocyte. Fig. 16, a septate sclerotic cell in a foreign body giant cell. A conidial bud is seen on one of the cells.

Fig. 17. — Shows the morphology of unstained hyphæ. Note the thick wall, the septa, and the fat droplets.



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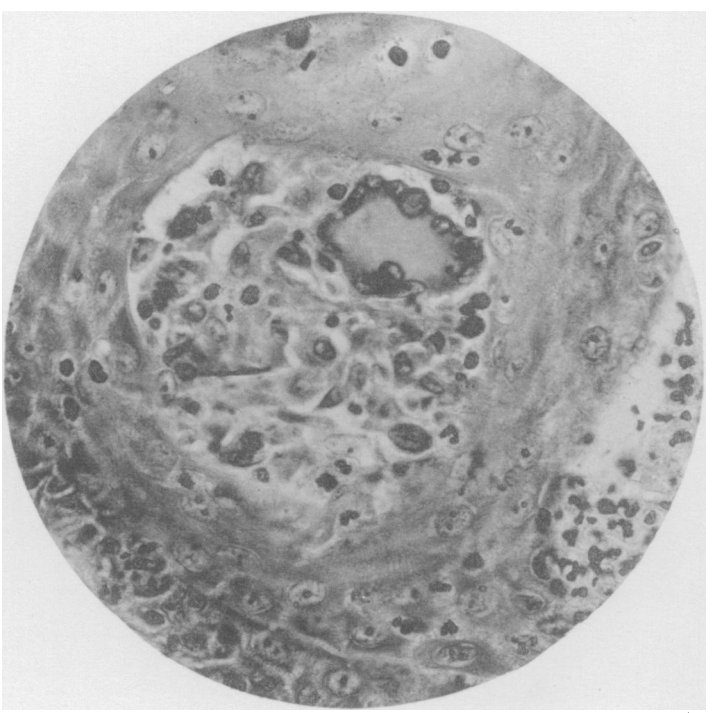

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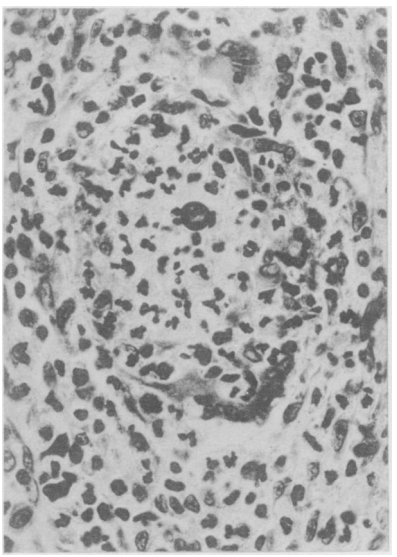
Medlar

Phialophora verrucosa



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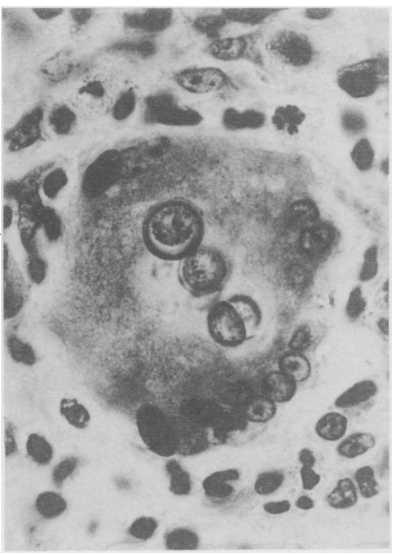

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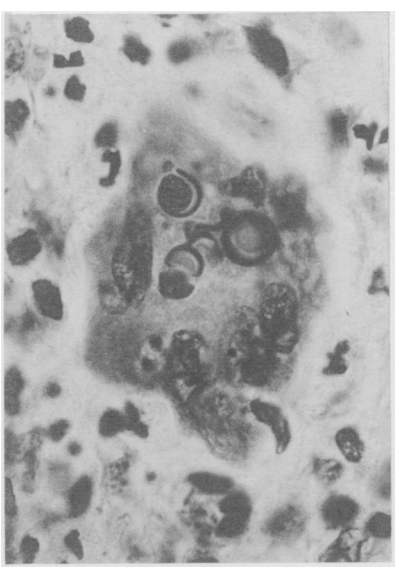

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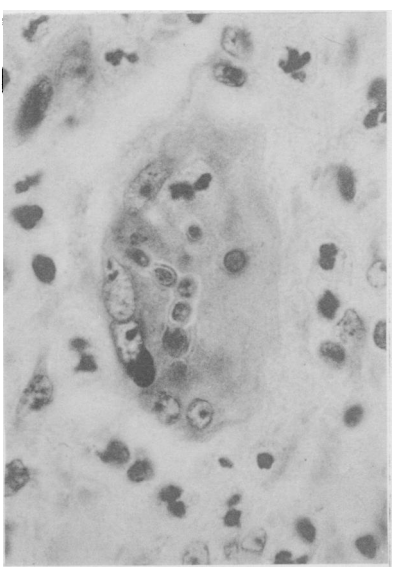

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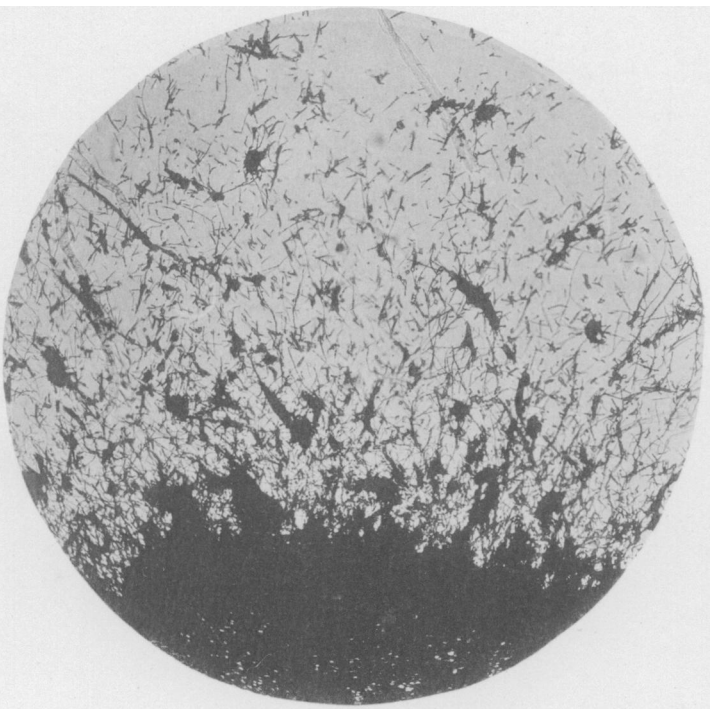
Medlar

Phialophora verrucosa



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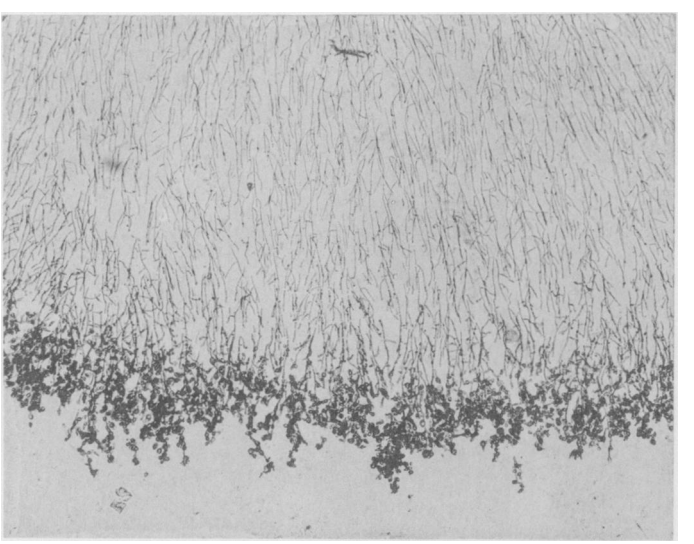

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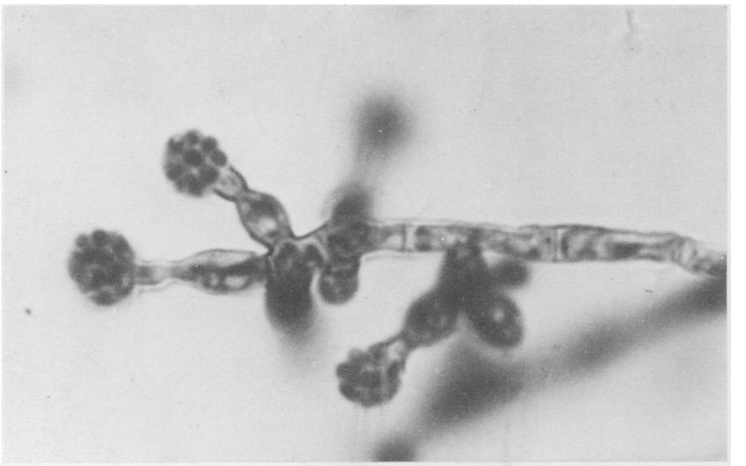
Phialophora verrucosa



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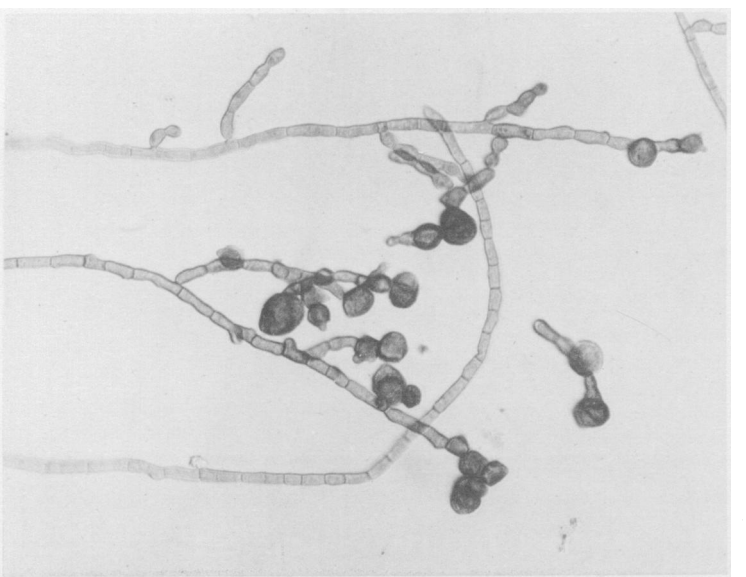

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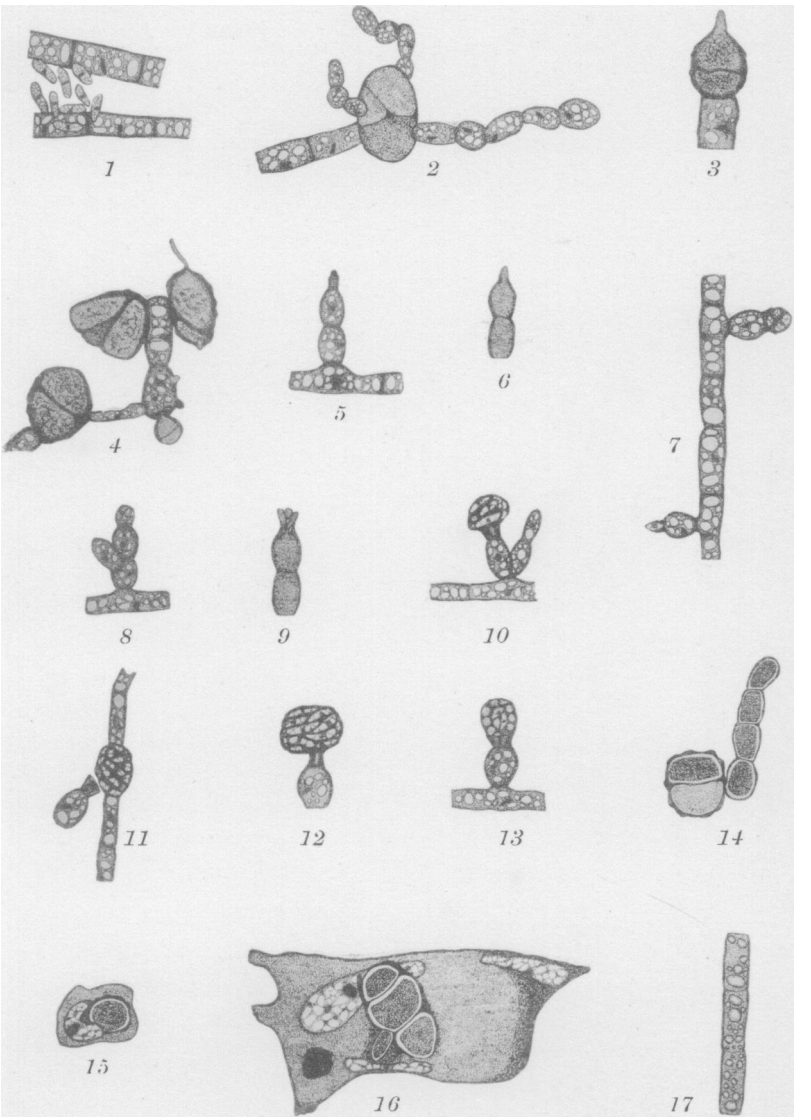
Medlar Phialophora verrucosa



Medlar Phialophora verrucosa