THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Bertha Cecelia Fineman final oral examination for the degree of Master of Arts . We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

June 10 1920 Arthur T. Henrici

Chairman

Josephine E. Tilden

E. J. Bell

W. P. Lawson

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Bertha Cecelia Fineman for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

Arthur T. Hamrici
Chairman

C. P. Parsons

E. T. Bell

A. H. Sanford

June 10 1920

THESIS

A STUDY OF THE THRUSH PARASITE.

A Thesis submitted to the

Faculty of the Graduate School of the

University of Minnesota

by

Bertha Cecelia Fineman

In partial fulfillment of the requirement

for the degree of

MASTER of ARTS

June

1920

A STUDY OF THE THRUSH PARASITE.

Introduction.

The disease called thrush, which especially manifests itself in the form of white patches in the mouths of children, has been known since the days of Hippocrates. At that time thrush, together with all other infections of the mouth membrane, were called "Aphthae". Thrush though primarily a children's disease is also common in debilitated people either as an acute or chronic infection. Oliver[22] describes a case of the tongue of twelve years duration. We hear of it in adults sick with tuberculosis, typhoid, diabetes, and on the mucous membrane of the vagina of pregnant women.

It appears in the form of white cheesy-like patches on the mucous membrane. The surrounding area is reddened and the mucous membrane underneath is ulcerated. A piece of this cheesy-like mass reveals under the microscope a mixture of dead epithelial cells, yeast-like cells, and long mycelium.

It is claimed by some that the mycelium is capable of penetrating the tissue, entering the blood, and of forming metastatic infections. Virchow[34] claimed to have discovered thrush mycelium in the submucous tissue of the oesophagus. Zenker[37] mentioned metastatic infections in the brain of an old man, who suffered with thrush of the mouth. Schmorl[30] carefully described a case of metastatic infection in the kidney of a nine year old girl.

In 1839 the thrush organism was discovered by Berg[2] and recognized as the agent which caused the thrush disease. Robin

in 1847 classified the parasite as an Oidium and named it Oidium albicans, a name it has held most constantly up to the present day. The discovery led immediately to botanical studies of the organism. Many works have been published. Unfortunately, however, one realizes upon reading these, that there are important aspects upon which there is as yet, no agreement. A glance into bacteriological text books reveals but meager descriptions of this organism.

Points upon which reports vary most widely are (a) in regard to its morphology and botanical position, and (b) in regard to the unity or plurality of species.

A. Morphology.

I. General Morphology.

Microscopically the thrush organism may appear in yeast-like or filamentous form. The filaments are simple or branched. They are definitely septate showing thick cross walls. The cells contain protoplasm, vacuoles, granules, nucleus and fat globules. The contents in young cultures are homogeneous. In older cultures they are vacuolated. Simple filaments may give rise to short globular buds at their sides. These buds may in turn elongate and form branches, and these branches may again bud and again branch out. This often leads to such an interlacing of filaments that it is impossible to follow any one filament.

The formation of filaments according to Linossier[20a] may take place by two distinct processes.

1. Sometimes a bud appears on the cell which separates off immediately by a manifest septum. It does not remain round but lengthens becoming the segment of a hypha, and reproduces other segments by a similar mechanism.

Or 2. the initial yeast cell may push out a prolongation like a finger of a glove which is not separated by a septum and retains the same protoplasm.

The yeast globules may be round or oval 4-9mic.in diameter. They bud actively. As a rule, the buds separate upon attaining mature size and form new daughter cells. Sometimes they remain attached presenting the appearance of beads or bouquets.

In the false membranes or "plaques" found in the mouth, the yeast-like cells are found attached to the filaments, or free. The attachments are lateral and terminal and look so much like conidia that many of the authors use the term "conidia" in speaking of the globular forms.

In cultures the organism appears either in the pure yeast form, or in the pure mycelial form(though this is very rare) and frequently in both forms. The appearance of any or both these forms depends upon the composition of the media and other factors which will be discussed later.

II. Botanical Classification.

The first obstacle which presents itself in the study of the parasite is that of classifying it and giving it a true botanical position. Since its discovery the organism has been classified as follows:

Sporotrichum by Gruby[13]

Oomphylium polymorphum by Hallier[15]

Mycoderma Vini by Grawitz[12]

Dematuim albicans by Laurent[19]

Mucor by Linossier and Roux[20a]

Syringospora by Quinquad,[24]

Saccharomyces by Guidi[14], Reess,[26] Brebeck[4] Fisher[7], and Audry[1]

Endomyces Albicans by Vuillemin[35]

Oidium lactis albicans by Robin[28]

But as, Plant points out, Gruby[13] named it Sporotrichum because of the side ectospores, but almost all fungi contain ectospores and we would therefore, have to name all fungi sporotrichum. Stemphylium has brown or black spores and in no way resembles thrush. Mycoderma vini is out of the question because it is non-pathogenic. Thrush differs from Hematium in the manner of spore formation. The mere finding of chlamydospores in thrush is not sufficient to name it Mucor, for chlamydospores are common to many fungi. The distinct septation of the thrush filaments separates it from the Phycomycetes. Its action toward chemicals, its pleomorphic forms, and the presence of chlamydospores removes it from the Saccharomyces.

The problem narrows down to the question of classifying the organism in the genus Endomyces or the genus Oidium.

According to Stevens[32] the genus Endomyces belongs to the family Endomycetaceae, order Saccharomycetales. The characteristic features of the family are a mycelium usually well developed, producing a luxuriant growth, multiseptate; asci borne singly on branches or intercalary 4-8 spored, and unicellular conidia produced apically. The genus Endomyces is characterized by asci 4 spored. The genus Oidium belongs to the family Oösporcae, order Moniliales, of the Fungi Imperfecte, since it produces no ascospores.

If the presence of asci and ascospores as mentioned by Fisher

and Brebeck can be verified we are justified in classifying thrush with the Endomyces.

The absence of these structures leaves for the organism no other classification but that of Oidium under Fungi Imperfecti.

Realizing the importance of the nature of the spores of thrush in its classification I paid special attention in the study of these.

III. Nature of Spores.

1. "Conidia"

The yeast-like globules or "conidial" form is surrounded by a membrane slightly thinner than that of pure yeasts. This membrane gives no celluluse reaction. Within the membrane are cytoplasm and nucleus. The cytoplasm contains granules and vacuoles. Within the vacuoles can be seen the little dancing granules which are so characteristic of yeast cells. In young cultures the protoplasm is hyaline and homogeneous. In older ones it becomes vacuolated and granular. It takes the basic aniline dyes and retains Gram's stain.

The nucleus is small but can be distinctly seen with the iron haemotoxylin stain. The slide is fixed in Fleming solution, put over night into a 2 1-2% solution of iron ammonia alum, washed in water, placed in a 1-2% aqueous solution of haemotoxylin for four hours, washed and finally decolorized with the iron alum. This stains the nucleus brown very nicely. It is surprising to read Linossiers'[20a] & Roux's statements that they could detect no nucleus.

According to Linossier[20a] these globules are not spores but the vegetative phase of the plant, which can adapt themselves to all

sorts of media.

Vuillemin[35] believes them to have the functions both of spores and vegetative cells.

According to Daïreuva[7] the "conidia" bud when conditions are favorable; when unfavorable these are able to resist drying, abnormal temperature, and lack of food by changing into mycelium. This observation sounds reasonable.

2. Chlamydospores - These are described by Linossier and Roux as follows: "The form is characterized by certain filaments at extremities of which are spherical cells. These cells or chlamydospores are larger than the conidia, sizes ranging from 9-24 mic. They may also be found between two segments. They are very spherical, their protoplasm is more refringent and granular than that of the "conidia" or filaments. Their membrane is thicker. The protoplasm in the chlamydospores is at first finely granular and little refractile, later it becomes coarsely granular. The granules are either arranged like a necklace or bunched, surrounding a central hyaline globule. The surrounding membrane of the terminal cell thickens and becomes glassy. On squeezing, it always opens at the same point by a v shaped rent thru which the granules with the central globule escapes. Preceding this process glycogen accumulates in the preterminal cells, as shown by staining with iodine. These globules as they escape remain indefinitely in the media, but have been made to germinate on raw cherries. They are believed to be chlamydospores which germinate in order that the parasite may develop in new habitat."

The medium in which, according to Linossier, the chlamydospores appear constantly, consists of a liquid, each liter of which con-

tains saccharose 20 gms, ammonium tartrate 10 gm., potassium phosphate 1 gm., magnesium sulphate 2/10 gm. and calcium chloride 1/10 gm.

Plaut[23a] considered chlamydospores as involution forms of thrush. Burchardt[4] described some capsules which he found in emulsified false membrane. He described them as being round, 1/50-1/12 mm. in diameter, double contoured and full of small spores. He considered them to be sporangia. Hausman[16] and Hallier[15] also mentioned capsules which they called Sporangia. Grasset[11] found chlamydospores in old boullion cultures. Ostrowsky[6] found some in dextrose boullion cultures. Hickel[17] described them as round cells three times the size of the ordinary cells, full of reserve food and surrounded with a thick refractile membrane. Vaillemin[35] and Daïreuva[7] have found them in old cultures and believe that they arise when conditions are unfavorable, such as lack of nutritive media, presence of bacteria, or chemical influences. They observed their germination upon transplanting them from beets to broth.

I found them in Linossier's media in two of my strains.

3. Ascospores. Vaillemin[35] and Fisher and Brebeck[9] have described Ascospores. Vaillemin states that they are numerous in old cultures on various media and that asci 4-5mic in diameter contain four ascospores.

Fisher and Brebeck found them in five cases of thrush. The strains formed a pellicle on milk. Fourteen day old cultures of these showed endospores. They also isolated a nonpellicle forming kind from a sixth case of thrush but in this case they could find no endospores.

IV. Factors determining mycelium or unicellular form.

In infected tissue the two fold form of "conidia" and mycelium can be seen.

In artifical media we come upon the interesting phenomenon of obtaining the globular form alone, under certain conditions and of obtaining the mycelial or filamentous form under other conditions. All of the authors except Stumpf,[31] agree upon the existence of a globular as well as a filamentous form, but they do not all agree as to where and why each form appears.

1. Composition of Medium.

Grawitz,[12] in his research, used a liquid medium made by adding to dextrose solutions, ammonium tartrate and 2% of a mineral salt obtained from ashes. He used various concentrations of sugar and claimed that the greater the concentration of sugar in the medium, the more the organism takes on the yeast form.

Plaut[23e] stated that he obtained mycelium in sugar free nitrogenous media, and yeasts in rich sugar media.

Audry[1] has formulated the theory that solid media cause the growth of yeast form while liquid media cause the growth of the mycelial form. He planted the organism on lemons and obtained the pure globular forms, some free and some attached like pearls on a string. In boullion, he said the round cells became oval, elongated, and attached. Some gave rise to long filaments which bulged at one end. This bulging part pediculated in some cases and formed small adherent cells. The filaments were septate and rounded at their extremities.

Nearly all the authors are in agreement that yeast like cells alone appear on the surface of solid media, while mycelium

may develop in some liquid media. The most elaborate research upon this aspect has been done by Linossier and Roux.[20a] After a most thorough study of the organism upon a variety of media they have arrived at the theory that "the complexity of form of the thrush organism is proportional to the increase in the molecular weight of the food elements in the medium." As a proof of their theory they cite the following experiment:

They planted the organism in a mineral liquid consisting of water 1000 cc., potassium phosphate.75 gm., Mg. sulphate. 05gm. ferrous sulphate .02 gm.,zn. sulphate .02gm., a trace of sodium silicate and ammonium sulphate one gram and to this were added carbohydrates of various molecular weights. In the media of low molecular weight such as lactose, glucose, or glycerine, they obtained only yeast forms. In media of high molecular weight such as dextrin or gum arabic they obtained long abundant filaments.

They do, however, admit that this rule is not without exceptions; for they obtained filaments in acid, alkaline and nitrogenous media of low food value, and under conditions which were unfavorable such as lack of food. They explained the presence of yeast like cells upon the surface of their solid media, especially upon media of high molecular weight by saying that cells separated from the medium by other cells receive that medium by diffusion; only simple foods are diffusable and therefore, the organisms grow in the simple or yeast like form. Cells directly in contact with the medium, namely in liquid medium develop at its expense and consequently grow in mycelial form.

2. Aerotropism.

Hickel[17] adopted two theories. He accepted Linossier's

theory as"to the complexity of form in proportion to increase of food value" and carried out a similar experiment. He used a medium containing 500 gms. of water, .25 gms. of magnesium sulphate., .25 gms. of potassium phosphate, a trace of iron sulphate, and .25 gms. of ammonium sulphate.

To some of this medium he added monoses such as Glucose, Levulose, Fructose and Galactose.

To some more of it he added bioses such as Maltose, Lactose, and Saccharose, and to some he added two polyoses, namely dextrin and glycogen.

Into these he planted the thrush organisms. He says that he obtained mycelium in the bioses and polyoses but obtained no mycelium in the monoses.

He also believed that aerotropism brings about the mycelial forms. He said that all his stab cultures showed a characteristic curve; where the oxygen tension was high he obtained only the yeast form, where the tension was low,namely towards the bottom of the tube, he obtained the mycelial form.

He further illustrated this theory by the following experiment: He dissolved a tiny piece of sugar in several cubic centimeters of saliva. To this he added a small amount of the yeast like cells from a fresh malt culture. He then took a drop of this emulsion and placed it on a slide. The slide was covered with a cover glass and placed in a dish surrounded with wet filter paper so as to provide sufficient moisture. This was then placed in the incubator. At the end of twelve hours Hiekel noticed definite aerotropism. Toward the center of the drop were found mycelial forms for here the oxygen tension was low. At the

edges of the drop could be seen only yeast like forms.

B. Plurality or Unity of Species.

Stumpf[31] declared that the filamentous and the globular forms were two different species of fungi. However, no other observer has agreed with this view.

Fischer and Brebeck[9] claimed that there were two varieties One - a large-spored variety; it liquified beer wort gelatin, formed a pellicle on milk and wort and formed endospores. The other - a small spore kind; it does not liquify gelatin nor does it form pellicles or endospores.

Daïreuva[7] has come to the conclusion that "neither the microscopic, pathologic or cultural aspects warrant the establishing of specie differences."

Sugars have been found useful in classifying bacteria, such as the coli typhoid group. No attempt, that we know of, has been made to classify the thrush organisms by this means. The authors who have mentioned sugars are Cao,[5] Fisher and Brebeck,[9] Daïreva,[7] Trossier and Achalme[33] and Denecke.[8]

Cao in describing the thrush organism, best known in literature as Oidium Abbicans, stated that it attacked no sugars.

Fisher and Brebeck said that the liquefying variety fermented dextrose, levulose and maltose but not saccharose.

Daïreuva stated that the parasite fermented glucose, levulose and maltose, that it consumed dextrin mannite and glycerin without fermenting, did not utilize or ferment lactose, and utilized saccharose without inverting or fermenting. Denecke claimed that it fermented levulose, maltose, lactose but not saccharose.

Troisser and Achalme described a case clinically diagnosed as thrush. They did not, however, believe the organism isolated from the case to be the thrush parasite, but rather a yeast. One of the points upon which they based this decision was from the fact that their organism fermented saccharose and showed strong alcoholic fermentation. They believed that the thrush parasite did not do this.

Agglutination is another means in use in modern bacteriology for establishing specie differences. Noisette[21] has carried out some agglutination tests with his thrush cultures. He says that upon immunizing an animal with a thrush strain, the serum of that animal develops agglutinins, which agglutinate that specific strain which has been used for immunizing. He has tried this serum upon his various strains but has found that the serums will agglutinate their own specific antigens. He has, therefore, concluded that there is not only a single saccharomycis albicans, but an entire class, which contains varieties.

Roger[29] succeeded in obtaining agglutinins in immunized rabbits.

Recently Widal[36] and his colleagues in studying agglutination with the serum of sporotrichosis cases have discovered that the serum of patients, who suffered with thrush will agglutinate thrush "conidia" but only in low dilutions 1:10,- 1:50 and that the same serum agglutinates more markedly Sporotrichum spores in dilutions of 1:50 to 1:150.

This reaction with the spores of Sporothrichum Beurmanni is so constant that they were able to use it in the diagnosis of thrush.

With the exception of Noisette no other author has tried to establish the unity or plurality of species by means of agglutination.

Personal observations and experiments.

A. Morphology.

I began the study of the thrush parasite with two strains on hand, T2 and T9; the first from the Army Medical School, and the other from the university clinic. These were from clinically diagnosed thrush cases. Within six months there were added to this number fifteen more strains; two, T26 and T 27 were from vaginal cases in pregnant women; one, T13, from a case of conjunctivitis; three, T11, T12 and T M, were from typical cases of mouth thrush; one, T L, from an ulcer in the mouth; and the remainder, T14, T16, T18, T21, T22, T23, T24, T25, were obtained from throat cultures sent in to the laboratory of the State Board of Health. The latter were cultures taken for diagnosis of diphtheria. For purposes of comparison yeast strains were used. Of these Y1 was a cultivated yeast, Y2, Y3, Y4, and Y5 were wild yeasts and YG, Y17 and Y19 were yeasts isolated from the throat cultures sent into the laboratory of the State Board of Health. A Monilia, from a case of sprue, obtained from the Army Medical School, was also used.

Pure cultures of these strains were obtained by plating them out on dextrose-tartaric acid agar. This medium is made by adding to ten cubic centimeters of melted agar one cubic centimeter of a sterile dextrose tartaric acid solution (prepared by adding 50% of dextrose and 5% of tartaric acid to water, sterilized in the autoclave). The addition of the dextrose tartaric solution inhibits the growth of bacteria and makes easy the obtaining of pure cultures. A good growth is obtained within two days.

The surface colonies are round, wax-like, creamy, elevated and granular; while the deep colonies are irregularly surrounded with radiating mycelium. Some of these deep colonies are round and are surrounded with a fine branching mycelium so that they present a stellate appearance, others are torpedo shaped with the mycelium extending from one side.

On routine media my strains of the thrush organism gave no characteristic growth. They grew at the bottom of broth in flocculent form. Upon milk there was no change, nor did any pellicle form. On gelatin there was a smooth white growth on top. None of my strains liquefied maltose gelatin. On agar the growth was fine and whitish; on potato it was grey. The organism grows most easily and abundantly upon Sabourand agar. I also planted my strains, as well as some yeasts, upon carrots. Both gave a snow white growth. This, therefore, cannot be used for the differentiation of the thrush organism as Linossier[20b] claims. The morphology of the organism has been described in the earlier part of this paper. As endospores were not observed in the numerous strains and wet preparations which I made from various media, I tried the gypsum block method, hoping to obtain them that way. Plaster of Paris was hardened, slanted, fashioned to fit into glass tubes, moistened with peptone solution or distilled water and autoclaved. The various strains of the parasite were inoculated upon the slanted surface of the gypsum. No nutritive material was placed in the tubes for I wanted to create unfavorable conditions which would result in sporulation, just as is the case with yeasts. The tubes were kept at a temperature of 20° for several days.

At the end of that time it could be seen that the cells became oval, larger and swollen. They became free from granules, the dancing figures were enlarged, and the vacuoles distended so that they looked like spores. Careful spore stains, using Moeller's method, showed none. The vacuoles appear so much like spores that one can easily be misled into believing them to be such.

An attempt was also made to verify the observation of chlamydospores so carefully and elaborately described by Linossier. I prepared the medium which he recommended, namely, water 100 cc. ammonium tartrate 1 gram., potassium phosphate 1/10 gram, magnesium sulphate .02 gm., calcium chloride .01 gm. To this was added 3% saccharose. Into this medium, tubed and sterilized, I planted my strains. I found chlamydospores in two of them, namely, T L and T2. Their appearance corresponds to the description given by Linossier. They were spherical, enclosed by a heavy membrane. Within could be seen the highly refractile globule surrounded by a corona of tiny granules. The chlamydospores were at the extremities of short hyphae. I was unable to squeeze out the globules or observe the germination of these chlamydospores. Chlamydospores are common to many of the fungi, therefore, the finding of these is of no great significance in aiding in the classifying of the organism.

The complete absence of ascospores, or in fact, of any sort of endospores in all of the strains studied, no matter what the medium, leads me to believe that such structures are not formed by the thrush parasite and that it should, therefore, be retained in the genus Oidium rather than the genus Endomyces.

Chlamydospores are not typical for any genus. They are common to many fungi, hence the finding of them in two of the strains does not affect the classification. I believe them to be a resistant form of spore arising when conditions become unfavorable.

B. Pleomorphism of organism.

Thruout the experiments I found, as a rule, yeast-like cells upon the surface of the solid media. However, there were exceptions. Some of the old agar slant cultures developed deep radiating mycelium. Certain of my dextrin and dextrose agar slants upon being subjected to anaerobiosis (an experiment described a little later) showed mycelium.

Liquid media, as a rule, showed the mycelial form. Here again were exceptions. I have found only yeasts in Linossier's dextrose liquid medium and in another medium devised by Retteger[27] and containing: Water 1000cc., sodium chloride 4 gm., KCl, 1 gram., $M9SO_4$ .2gm, $CaCl_2$ .05 gm., KH_2PO_4 1 gram., $(NH_4)_2$ PO_4 1 gram., glycerine 30 gm. As already stated mycelium was also found in the solid media.

The addition of carbohydrates to the liquid media showed marked results. Linossier's medium was used, to which were added dextrose, galactose, or dextrin. These were made up in liquid form, or solidified with agar. The solid media gave only the yeast-like cells. Of the liquid media dextrin and galactose gave profuse mycelium and dextrose gave only the globular form. Galactose is of about the same molecular weight as dextrose and therefore, in accordance with Linossier's theory, should give the yeast cells only. I have also obtained mycelium in sugar free media such as plain broth and peptone. These results seem to show that sugars

have some influence upon the formation of mycelium, but not to the extent to which Linossier would have us believe.

Two other experiments which I have carried out seem to indicate that other factors besides the composition of the medium influence the morphology of the organism. These experiments were carried out to observe the influence of oxygen, and the surface tension of the medium.

1. Oxygen tension experiment.

Linossier's mineral liquid medium was made up with dextrose, dextrin and galactose, and as dextrin and dextrose agar slants. Upon these were planted five strains, namely, T9, T12, T26, TM and T16. The tubes were immediately placed in jars which were connected by means of glass tubing to a flask containing calcium carbonate. Just before adding hydrochloric acid to the calcium carbonate for the purpose of generating carbon dioxide, there was added to each jar some pyrogallic acid and sodium hydroxide solution. Carbon dioxide was then passed through the jars. When a match would no longer burn in the gas escaping from the last jar in the series, the apparatus was disconnected and the glass tubing quickly sealed. The jars together with some controls grown aerobically, were placed in the incubator. After two days the cultures were examined. Table C shows the results. It shows that anaerobiosis brought about a mycelial growth on the solid media of most of the strains planted. There are some exceptions, and it is these exceptions that make it hard to assume that anaerobiosis is the only explanation for the two-fold form of the thrush parasite. The experiment does, however, show that the oxygen tension of the medium may influence the growth of the organism.

Surface tension experiment.

The work of Larson, Cantwell and Hartzell[18a] in this laboratory upon surface tension has given interesting results with bacteria. It was thought that the surface tension of the medium might have some influence on the growth of the thrush parasite, so the following experiment was performed. Linossier's liquid medium was again used. The surface tension was depressed by the addition of castor oil soap prepared as described by Larson, Cantwell and Hartzell.[18a] The dextrin solution without the soap had a surface tension of 50 dynes. The addition of 1% of a 2% solution of the castor oil soap depressed the tension to 44 dynes. The galactose medium had a tension of 48 dynes, and 43 when depressed as above. Dextrose changed from 58 to 43 dynes. Seven strains, T9, T12, T16, T.26, T M, T2, and T L were inoculated simultaneously into tubes of these media, both of normal tension and of low tension as described, and incubated at 37°. Table C shows the results after two days' growth. It shows that in the case of dextrin the filamentous form was present in the controls, while yeast-like forms alone appeared when the tension was depressed. The results were most striking and definite. They are presented in Plate I.

With galactose, however, no such differentiation was observed, mycelium appearing in fluid of low tension as well as in the normal control tubes, while with dextrose also the addition of soap was without influence, the yeast form alone appearing in both series of tubes. After making these microscopic observations the tubes of each of the inoculated media both those of the normal tension and those of low tension were centrifuged and the surface tention of the clear supernatant fluid was read.* The results are

*The surface tensions were determined by Mr. Green on Dü Nuoy's apparatus.

shown in the following table:

Uninoculated normal medium	Uninoculated depressed medium.	Inoculated normal medium	Inoculated depressed medium
Dextrin 50 dynes	44 dynes	54.5 dynes	48 dynes
Galactose 48 "	43 "	55 "	49.5 "
Dextrose 53 "	43	58 "	46.5 "

It will be seen that the growth of the organism in every case raised the surface tension of the medium. This increase, however, is much more marked in the case of galactose and dextrose than with dextrin, and is probably to be explained by the acid produced from the simpler sugars, which will precipitate soap.

Unfortunately lack of time prevented carrying these studies further. It is quite clear however, from the work done that a multiplicity of factors determine the form which the thrush parasite shall assume. In general we may state that the yeast or unicellular form occurs in the optimum media. The organism grows most rapidly when the medium contains an abundance of the simpler fermentable carbohydrate. It is aerophilic and produces a more luxuriant growth on the surface of solid media than in the depths of liquid media. The unicellular form, the cells being spherical or oval, offers a smaller surface in proportion to the volume of the protoplasm than the cylindrical mycelium but also affords the most rapid means of reproduction and dissemination of the organism.

It would seem that the yeast-like form is, therefore, the optimum form and that the mycelial form is only assumed in media poor in oxygen, or in readily assimilable carbohydrates; this form possibly being better adapted by virtue of its larger surface for

absorption and respiration.

C. Unity of Species.

Bacteriology's task of differentiating species of bacteria has been greatly aided by the use of sugars and agglutination tests. I resorted to these means in an attempt to determine the unity or plurality of species of thrush organisms.

Sugar broth media were made by dissolving one gram of the sugars to each 100 cc. of beef extract broth, using Andrade's indicator. The following carbohydrates were used at first: dextrose, maltose, lactose, saccharose, mannite, glycerin, salicin, inulin, galactose, arabinose, levulose, amygdalin, xylose, erythrite, raffinose, dulcit and starch. Into these were planted some strains of the thrush organism and some strains of yeasts as controls. Table A shows the results obtained. They seemed encouraging, for whereas the yeasts showed a variety of fermentations, the thrush strains presented uniform results. A fresh lot of medium was made. The list of sugars was limited to dextrose, galactose, levulose, maltose and saccharose since upon the other sugars there was no action. Again the thrush strains fermented the same sugars. Whenever I obtained a new strain of clinically diagnosed thrush I planted it in the sugars, and invariably obtained the same results. All of the strains studied fermented the following sugars with the results indicated. Dextrose acid and gas, galactose acid without gas, levulose acid and gas, maltose acid and gas, saccharose acid without gas.

On repetition of the experiment with all of the strains after several months' cultivation, the same results were obtained. From this experiment it is clear that the thrush parasite is constant

in its sugar reactions and that these reactions do not indicate a plurality of species. All of the yeasts studied gave sugar reactions different from those of the thrush strains, so that the sugar reactions can be utilized for the identification of the Oidium albicans. They are the more useful in that typical mycelium is formed very readily in many of the sugar broths, most constantly and abundantly in galactose.

Agglutination tests.

Vaccines were made from several strains as follows: Ten cubic centimeters of a sterile 0.8% salt solution with 0.25% of tricresol was added to each of the Sabouraud agar slants. The growth was gently emulsified. The emulsions were transferred into clean tubes. They were heated for one hour at 56° in the water bath. A little of each emulsion was planted upon a fresh medium and inspected the next day for growth. If they showed growth the emulsions were again heated and tested. Rabbits were then immunized with these vaccines. Two cubic centimeters of vaccine was injected intraperitoneally into a rabbit. The rabbits were given four such injections, three days apart. One week after the last injection they were bled. The serums collected were used for microscopic agglutination tests in dilutions of 1:10, 1:20 and 1:50. The emulsions used as antigens were prepared by scraping the growth from Sabouraud agar slants into salt solution. This gave emulsions which slowly sedimented but became uniformly turbid on very slight agitation. After setting up the agglutinations they were incubated at 37° fortwo hours and then placed in the icebox over night. In the first experiment the following strains were used for immunization: T2, T2, T9, T11 and TL. The same strains were used as anti-

gens. Serum T L agglutinated the homologous strain and strain T 9, both in a dilution of 1:20. Serum T9 agglutinated its homologous strain at a dilution of 1:50 and strain TL at 1:10. Otherwise no agglutination occurred.

After one more injection the serum of the rabbit inoculated with T9 was used for a further experiment using in addition to the yeast and thrush strains mentioned two emulsions of spores of Sporotrichum Schenckii. These were both isolated from cutaneous cases of sporotrichosis.

A further study was made using the serums of the two cases of vaginal thrush with their homologous strains and with emulsions of Sporotrichum spores, with negative results.

It would appear from these experiments that agglutinins are not formed in sufficient quantity either in experimentally inoculated animals or in clinical cases of thrush to be of diagnostic or differential value. I am unable to confirm with Sporotrichum Schenckii the observation of Widal[36] with Sporotrichum Beurmanni.

Summary and Conclusions.

1. Seventeen strains of the thrush parasite proved identical and constant in their morphological and cultural characters. They all corresponded to the non-liquefying type of Fischer and Brebeck.

2. Carbohydrate media were fermented uniformly and constantly by all strains. They are of value in the identification of the species.

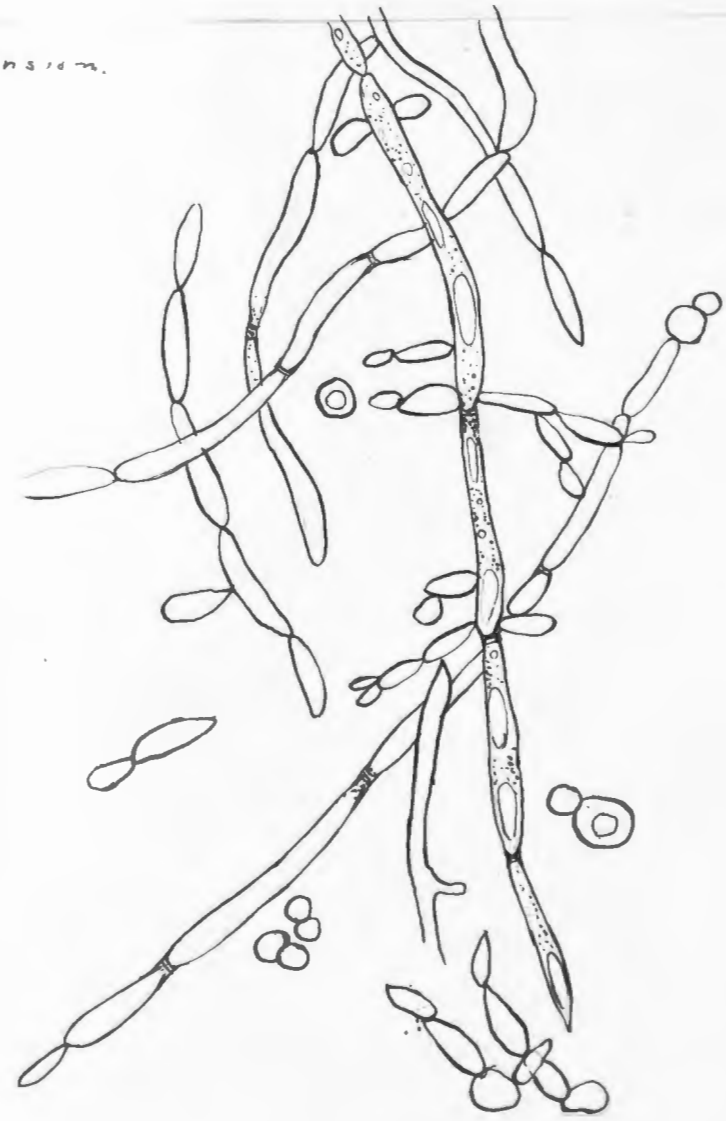
3. Agglutinins are not produced by the thrush parasite in sufficient quantity to be of diagnostic or differential value.

4. The thrush parasite produces chlamydospores but not asco-spores. It is correctly placed in the genus Oidium.

5. The organism tends to assume mycelial form in liquid media, in media containing complex carbohydrate, in media of low oxygen tension, and in media of low surface tension, while the unicellular or yeast-like form occurs in solid media, in the presence of simple carbohydrates, an abundance of oxygen, or media of higher surface tension. These factors may be interellated while other factors as yet unknown may affect the morphology. It is suggested that pleomorphism of this organism is an attempt at adaptation, the mycelial form developing in relatively unfavorable conditions.

Plate I.

(A) Normal tension.



(B) Depressed tension.

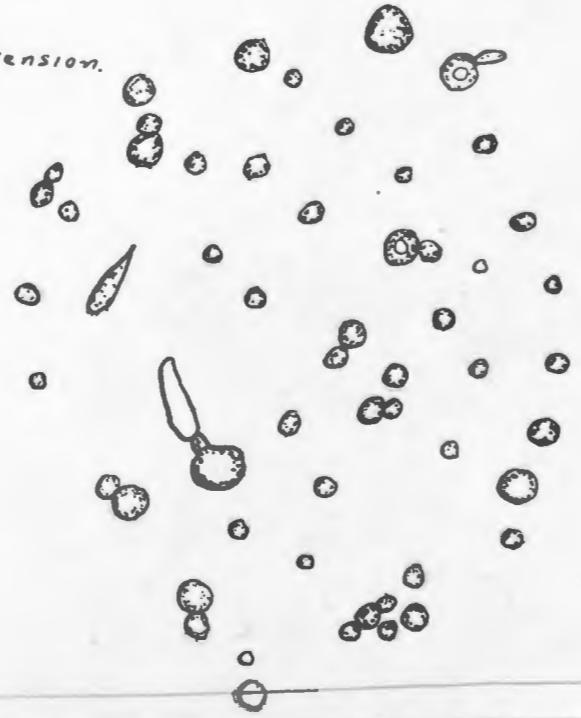


Table A

SUGAR RESULTS

	Arabinose		Amygdalin		Dextrose		Dulcit		Erythrite		Galactose		Glycerin		Inulin		Lactose		Levulose		Maltose		Mannite		Raffinose		Saccharose		Starch		Sulcin		Xylose	
	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas
Y1	+	−	+	−	+	+	−	−	−	−	+	+	+	−	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	−	+	−
Y2	+	−	−	−	+	+	−	−	−	−	+	−	+*	−	+	−	−	−	+	+	+	+	+	−	+	−	+	+	+	+	−	−	−	−
Y3	−	−	+	−	+	+	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−
Y4	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−
Y5	+	−	+	−	+	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	−	−	−	+	−	+	+	+	−	−	−	+	−
T2	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
T9	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
T11	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
TL	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
TM	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
Results from Spirit	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−

N.B. 'Y' indicates yeast strains

'T' indicates thrush strains

Table 8

SUGAR RESULTS

	Dextrose		Galatose		Lactose		Levulose		Maltose		Mannite		Raffinose		Saccharose		Presence of Mycelium
	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas	
YG	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Y17	+	+	+	+	−	−	+	+	+	+	−	−	+	−	+	+	−
Y19	+	−	+	−	−	−	+	−	+°	−	−	−	−	−	−	−	−
T2	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T9	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T11	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T12	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T13	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T14	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T16	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T18	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+°
T21	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T22	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T23	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T24	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T25	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T26	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
T27	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
TL	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
TM	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	−	+
Manilia from Sprue	+	+	+	+	−	−	+	+	+	+	−	−	+	−	+	+	+

N.B. 'Y' indicates yeast strains

'T' indicates thrush strains

Table C

LINOSSIER'S MEDIA

	AEROBIC					ANAEROBIC					LIQUID					
	SOLID		LIQUID			SOLID		LIQUID			NORMAL TENSION			LOW TENSION		
	Dextrin	Dextrose	Dextrin	Galactose	Dextrose	Dextrin	Dextrose	Dextrin	Galactose	Dextrose	Dextrin	Galactose	Dextrose	Dextrin	Galactose	Dextrose
T 9	Y	Y	M	M	Y	M contaminated	M	M	M	Y	M	M	Y	Y	M	Y
T 12		Y	M	M	Y	M	M	M	M	Y	M	Y?	Y	Y	M	Y
T 26		Y	M	M	Y	M	M	M	M	Y	M	M	Y	Y	M	Y
T M		Y	M	M	Y	tube broken	M	M	M	Y	M	M	Y	Y	M	Y
T 16		Y	M	M	Y	M	M	M	M	Y						
T 2											M	M	Y	Y	M	Y
T 21											M	M	Y	Y	M	Y
T L											M	M	Y	Y	M	Y

N.B. 'Y' indicates yeast cells
'M' indicates mycelium

BIBLIOGRAPHY.

1. Audry, - Sur l'evolution du champignon du muguet. Rev. de med., 1887.
2. Berg, - Ueber die Schwämmchen bei Kindern, Bremen. 1842.
3. Baginsky, - Ueber Soorkulturen. Deutsche med Wochenschr. 1885.
4. Burchardt, - Charite - Annal, 1864, quoted by Plaut.
5. Cao, - Oidien und Oidiomykosen, Zeitschr. f. Hyg., 34 Bande, 1900.
6. Charrin and Ostrowsky, - L'Oidium Albicans, agent pathogene general, Soc. de biol., Paris 1896.
7. Dafreuva, - Rech sur le champignon du muguet et son pouvoir pathogene. Nancy 1899.
8. Denecke, -Deutsche Zeitschr. f. Chir. Bd. 62. 1902 - quoted from Plaut.
9. Fischer and Brebeck, - Zur Morphologie, Biologie und Systematic der Kahmpilze, der Monilia Candida und des Soorerregers, Jena, Gustav Fischer, 1894.
10. Galli Valerio, - Zur une variete' d' Oidium albicans isolee des selles d'un enfant, Arch. de parasit., 1898, T. 1, Nr.4.
11. Gasset, - Arch. de med. exper., 1893 - quoted by Plaut.
12. Grawitz, - Ueber die Parasiten des Soors, des Favus und Herpes tonsurans, Virch. Arch. 1886. Bd. 70.
13. Grundy, - Comptes rendus de seances de l'acad. d. sc. de. Paris, 1842. T.14 - quoted from Plaut.
14. Guidi, - Firenze 1896. - quoted from Plaut.
15. Hallier, - Botan. Ztg., 1865.
16. Hausman, - Die Parasiten der weiblichen Geschlechtsorgare, 1870, quoted from Plaut.

17. Hickel, - Beiträge zur Morphologie und Physiologie des Soorerregers, Sitzungsber. d. math. - naturwiss Klasse der Akad. d. Wissensch. in Wien, 1907, 1 Abt., Bd. 115.

18. Klemperer, - Ueber den Soorpilz. Diss. Berlin, 1886

18.a. Larson, Cantwell & Hartzell, - Influence of the Surface Tension of the Culture Medium on the Growth of Bacteria. J. of Infectious Diseases, 1919, Vol. 25.

19. Laurent, - Observations sur le champignon du muguet. Bull. de la soc. belge de micr., 1890.

20a. Linossier and Roux, - Sur la morphologie et la biologie du champignon du muguet. Compt rend. de l'acad. d. sc., 1889.

20b. Recherches biologiques sur le champignon du muguet. Arch. de med. experim. et d'anatomie pathologique. 1890.

21. Noisette, - These de Paris, 1898, quoted from Plaut.

22. Oliver, - Preliminary report on a peculiar infection of the mouth and throat, with new variety of oidium resembling thrush. Calif. State Journal of Med., San Francisco, Aug. 1904.

23.a. Plaut, - Die Soorpilzgruppe, Hanbuch der pathogenen Microorganismen, herausgegeben von Kolle und Wasserman, 1913. Band 5, Jena, Gustav Fischer. p. 42

23b. Plaut, - Studien zur bacteriellen Diagnostik der Anginen, Deutsche med. Wochenschr., 1894, No. 49.

24. Quinquad, - quoted from Plaut.

25. Rabinowitsch, Lydia, - Untersuch uber path. Hefearten., Zeitschr. f. Hyg. 1896, Bd. 21.

26. Reess, - quoted from Plaut.

27. Retteger, - J. of Inf. Diseases, 1919, April, Vol.24.

28. Robin, - Paris 1853, quoted from Plaut.

29. Roger, - Modifications du serum chex les animaux vaccines contre l'Oidium albicans, Societe de' biologie, Paris 1896.

30. Schmœrl, - Ein Fall von Soormetastase in der Niere. Centralbl. f. Bakt., 1890, Bd. 7.

31. Stumpf, - Untersuchung über die Natur des Soorpilzes, Cemtralbl. f. klin. Med., 1886.

32. Stevens, - Fungi Which Cause Plant Disease, The Macmillan Co. N.Y. 1913.

33. Troisier and Achalme, - Sur un cas cliniquement identique au muguet et cause par une levure veritable. Archives de' medicine experimentale, 1893.

34. Virchow, - Splz. Path. u Ther., Bd. 1, 358, quoted from Plant.

35. Vuillemin, - Les formes du champignon des muguet. Revu mycologique. T. 21, 1899.

36. Widal, Abram, Joltrain, - Briss und Weill, Serodiagnostic mycosique. Ann. d. l'Inst. Past., 1910.

37. Zenker, - Ber d. Ger. f. Natur - u. Heilk., 1861, quoted from Plaut.