Morphology and Cultural History of *Plasmodiophora brassicae.*

By
Philip M. Jones.

(With 1 figure in text and Plates 15—21.)

Introduction.

The purpose of this paper is to give a description of the life history of *Plasmodiophora brassicae* in culture as obtained from galls on the roots of affected cabbage seedlings. Special attention is devoted to cultural methods, morphology, and reproduction.

Cultural methods.

To demonstrate the presence of the organism in culture, the following methods were used. On July 15, 1926, I took eight galls from roots of cabbage seedlings, washed them in tap water, then in mercuric chloride, one to one thousand, for one or two minutes, then in a flask containing sterile water. Next, each gall was placed in and allowed to stand for two days in a 250 cc. flask containing 50 cc. of water, previously sterilized. The galls were then transferred to new flasks containing cover glasses and 50 cc. of sterile water, and allowed to stand for two more days. Then the cover glasses were removed and studied under the microscope, after which the organisms that had collected on them were killed in osmic acid fumes and fixed in Schaudinn solution without acetic acid. They were stained in HEIDENHAIN'S iron haematoxylin (short method) and mounted in balsam.

Observations on the living cultures were continued for two months.

Life history and morphology.

Spores.

The mature spores are small, their greatest diameter never being over four microns. Almost without exception they are spherical in shape, although occasionally biscuit-shaped and double forms are seen. On germination, the spores increase in size and numerous large vacuoles appear and break in rapid succession. Soon after this the protoplasm is seen oozing out as the spore wall disintegrates where a vacuole has broken. Figs. 3 and 8 show the protoplasm very much vacuolated and in Fig. 11 it flows out on one side, and withdraws from near the opposite wall leaving a nearly hyaline semicircle, about two thirds the distance from the center. A considerable part of the contents is left within the old spore wall, so that when the broken part is turned upward it has the appearance of a circle bounded by a darker band, whose width is about one-third of the radius. If, however, the open part is on the side, the residue within the spore wall more nearly resembles a crescent.

Gametes are of two kinds, but arise from the same source. First the contents of the spore, enlarge by vacuolization, may produce either (1) a single, large, pyriform, uniflagellate gamete or (2) a number of very small uniflagellate gametes. When a single large gamete is formed from a spore it has a single nucleus surrounded by opaque cytoplasm, arising from the nucleus a single flagellum about the length of the amoeba and situated at the anterior end, and a vacuole near the posterior end. The body of these gametes is about 4 microns long and 2 microns wide. They move about with a jerky motion for a time, then conjugate (Fig. 58), the anterior end of one with the posterior end of the other, thus forming a zygote (Figs. 5, 58). They can withdraw one flagellum and move with the other or whirl about until apparently exhausted, then settle to the bottom and assume the amoeboid movement. The nuclei do not fuse at once, but when the fusion takes place a nuclear membrane is formed. They may continue to grow into mature amoebae or encyst, depending upon the conditions of the culture. In the second way the small gametes may occur in two ways: (1) As the spore contents are being extruded small gametes about a micron in length and one-half micron in width, are formed by division the of nucleus and constriction of the cytoplasm of the spore contents. As the division takes place these gametes enlarge and divide. Although

the division of the nucleus is complete and the constriction of cytoplasm has taken place, they do not necessarily separate. I have counted as many as 20 gametes formed from one spore, which may or may not separate. When they do separate they have either amoeboid or flagellate movement. In the case of the flagellate the nucleus is always at the anterior end and a flagellum arises from it. These amoeboid or flagellated gametes either fuse with like gametes or with a large number of gametes which have not separated, thus forming a plasmodium (Figs. 29, 30) also text (Fig. i'). (2) During chromidia extrusion in the plasmodium, the whole plasmodium breaks up into gametes, similar to those described, by the rapid division of spore contents (Fig. 56).

All of these conditions are governed by the condition of culture such as the amount of food, and dryness of culture.

The amoeboid stage.

Morphology: After the fusion of the gametes to form the zygotes, the flagella are lost. The amoebulae formed in this manner begin feeding rapidly, and the endoplasm becomes filled with food vacuoles, which contain bacteria in process of digestion. These bacteria were probably carried into the culture by the plant tissue (Figs. 49, 70). For discussion of bacteria see page 307. The contractile vacuole is generally found very close to the nucleus (Figs. 57, 59); the resting nucleus is spherical or egg-shaped, with a distinct nuclear membrane (Fig. 47); between this and the large karyosome lies a clear area; the karyosome is centrally located and appears as a large, compact sphere, which stains homogeneously.

Movements: The body of the amoeba has one constant character, namely, it progresses by one broad, fan-shaped pseudopodium, (Fig. 49, 57). While the general direction of movement is in a straight line, there is not a constant flowing movement, but rather an alternation of wave-like pseudopodia formation (Fig. 13). In the following form, the cytoplasm is clearly differentiated into ectosarc and endosarc. I often find long stringy pseudopodia, which resemble the pseudopodia of *Amoeba radiosa*. Dr. Cupp thinks they are used in penetrating the call wall (Fig. 48).

Binary fission: During division, the amoeba does not necessarily round up (Fig. 49); the division of the nucleus is promitotic, that is to say, large chromatic masses are formed within the nuclear membrane with fibrils between them, on which the chromosomes may be seen. The beginning of nuclear division is characterized

by an increase in nuclear size. The nuclear membrane becomes more distinct. The central karyosome then increases in size, elongates, and assumes a dumb-bell shape. Spindle fibres then appear between the chromatic masses (Fig. n, text diagram). The nuclear membrane constricts in the middle and the two daughter nuclei separate. The polar masses then assume the shape of karyosomes (Fig. 16), and the daughter amoebulae then become vacuolated, prior to the formation of plasmodia.

There are certain inclusions which may be parasites, found in the endosarc of the amoeboid and preplasmodial stage of *P. brassicae* (Figs. 44, 45, 49, 78), that may play a part in the entrance of *P. brassicae* into the host plant, and its passage from one cell to another. Although due to their small size I have not seen these inclusions in living conditions, in stained preparations they resemble the parasite *Calkinsi* I discussed in my previous papers on *Plasmodiophora tabaci.*

The plasmodial stage.

The plasmodium may be formed in two ways: (1), fusion of zygotes, which arose by conjugation of gametes; (2), vacuolization of amoebulae after the fusion of the gametes.

The zygotes formed by union of the gametes fuse to form a plasmodium, which moves like an amoeba. Most of the plasmodia are formed in this manner, but a plasmodium may be formed from a single zygote (Figs. 29, 30). The chromatin masses then break up to form chromidia, around which the epiplasm extends (Figs. 22, 23, 24). As it increases in size we have this chromidial mass becoming vacuolated with chromidia distributed around the vacuole (Figs. 25, 39). A nucleus is then pinched off (Fig. 25), which will undergo true mitotic division. All stages of this process are shown: resting stage (Fig. 36), prophase (Fig. 35), metaphase (Fig. 39), anaphase (Fig. 36), and telophase (Fig. 40). During this division, a bud begins to form which takes one of the new nuclei with it (Fig. 41). The process is similar to endogenous budding. These buds may undergo rapid division (Fig. 68), and then go into a cyst (Fig. 66). When the cyst germinates, it gives rise to a plasmodium (Fig. 64). In the old form, from which the bud arose, the nucleus becomes vacuolated and the chromidia are distributed through the epiplasm (Figs. 41, 43). In this condition the chromidia either collect in vacuoles to form nuclei (Fig. 62), or the whole plasmodium breaks up to form gametes (Fig. 56), which conjugate (Fig. 20).

The new nuclei, formed by chromidia collecting in vacuoles, undergo division which is truly mitotic (Fig. 61). I counted eight chromosomes. While the nucleus was becoming vacuolated and the chromidia were being distributed, a dark line appeared around the vacuolated portion (Figs. 42, 52, 60). The chromidia never go beyond this line, thus forming an "island", with growing protoplasm around the island (Fig. 60). The spores are found in this island, while the protoplasm continues to grow on the outside (Fig. 54). I have observed one plasmodium with eight of these islands in it. The plasmodium has power to encyst, forming a thick wall around itself (Fig. 38). Under favorable conditions, the plasmodium ruptures the thick wall and flows out, the entosarc preceding the endosarc with a long, finger-like projection. The whole plasmodium may break up into small amoebulae (Figs. 37, 64).

I have never been able to find nuclei in the old portion of a plasmodium, but find them frequently in the growing portion (Fig. 36).

The plasmodium of *Plasmodiophora brassicae* is similar to that of other mycetozoa, in that it is a jelly-like, amorphous substance, in which are imbedded small oil droplets, which make the organisms stain very black after being killed by osmic fumes. In the young stages (Figs. 22, 23, 24), the protoplasm is pale and colorless, but gradually becomes more and more opaque (Fig. 46). Movement is very slow, proceeding with the production of a watery fluid and a gelatinous substance of a soapy consistency.

In spore formation, round vacuoles appear in the protoplasm at some distance from each other, giving the plasmodium a reticulated appearance (Fig. 46). Between these vacuoles, the chromidia, which have been distributed in the cytoplasm, gather gradually into small masses which form nuclei (Fig. 62). These nuclei undergo division which I believe to be reduction division, to form spores, which seem to be bound together by a sort of clear, slimy substance — possibly a capillitium. The ripe spore is composed of a thin, transparent, refractive outer portion enclosing a more or less granular matrix (Fig. 2), in which is embedded a nucleus. Ripe spores generally break off from the spore mass. I have observed the contents of these spores to fuse, forming a plasmodium before breaking up into gametes, although segmentation had already started.

The mature amoeba becomes vacuolated (Fig. 59), to form a preplasmodium with one nucleus (Fig. 69). This begins feeding rapidly, the cytoplasm as well as the nucleus increasing in size (Fig. 70). In this stage we have rapid movement. Foreign bodies,

which stain black when killed with osmic acid fumes, appear all through the endosarc. I believe these bodies are fat globules. After the nucleus has become very large (Fig. 70), the karyosome moves to one side (Figs. 69, 72), and then excapes from the nucleus. The karyosome, during this movement, assumes a dumb-bell shape and starts dividing by promitosis (Fig. 74). When the karyosome has completely left the nucleus (Figs. 71, 73), it undergoes rapid division, by mitosis, until the plasmodium becomes filled with little nuclei. These nuclei increase in size to form a multinuclear plasmodium. The plasmodium stops feeding and assumes a frothy appearance. The nuclei become vacuolated, chromidia are distributed around the vacuoles, and collect into new vacuoles to form new nuclei, which now follows Text-Figure (1). See diagram.

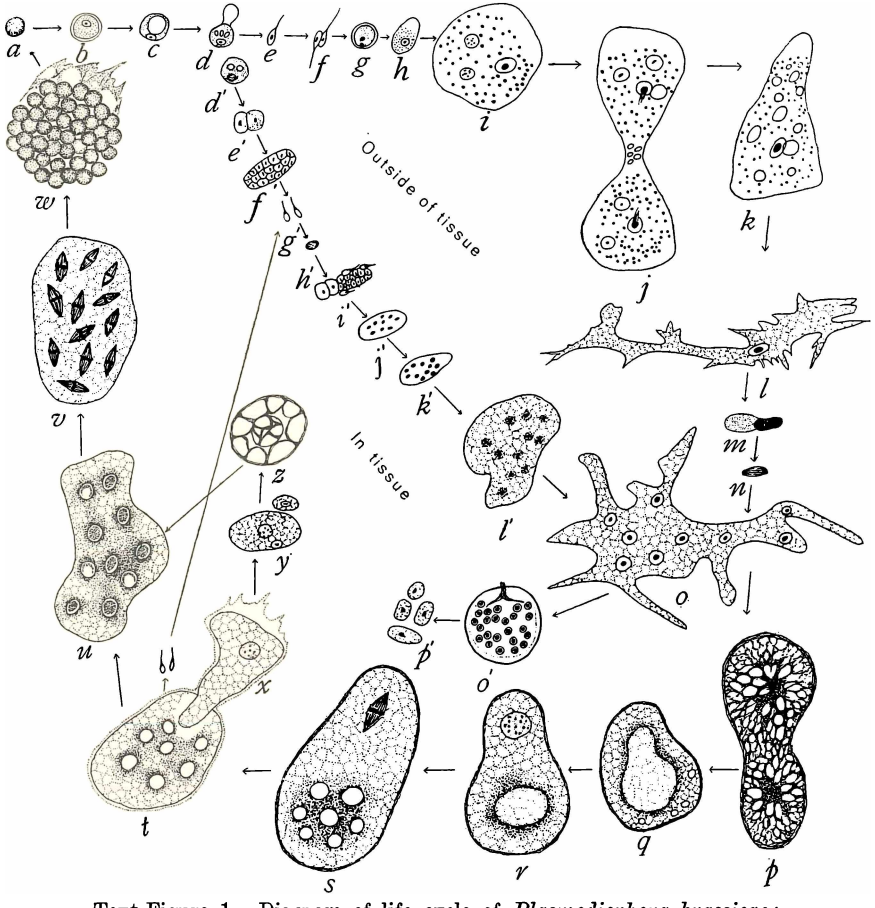
Penetration and pathogenicity to turnip.

Several cultures of *Plasmodiophora brassicae* were prepared at the University of Chicago on June 17th, 1926, and subsequently brought to the University of Virginia and left in the laboratory at room temperature until Feb. 7th, 1927, on which date they were examined and found to contain the organisms in encysted condition.

From Dr. BRUCE REYNOLDS I obtained several turnips, grown in his garden, which were washed in tap water and then in mecuric chloride (1—1000). I then cut some small pieces about a quarter of an inch square from one turnip and put two pieces in each culture brought from the University of Chicago. I allowed these cultures to stand until March 1st, 1927, when examination showed the occurrence of the organism in the amoeboid stage.

One of the turnips was planted in some sterile soil and allowed to grow. On March 1st, the turnip was almost in full bloom. On this date I poured the contents of the previously mentioned culture on the soil, near the fleshy part of the turnip. By March 14th, the turnip and its roots were dotted with small white galls, in size about 5 mm in diameter. The galls appeared in small crevices both on the turnip and on its roots. Many of them appeared on the turnip at the base of the small root. The majority of the galls occurring on the roots contained numerous hairy-like roots.

The galls were removed, some fixed for sectioning, and stained in HEIDENHAIN'S iron haematoxylin (long method). In most cases I found that a uninucleated amoeba (Figs. 44, 45, 49, 57), had entered the turnip at the base of a young root and worked its way up the young root until it was near the growing region. The



Text-Figure 1. Diagram of life cycle of *Plasmodiophora brassicae:*

Spore stage (a); ripe spore composed of a thin, transparent, refractive outer portion, enclosing the more or less granular matrix (b); vacuolated spore (c); contents flowing out of a mashed spore (d). From outside of the plant the line of development will be indicated by Figs. (e) to (o). In the plant the method of development will be that indicated by Figs. (d') to (l') to (o) to (z). Uniflagellate gamete (e); conjugation of gametes to form zygotes (f); young cyst, nuclei not yet fused (g); young amoebula arising from zygote (h); mature amoeba (i); mature amoebula undergoing division (j); amoebula becoming vacuolated prior to changing into a preplasmodium (k); preplasmodium stage with one nucleus (l); karyosome leaving the nucleus also undergoing division (n); karyosome undergoing further division (n); plasmodium with many nuclei, which resulted from rapid division of the old karyosome (o); plasmodium nucleus breaking up into chromidia (p). Encysted plasmodium (o'); plasmodium, after coming out of cyst, may break up into small amoebulae (p'); vacuolization of the nucleus with chromatin granules around the vacuole, new nucleus being pinched off (q); definite nucleus formed (r); nucleus in metaphase

prior to bud formation, other end becoming more vacuolated (s); bud flowing out while the chromidia are being distributed throughout the cytoplasm of old form (t). From this point development may be along either of three lines; viz. (1), from (u) to (w); (2), from (x) to (z); (3), from (t) to (g'). New nuclei being formed from chromidia (u); new nuclei undergoing reduction division prior to the formation of spores (v); mature spores (w); bud flowing out of old form (x); bud undergoing rapid division (y); bud forming a resistant cyst (z); nucleus of spore undergoing schizogony (f'); small gametes as result of rapid division (g'); zygote (h'); fusion of gametes to form a plasmodial mass (i'); motile plasmodial mass (j') and (k'); new nuclei being formed (l').

place of entrance is furnished by the distorted parenchyma cells caused by the pushing out of the young root from the fleshy part of the turnip (Figs. 75, 76, 77). In Fig. 77 one may trace the place of entrance. As soon as the amoeba enters it turns into what I call a preplasmodium (Figs. 70, 74), and pushes towards the center of the root by actually penetrating the host cell wall. The movement is accomplished by an actual amoeboid creeping and an elongation of the forward end. The plasmodium cytoplasm at this stage stains heavier than cytoplasm of the healthy host cells. The karyosome of the host nucleus increases in size and may divide (Fig. 78), or merely increase in size, while the adjoining healthy cells show abnormal division. NAWASCHIN (1899) explains this hyperplasia on the part of non-invaded cells as due to the mechanical outward pressure of the much-enlarged diseased cells. The cytoplasm of the preplasmodium seems to be running wild but follows the line of least resistance. It becomes vacuolated, clear and almost transparent at first, the outline being difficult to distinguish as it blends with the cytoplasm of the host. Since the cytoplasm of the cell and that of the plasmodium are so nearly of the same consistency they fuse with one another so quickly when the plasmodium enters the cell, that I find it hard to get a stain to show the difference between the infected and healthy cells.

Discussion.

I do not believe this is the only stage of entrance for the simple reason that this culture was made by germinating encysted amoebae and not from the spore contents. Other authors have described with some assurance various ways in which the organism may enter the host, but no one has observed the real process. EVEN WORONIN (1878), who believed that the organism enters through the root hair, was never able to demonstrate this clearly. Nevertheless he

felt assured that it enters in the form of a uninucleate amoeba, and his opinion has been accepted by most investigators. A few workers, as W. G. Smith (1884), maintain that the organism enters the root in the form of a plasmodium. The question was revived again when Kunkel (1915) studied the powdery scab of potato, in which the swarm-spores are found to fuse before attacking the host.

There seems to be no doubt in the minds of Marie and Tison (1911) and Schwartz (1914) that all the other known parasitic myxomyctes enter immediately after the swarm-spore stage. This conclusion is based on the fact that many of the slides of these investigators show the uninucleate forms in the apical cells. There is no other theory that would explain this phenomenon, unless a single uninucleate amoeba of an infected plasmodium passes through the intervening cell walls and spreads in this manner through the tissue.

From a study of Dr. Chupp's works, I believe that the zygote, formed by the fusion of the gametes (which arose by rapid division of spore contents, or by a breaking up of the plasmodium), is the stage of penetration. The reason for this is shown in drawings (a) and (*b*), plate 5, of Dr. Chupp's article, which I consider to be identical with the zygote stage that I described in drawings 28 and 29. His Fig. c is similar to my Fig. 30. His Fig. d shows the plasmodia, which did not fuse in the zygote stage because they did not come in contact with each other. I have never been able to get the plasmodia to fuse in cultures after they pass the zygote stage. His drawing g shows the results of the fusion of a number of zygotes to form a plasmodium. Whether the gametes enter the root and then conjugate, or conjugate first and then enter, is uncertain. I am inclined to believe the latter. I believe further, that the zygotes then fuse with each other after entrance.

Bacteria in relation to *Plasmodiophora brassicae.*

Pinoy's (1902, 1903, 1905, 1907) work with Mycetozoa in relation to bacteria, and his subsequent suggestion that there is a true symbiosis between the two, represent a very interesting phase in the study of *Plasmodiophora.* It has long been held that certain saprophytic slime molds feed on accompanying organisms, and the data at hand seem entirely plausible, Lister (1894) reported the ingestion of bacteria by active swarm-spores. The experiments of Vuillemin (1903), Nadson (1901), and Potts (1902) show that *Dictyostelium mucoroides* Bref. feeds directly on bacterial colonies and

destroys a large number of these at fruiting time. According to CHUPP, when the surfaces of the roots are sterilized and placed in a jar, they may show no indication of bacteria until they are cut in two and the fresh surface is placed in contact with the medium. Moreover, E. F. SMITH (1911) and EYCLESHYMER (1894), both careful workers, state that they saw these bacteria. This is also in accordance with MAIRE and TISON (1911) who claim it to be true for certain parasitic slime molds that are able to ingest unicellular algae present in their aquatic host; and with what KUNKEL (1915) has demonstrated in the case of *Spongospora subterranea* grown on agar in which pure cultures of plasmodia become abnormal and die, while those with which bacteria are present live and thrive.

All of PINOY'S (1905) experiments appear to corroborate his idea that a coccus form enters the root with a swarm-spore and lives in constant association with the parasite throughout its entire life cycle. He stained sections of the root and observed bacterial forms within the cells. They appeared so much like parts of the cell contents that the microscopical analysis had to be accompanied by cultural study. For this he procured diseased roots of *Brassica sinensis* measuring from eight to ten centimeters in diameter, seared the outside, and cut plugs from the interior by means of a flamed pipette. When these plugs were planted in agar media, numerous colonies of bacteria soon appeared. To prove that these organisms were necessary for the development of the myxomycete, PINOY placed spores of *Plasmodiophora brassicae* in a large number of test tubes containing sterilized extract of roots. In two tubes the spores were accidentally not associated with bacteria and they failed to germinate while in all the other tubes the spore did germinate.

Dr. CHUPP claims that no bacterial colonies were obtained from the roots with young swellings. From the medium-sized swellings occasional colonies develop; and from larger galls, especially when the epidermis had been broken, numerous colonies always appeared.

Summary.

1. Eight pure cultures of *Plasmodiophora brassicae* have been maintained under laboratory conditions for two months.

2. Two different types of nuclear division occur: (1), promitosis in amoebula and in preplasmodium stage, which arise from them; (2), typical mitosis in bud formation and before spore formation.

3. The amoebulae change into a preplasmodium, with one nucleus, by vacuolization.

4. A multi-nucleate plasmodium arises when the karyosome of the preplasmodium leaves the nucleus and undergoes division.

5. New nuclei as well as gametic nuclei are formed from chromidia.

6. No nuclei are formed in old parts of the plasmodium.

7. In culture, *Plasmodiophora brassicae* has two kinds of gametes; (1), those which fuse to give rise to amoebae, and (2), those which fuse and then coalesce to form a plasmodium. The latter gametes have the same origin as the former, but they undergo rapid division.

8. Gametes conjugate with reversed polarity to form a zygote.

9. *Plasmodiophora brassicae* has eight chromosomes during division of the nucleus in the young plasmodium.

10. In the life cycle of *Plasmodiophora brassicae* the following stages may occur: spores, gametes, zygotes, cysts, mature amoebulae, preplasmodia, and buds.

If conditions are favorable *Plasmodiophora brassicae* does not have to pass through all of these stages.

11. In plant tissues the following stages occur: gametes, zygotes, preplasmodia, plasmodia, buds, cysts, spores.

12. Endogenous buds may be formed by pinching off a portion of a nucleus followed by mitosis of the nucleus which is pinched off.

13. Chromidia are not distributed to all parts of the old plasmodium, but are restricted to certain areas.

14. Both young and old plasmodia contain foreign bodies, which may be oil globules.

15. In the amoebulae there is a contractile vacuole which is located near the nucleus.

16. *Plasmodiophora brassicae* taken from cabbage galls was cultured, and poured on soil containing a turnip. The turnip developed galls and the organism was cultured from these turnip galls.

17. The encysted organism, in culture, can be induced to come out of its encysted condition by adding several pieces of turnip.

18. Uninucleated amoebae penetrate the cells of young roots of turnip or the young root pushes out through the parenchyma cell. As soon as entrance is gained a preplasmodium is formed.

Literature cited.

Chupp, C. (1917): Studies on Club-root of Cruciferous Plants. Cornell Univ. Agri. Exp. Sta. Bul. p. 387.

Eyclesheymer, A. C. (1894): Club-root in the United States. Journ. Myc. Vol. 7 p. 79—88 Pl. 15—16.

JONES, P. M. (1926): „Structure and Cultural History of a Mycetozoan Found in Tobacco Plants with Mosaic-like Symptoms". Bot. Gaz. Vol. 81 p. 446—449.

KUNKEL, L. O. (1915): A contribution to the life history of Spongospora subterranea. Journ. Agr. Res. Vol. 4 p. 265—278 Pl. 39—43.

LISTER, ARTHUR (1894): A monograph of the Mycetozoa p. 1—224 (Reference on p. 4).

LUTMAN, B. F. (1913): Studies on club-root. I. The relation of Plasmodiophora Brassicae to its host and the structure and growth of its plasmodium. Vermont Agr. Exp. Sta. Bul. Vol. 175 p. 1—27 Pl. 1—4.

MAIRE, RENE, et TISON, ADRIEN (1909): La cytologie des Plasmodiophoracees et la classe des Phytomyxinae. Ann. Myc. Vol. 7 p. 226—253 Pl. 4—6.

— (1911): Nouvelles recherches sur les Plasmodiophoracées. Ann. Myc. Vol. 9 p. 226—246 Pl. 10—14.

NADSON, G. A. (1901): Des cultures du Dictyostelium mucoroides Bref. et des cultures pures des Amoebes en général. Scripta Botanica (St. Petersburg) Vol. 15 p. 1—38. Abstracted in JUST's Bot. Jahresber. Vol. 27 p. 86.

NAWASCHIN, S. (1899): Beobachtungen über den feineren Bau und Umwandlungen von Plasmodiophora Brassicae Woron, im Laufe ihres intracellulären Lebens. Flora, Vol. 86 p. 404—427 Pl. 20.

PINOY, ERNEST (1902): Nécessite de la présence d'une bactérie pour obtenir la culture de certains myxomycètes (Note préliminaire). Soc. Myc. France. Bul. Vol. 18 p. 288—290.

— (1903): Nécessité d'une symbiose microbienne pour obtenir la culture des Myxomycètes. Acad. Sci (Paris). Compt. Rend. Vol. 137 p. 580—581.

— (1905): Rôle des bactéries dans le développement du Plasmodiophora brassicae, myxomycète parasite produisant la hernie du chou. Soc. Biol. (Paris). Compt. rend Vol. 57 p. 1010—1012.

— (1907): Rôle des bactéries dans le développement de certains myxomycètes. Inst. Pasteur. Ann. Vol. 21 p. 622—656, 686—700 Pl. 13—16.

POTTS, GEORGE (1902): Zur Physiologie des Dictyostelium mucoroides. Flora, Vol. 91 p. 281—387.

PROWAZEK, S. (1905): Über den Erreger der Kohlhernie Plasmodiophora brassicae WORONIN und die Einschlüsse in den Carcinomzellen. Arb. a. d. Kaiserl. Gesundheitsamt Vol. 22 p. 396—410.

SCHWARTZ, E. J. (1911): The life-history and cytology of Sorosphaera Graminis. Ann. Bot. Vol. 25 p. 791—797 Pl. 66.

— (1910): Parasitic root diseases of the Juncaceae. Ann. Bot. Vol. 24 p. 511—522 Pl. 40.

— (1914): The Plasmodiophoraceae and their relationship to the Mycetozoa and the Chytrideae. Ann. Bot. Vol. 28 p. 227—240 Pl. 12.

SMITH, ERWIN, F. (1911): Bacteria with myxomycetes. In Bacteria in relation to plant diseases Vol. 2 p. 169—172.

SMITH, WORTHINTON G. (1894): Club-root of turnips, cabbages, mangels and allied plants. In Diseases of field and garden crops. p. 94—104.

VUILLEMIN, PAUL (1903): Une Acrasiée bactériophage. Acad. Sci. (Paris). Compt. Rend. Vol. 137 p. 387—389.

WORONIN, M. (1878): Plasmodiophora Brassicae, Urheber der Kohlpflanzenhernie. Jahrb. wiss. Bot. (PRINGSHEIM) Vol. 11 p. 548—574 Pl. 29—34.

Explanation of Plates.

Plate 15.

All drawings on plate 1 and 5 drawn on scale of 500 ×.

Fig. 1. Mature spore.

Fig. 2. Ripe spore showing granular matrix and nucleus.

Fig. 3. Contents of mashed spore flowing out.

Fig. 4. Uniflagellata gamete.

Fig. 5. Conjugation of gametes; notice the conjugates have their anterior ends pointing in an opposite direction.

Fig. 6. Encysted zygote.

Fig. 7. Young amoebula resulting from zygote.

Fig. 8, 11. Contents of spore becoming vacuolated while flowing out of spore wall.

Fig. 9. Contents of spore undergoing division.

Fig. 10. Plasmodium resulting from fusion of numerous gametes.

Figs. 12, 13, and 14. Young amoebulae feeding.

Fig. 15. Mature amoebula.

Fig. 16. Mature amoebula undergoing division.

Fig. 17. Mature spore; lower one becoming vacuolated.

Fig. 18. Contents of spore coalescing to form plasmodium.

Fig. 19, 20. Spore contents undergoing rapid division.

Fig. 21. Contents of spore undergoing rapid division.

Figs. 22, 23, and 24. Epiplasm growing out from a concrete chromatin mass which originated from fusion of zygotes.

Fig. 25. Nucleus becoming vacuolated and chromidia distributed around the vacuole; portion of vacuole being pinched off to form a nucleus.

Fig. 26. Preplasmodium formed by vacuolization from amoebula; notice two nuclei. This is very rare. Division occurs by promitosis.

Fig. 27. Gametes, which are formed from rapid division of spore contents or breaking up of plasmodium.

Fig. 28. Zygote resulting from conjugation of above gametes.

Fig. 29. Fusion of zygotes to form young plasmodium.

Fig. 30. Fusion of zygotic nuclei to form pasmodial nuclei.

Figs. 31, 32, and 33. Development of nuclei.

Fig. 34. Plasmodium with the karyosome undergoing rapid division.

Fig. 35. Nucleus in prophase.

Fig. 36. The old nucleus vacuolated, chromidia flowing out into cytoplasm, three nuclei in resting condition, one in anaphase.

Fig. 37. Small amoebulae which escape from encysted plasmodium when the latter ruptures.

Fig. 38. Encysted plasmodium.

Fig. 39. Nucleus in metaphase, eight chromosomes prior to budding.

Fig. 40. Telophase.

Fig. 41. Bud flowing out of old plasmodium.

Fig. 42. New bud; notice the island formed, nucleus vacuolated and chromidia flowing into cytoplasm but never beyond the island. The island is surrounded by young epiplasm.

Fig. 43. The plasmodium becoming more vacuolated preparing to form new gametes or nuclei.

Plate 16.

Fig. 44, 45. Photograph of mature amoebulae showing ectosarc, granular endosarc and karyosome. 750 ×.

Fig. 46, 50 and 52. Nucleus and cytoplasm becoming vacuolated, the chromidia flowing out into new vacuoles to form new nuclei. 500 ×.

Fig. 47. Photograph of amoebula showing elongated nucleus and karyosome. 500 ×.

Fig. 48. Showing the long, thin, pseudopodia, which are quite prevalent in feeding stage. At this stage are many amoeba, which appear as if they are fusing, but on close observation, it may be determined that they do not fuse. 500 ×.

Fig. 49. Amoebula dividing; the nucleus has divided, the cytoplasm has become vacuolated between the two. The food vacuoles are quite prominent. 750 ×.

Fig. 51. Young plasmodium which arose by budding.

Fig. 53. View of vacuolization of nucleus not in focus. 500 ×.

Fig. 54. Mass of spores being formed from plasmodium; notice that the plasmodium is still growing. 500 ×.

Fig. 55. Mature Spores. 500 ×.

Plate 17.

Fig. 56. Plasmodium breaking up into gametes. 1500 ×.

Fig. 57. Showing various shapes of mature amoebae. 800 ×.

Plate 18.

Fig. 58. Conjugation of vegetative gametes; notice the anterior end of one gamete joins to the posterior end of the other. You can see this by careful observation of the gametic flagella in the photograph. 1500 ×.

Fig. 59. Mature amoebulae becoming vacuolated just before they change into a preplasmodium. 400 ×.

Plate 19.

All drawings 500 ×.

Fig. 60. Vacuoles resulting from the nuclei being broken up into chromidia: notice the island formed around these vacuoles and the young growing portion around the island.

Fig. 61. New nuclei undergoing division (possible reduction).

Fig. 62. Chromidia collecting in new vacuole to form nuclei.

Fig. 63. Plasmodium just before spore formation.

Fig. 64. Plasmodium either flowing out or into a cell.

Fig. 65, 66. Showing the shape of chromatin during encystment.

Fig. 67. Cyst being formed from bud, although the nucleus is undergoing division.

Fig. 68. But undergoing rapid division.

Fig. (A), (B), (C), (D) and (G). Drawings from Dr. CHUPP's work, (my interpretation).

Fig. (A). Zygotes fusing.

Fig. (B). Zygotes fusing, zygotic nuclei not yed fused.

Fig. (C). Zygotic nuclei fused, possibley undergoing division.

Fig. (D). Plasmodia. They will not fuse after passing the zygote stage.

Fig. (G). Plasmodium formed by fusion of many zygotes, definite nucleus being formed.

Plate 20.

All magnified 1000 ×.

Fig. 69, 72. Karyosome moving to one side of the nucleus. The black bodies are oil globules (?).

Fig. 70. Uninculeated preplasmodium feeding on bacteria.

Fig. 71. First division of the karyosome after leaving the nucleus.

Fig. 73. Karyosome undergoing further division.

Fig. 74. Karyosome leaving the nucleus undergoing division at the same time.

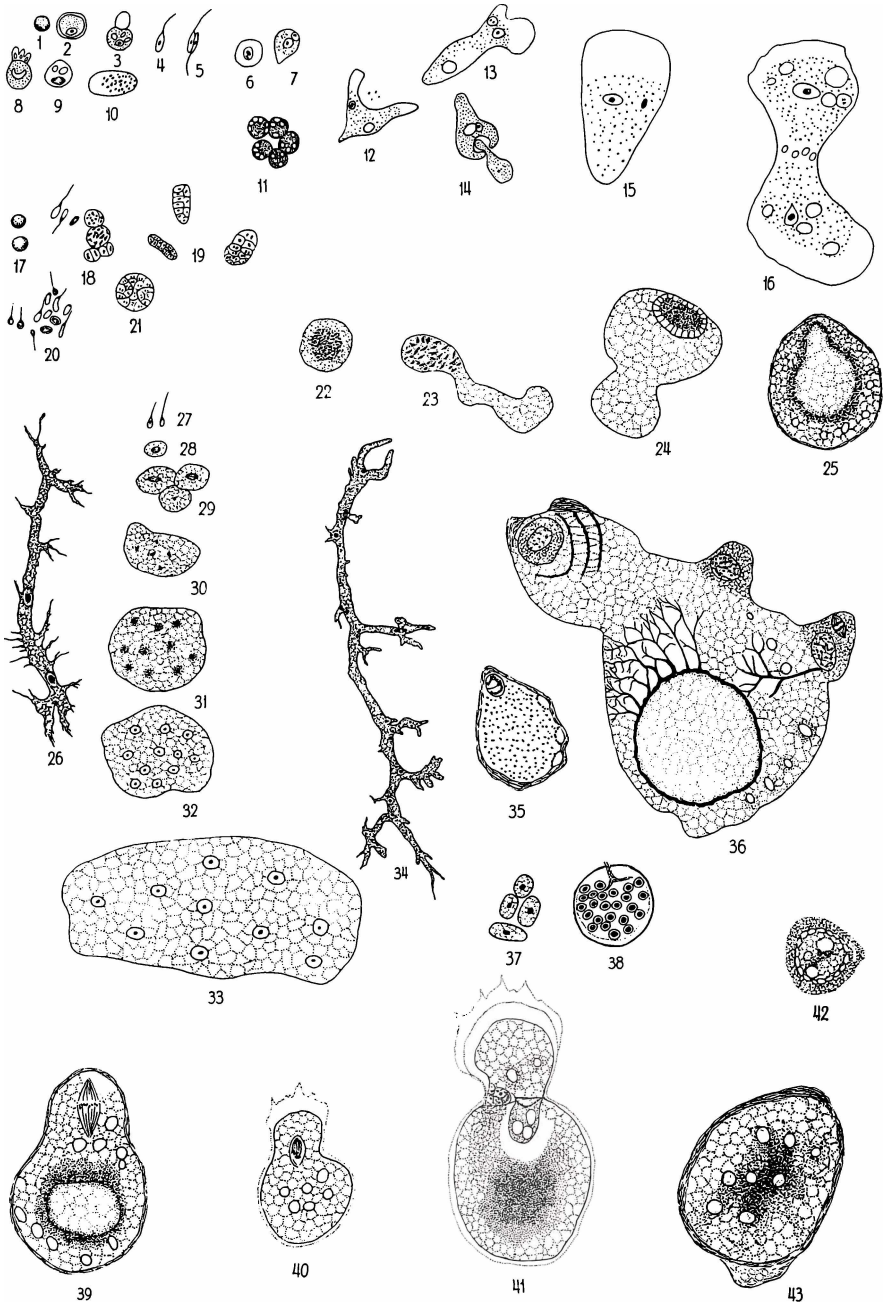
Plate 21.

Figs. 75, 76, 77 500 ×. Fig. 78 700 ×.

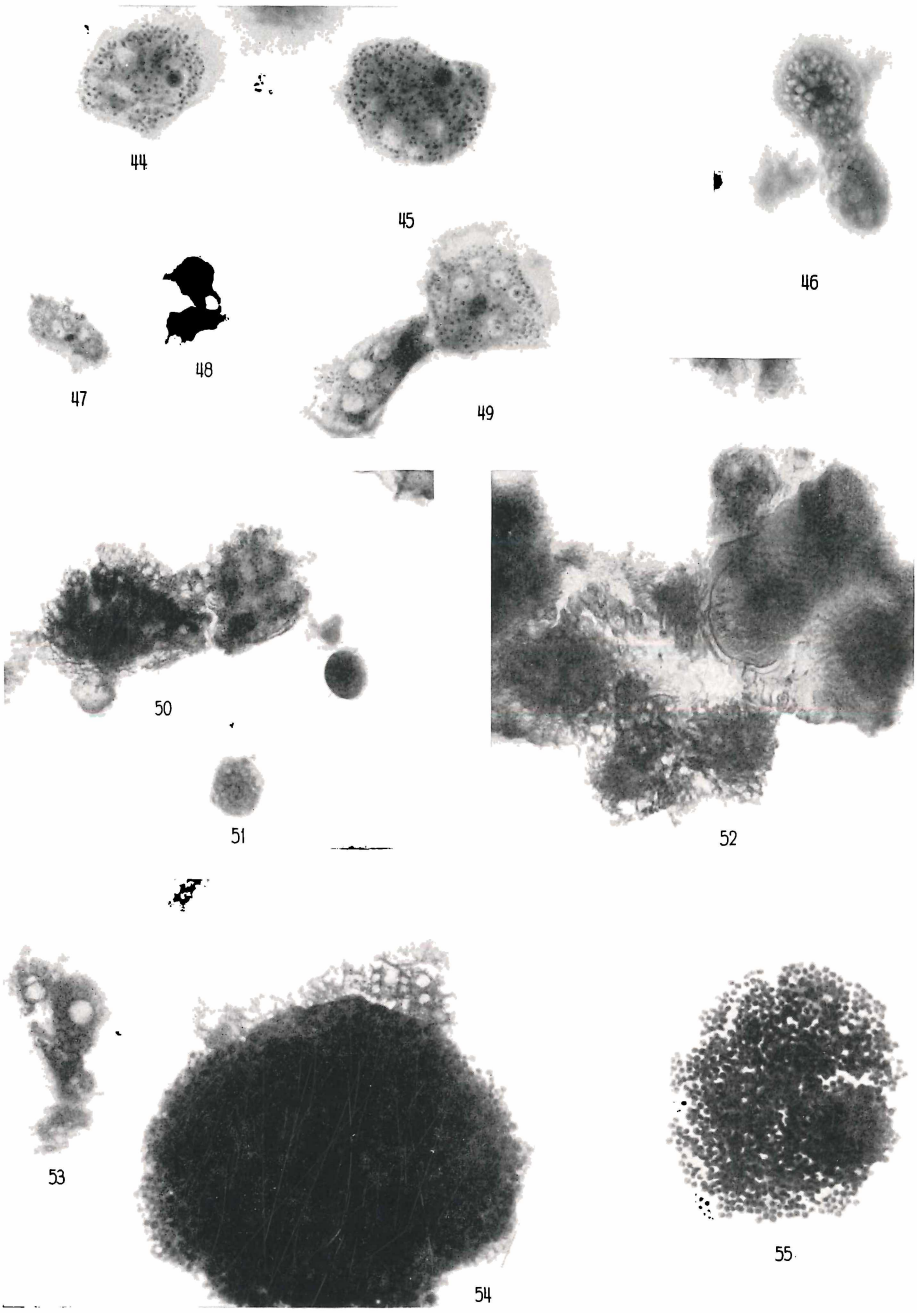
Fig. 75, 76. Preplasmodium posterior to the growing region of a young root of turnip.

Fig. 77. Showing entrance of the uninucleate amoeba into young root, and its passage from cell to cell. Notice the enlarged karyosome of host cell.

Fig. 78. Lower half shows the preplasmodium in the host cells as compared with healthy in the upper half.

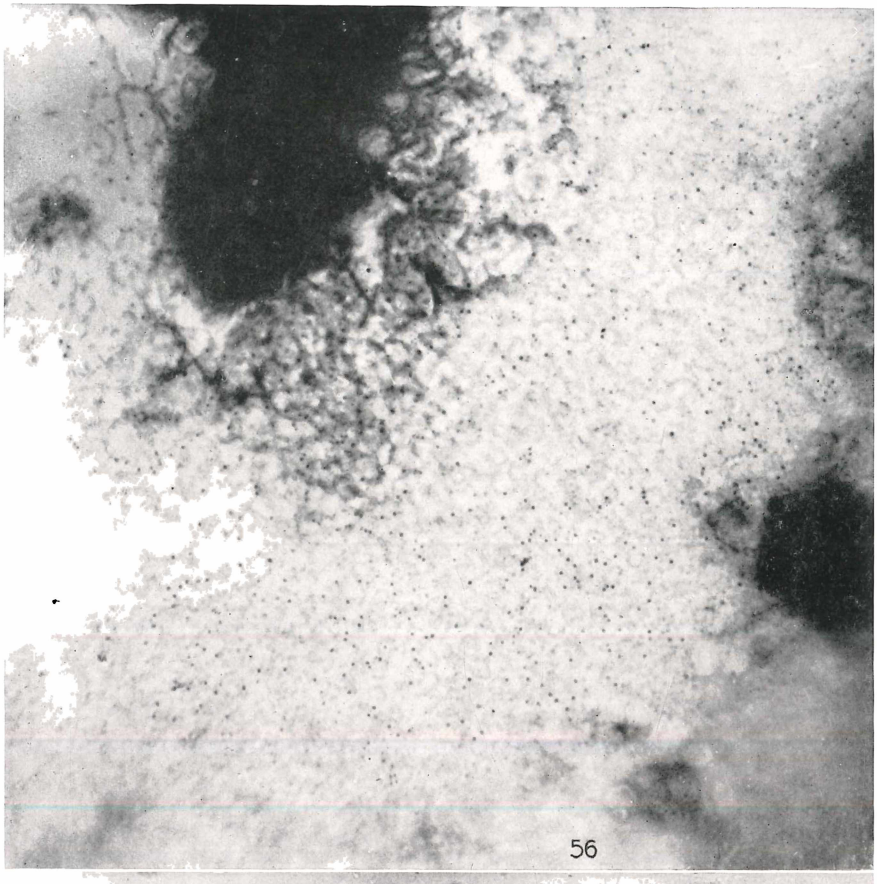


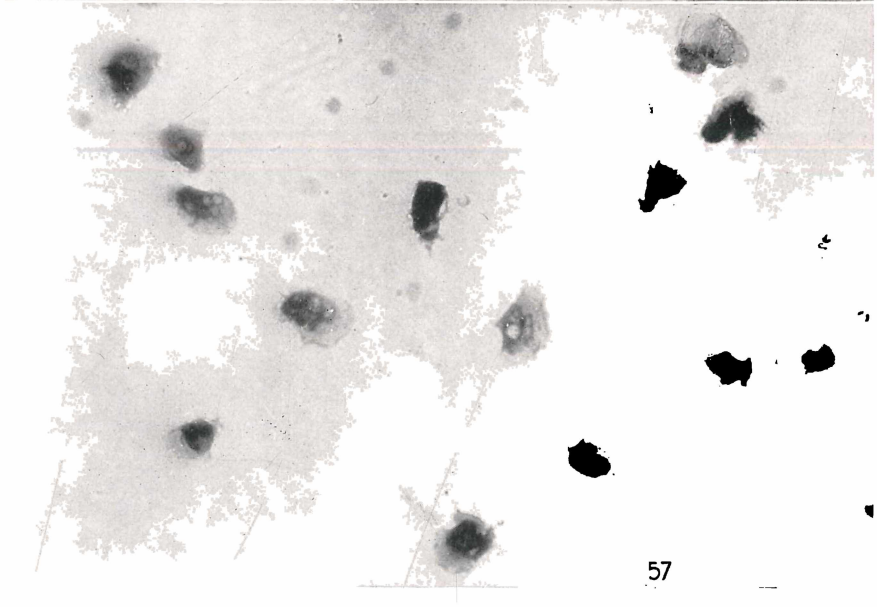
VERLAG VON GUSTAV FISCHER IN JENA.



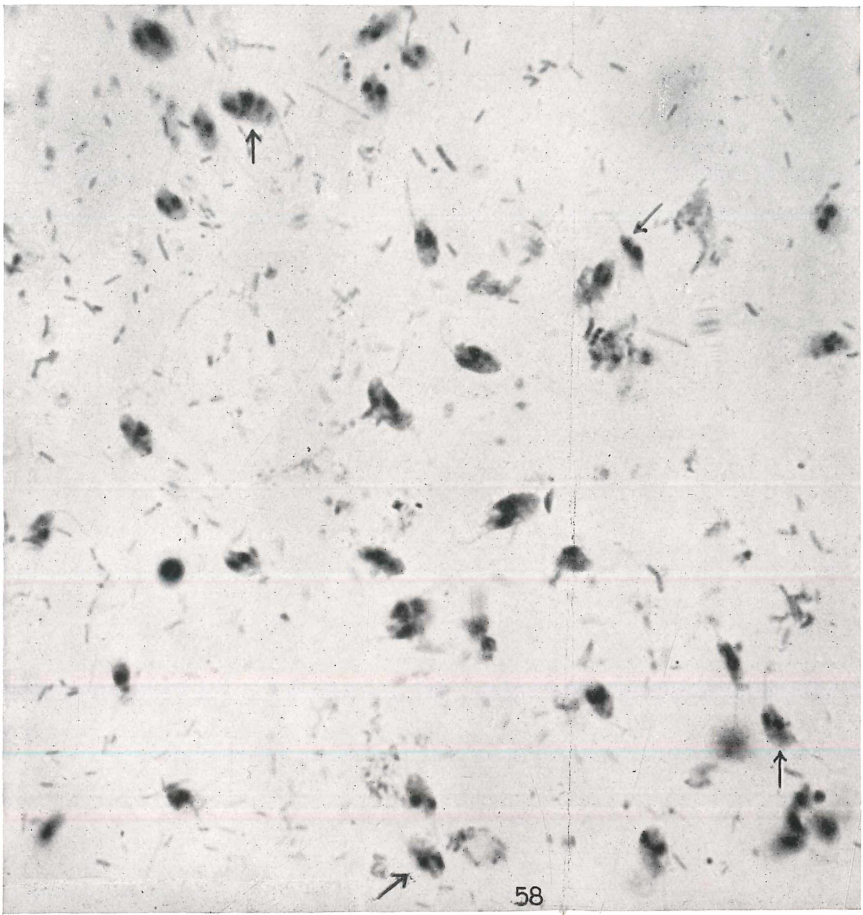
Jones.

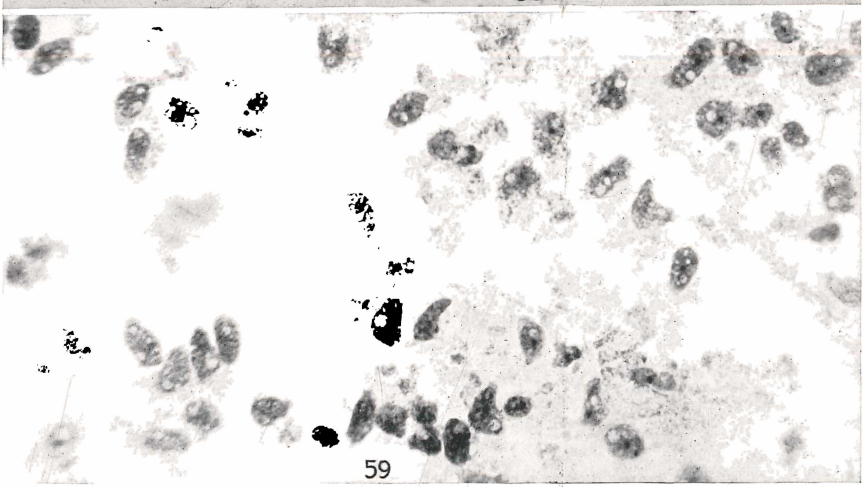
VERLAG VON GUSTAV FISCHER IN JENA.



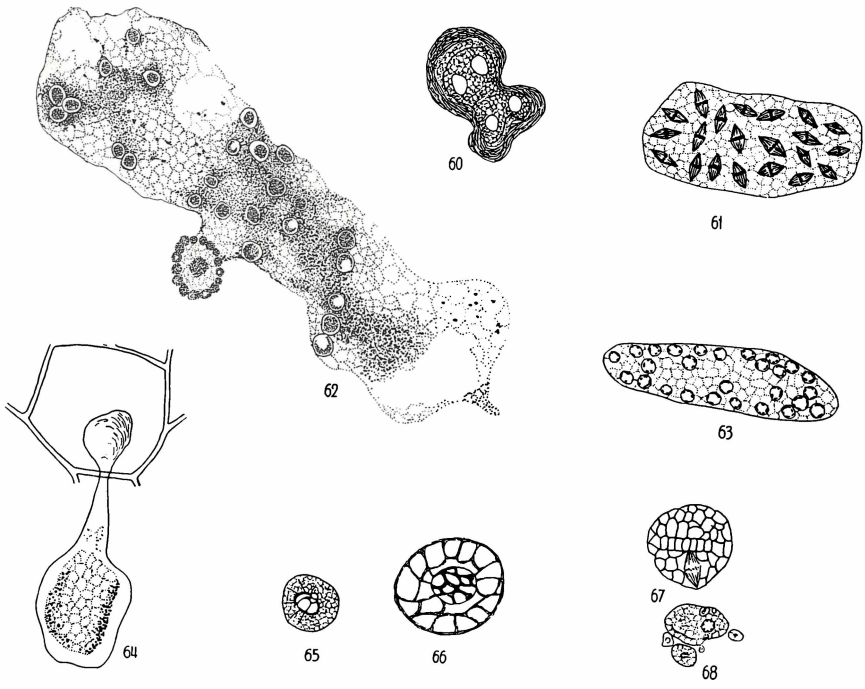


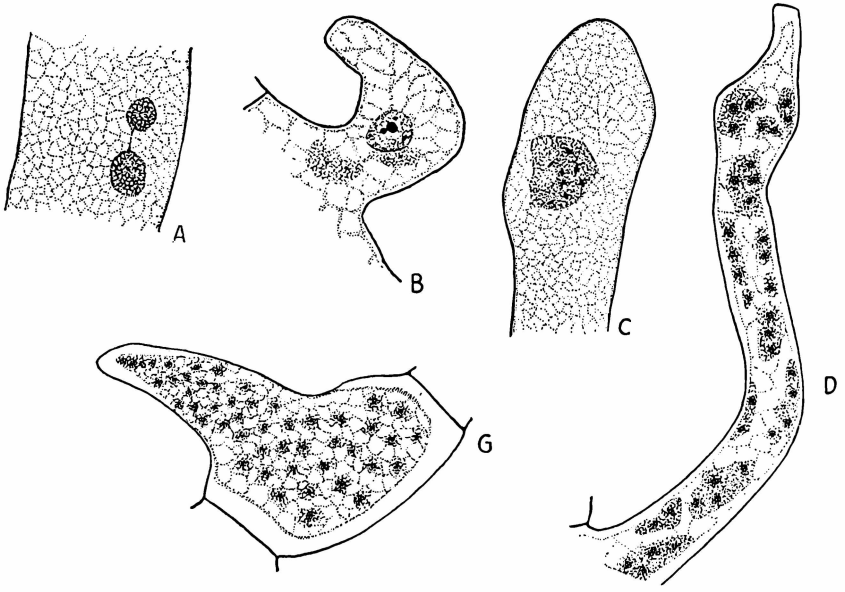
VERLAG VON GUSTAV FISCHER IN JENA.





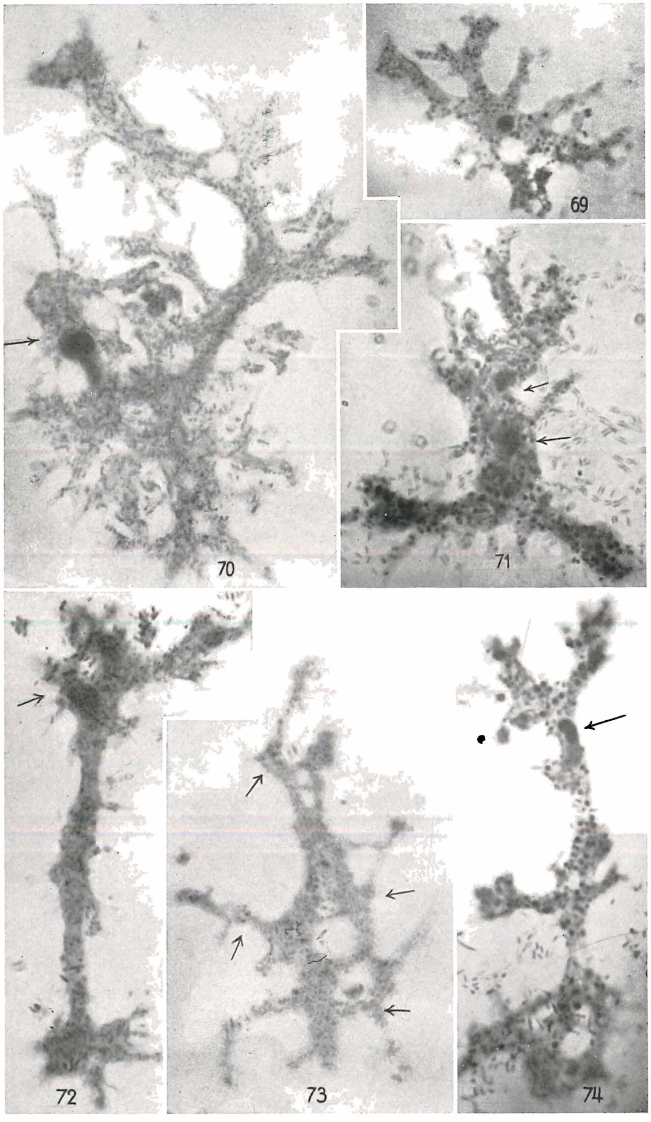
VERLAG VON GUSTAV FISCHER IN JENA.



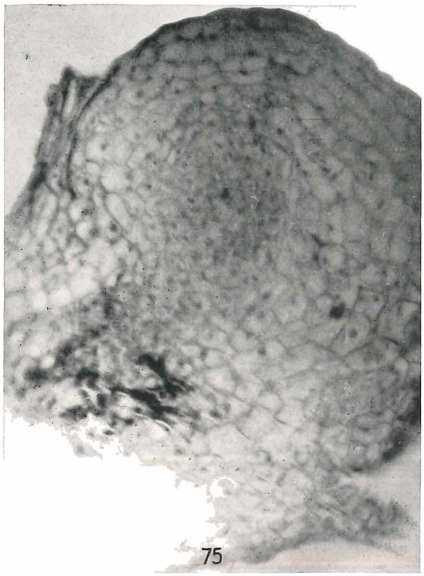


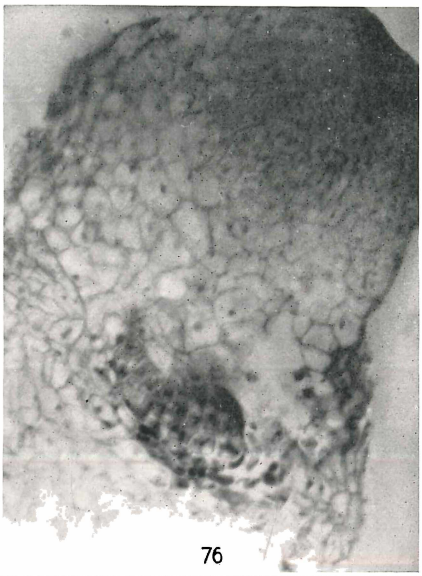
Jones.

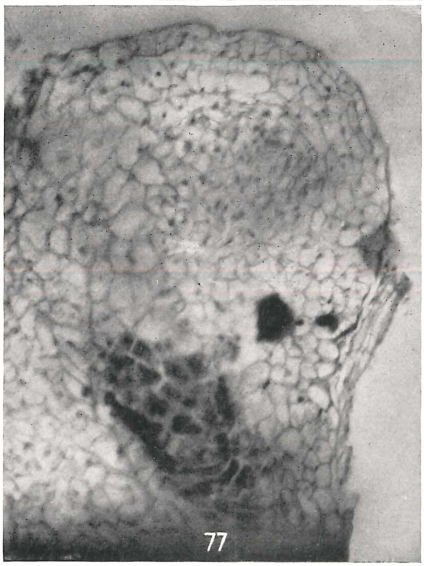
VERLAG VON GUSTAV FISCHER IN JENA.

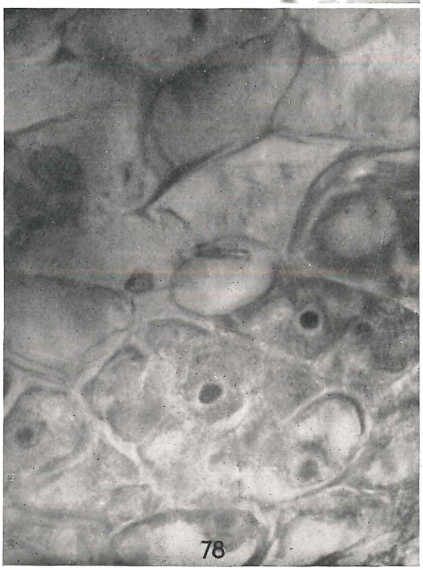


VERLAG VON GUSTAV FISCHER IN JENA.


75


76


77


78