Studies in Plasmopara Halstedii II.

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I. Introduction.

Two kinds of reproduction are exhibited in *Peronosporaceae*, namely, sexual and asexual. Sexual reproduction has been investigated by a number of students. In it oospores are formed in which both the egg and the sperm can be recognized. In *Plasmopara Halstedii*[1] the nuclear changes concomitant with fertilization are typical. A tube from the antheridium penetrates the oogonium and fertilization is accomplished. A large monocyst (receptive papillia) is formed during the nuclear division in the oosphere and penetrates

1) Nishimura, M. 1922, Studies in Plasmopara Halstedii. Jour. Col. Agric., Hokkaido Imp. Univ., Vol. XI, Part 3; 185-210.

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into the antheridial cell. The nature and structure of the monocyst is found to differ with the species and a knowledge of it is of vital importance in this study. Further it was found that some species have no monocysts.

Cytological interest centers chiefly in the oospore formation. In this discussion special attention has been given to the development of the oospore wall; that is, the wall formation of the free cell in the oogonium of *Peronosporaceae* and *Albuginaceae*. Although several scientists have investigated the process of oospore formation in some detail, the mode of wall formation has remained unknown. It is an important fact that the wall formation usually occurs during the so-called zonation stage. The chief characteristic of this stage is the formation of the plasma membrane of the oosphere with a film of granulated protoplasm and numerous nuclei in metaphase on the periphery of it.

Davis[1] is rather inclined to think that, though the nuclei in division frequently lie very close to the boundary of the ooplasm, there is no evidence that the kinoplasmic membrane has any relation to these mitotic figures. But he suggests that there must be an accumulation of vacuoles between the ooplasm and periplasm in order to form the delicate layer along the line where the probable splits occur. For the primary wall is certainly established between two plasma membranes because the secondary layers are added to it from both sides.

Stevens[2] has clearly demonstrated and given figures showing the spindle in metaphase lying across the film between the ooplasm and periplasm. He has also shown some nuclei in anaphase lying directly across the boundary film of the ooplasm.

The question is whether or not the nuclei which lie very close to the boundary of the ooplasm have any relation to the free cell wall formation in the oogonium, or whether their location is simply due to some physical causes, such as surface tension, the formation of vacuoles and the relative density of the cytoplasm in different portions of the oogonium. At any rate further studies on the significance of zonation will not only contribute to the understanding of

1) Davis, B. M. 1904, Studies on the Plant Cell. The American Naturalist. Vol. XXXVIII. No. 450.

2) Stevens, F. L. 1899, The Compound Oospore of Albugo Bliti. Bot. Gaz. Vol. 28. 149-176.

the cell wall formation, but will also aid in solving the problem of the relation of the periplasmic nuclei to the formation of the oospore membrane.

In discussing the fertilization process consideration will be given to the mode of closing the opening in the oosphere membrane after it has been penetrated by the fertilizing tube. This study will also give light upon the general subject under consideration.

Comparatively little is known of zoospores in general and even less of zoospore formation in *Peronosporales*. The most important investigation on the structure of the zoospore is that of Timberlake.[1] Strasburger[2] has written extensively on the subject in Histologische Beitrage, in one article reviewing the entire subject of cilia formation. Dangeard,[3] in an account of the *Chlamydomonadeae*, describes *Polytoma* in particular and compares its structure with that of animal spermatozoa.

In *Plasmopara Halstedii* it was found that in a number of cases conidia developed in the intercellular spaces of the spongy tissue and in substomatal cavities of leaves. Conidia were also found in the tissues of stems and roots, especially where small cavities had been formed by some nematode or insect.

Though cytological and physiological knowledge has advanced much recently, zoospore formation in *Peronosporales* has not been fully explained. Gregory[4] describes the various stages of germination in *Plasmopara viticola* mentioning, with *illustrations*, six stages of zoospore formation. But although he gives the fundamental ideas of germination, the cytologically important points, such as, the protoplasmic

1) Timberlake, 1902, Development and Structure of the Swarmspores of Hydrodictyon. Trans. Wis. Acad. of Sci. Arts and Letters 13: 486.

2) Strasburger, 1892, Schwärmsporen, Gameten Pflanzliche Spermatozoiden. Hist. Beit. 4: 49.

Strasburger, 1900, Ueber Reduktions-theilung, Spindeldung, Centrosomen und Cilienbildner im Pflanzenreich. Hist. Beitr. 6: 1-224.

3) Dangeard, 1899, Mémoire sur les Chlamydomonadinees on l'histoire d'une cellule. Le Bot. VI: 65.

Dangeard, 1901, Étude sur la structure de la cellule et ses functions. Le Polytoma uvella. Le Bot. VIII: 5.

4) Gregory, C.T. 1913, Spore Germination and Infection with Plasmopara viticola. Phytopath. 2: 235-249.

Gregory, C. T. 1915, Studies on Plasmopara viticola. The Session of the International Congress of Viticulture, P. P. I. E. San Francisco, California, July 12-13: 126-150.

activities, the modes of cleavage, the development of cilia and the structure of nuclei are still vague. In a study of the conidia of *Plasmopara Halstedii* in fresh living materials the above points were observed. Using imbedded materials formed in the large intercellular spaces of leaves and roots, the zoospores were satisfactorily sectioned and stained. Thus many inportant facts of cytological interests, lacking in Gregory's work, have been made clear.

In addition to the above the development of haustoria and nuclear division in them is discussed.

Also attention is paid to the nuclei and centrosomes in both reproductive and vegetable cells.

II. Materials and Method.

For the study of free cell wall formation it is necessary to select materials which show many oogonia. Practically the same material which had been used previously in studying the oospore formation and fertilization method was used again. The best materials were collected from young sunflower seedlings infected with *Plasmopara Halstedii*. Especial attention was focused upon the zonation stage in oogonia. The study of this stage is very important for a clear understanding of free cell wall formation.

It many cases it was impossible to get oogonia in a good stage of development from the infected leaves, but usually excellent material was obtained from infected roots and underground stems. Late in April in sunflower seedlings contaminated with *Plasmopara Halstedii* a number of oospores were to be found after four or six weeks. In July and August the sexual stage was not common. As a rule, the oospores were best formed in autumn when the vitality of the host had declined.

Sunflower seeds were sown in a field where *Plasmopara Halstedii* had attacked the plants the preceding year. These seeds germinated after 8 or 10 days. Every third day seedlings showing symptoms of the disease were taken up for examination. Six weeks later, both the main and secondary roots of these diseased and dying plants were found upon microscopic examination to harbor an immense number of oospores.

It is said that the conidiophores of *Plasmopara* develop only from the stomata of leaves; for example, *Plasmopara viticola* on grape, *Plasmopara nivea* on various species of *Umbelliferae, Plasmopara*

obducens on *Impatiens*, *Plasmopara pygmea* on various *Ranunclaceae*, etc.

The branching non-septate mycelia of these mildews wander between the host cells and draw nutriment from them by haustoria, eventually producing conidiophores which protrude through the stomata. The same is true of the conidiophores of *Plasmopara Halstedii* on *Helianthus annuus L.* In most cases they are developed on the leaves (Plate V, Fig. 61), emerging from the stomata on both sides but in some of the above mentioned species they appear only on the upper surface.

The younger seedlings of the sunflower are easily attacked on their leaves by this fungus. The stem being short the leaves of the seedlings are quite near the surface of. the earth, and thus are always kept moist by water evaporating from the soil.

It has been proved already that the roots and underground stems are also attacked by this fungus and that in such cases conidiophores are produced much better on the concave surface of a twisted root or on the wounded portions caused by nematodas or injurious insects.

In sunflowers the formation of conidiophores could occasionally be detected in the larger inter-cellular spores on some lysigenous cavities of the Pith, Cortex, etc., of the underground stem, or even in the substomatal cavities and larger intercellular spaces of the spongy mesophyll of the host leaves (Plate V, Figs. 60, 62 and 63). Evidently light is not essential to the formation of conidiophores.

Hitherto no one seems to have observed the formation and presence of the conidiophores and mycelium of *Plasmopara Halstedii* developing on the roots and underground stems of the plant. De Bary[1] pointed out the ability of conidia formed on the potato tuber to reach the surface of the soil and cause foliage infection. Jensen[2] claimed to have found a case where the shoots were killed by the mycelia of a potato root fungus before they reached the surface of the soil and stated that the spores, formed on these shoots, infected the stem of a healthy plant which grew in close proximity. Hecke[3]

1) De Bary, A. 1876, Researches into the Nature of the Potato Phytophthora Infestance. Journ. Bot., N. S., 5: 105-26, 149-54. (Illust.)

2) Jensen, J. L. 1887, Moyens de combattre et de détruire Le Peronospora de la Pomme de Terre. In Mem. Soc. Nat. Agr. France, L. 131, P. 31-156.

3) Hecke, L. 1898, Untersuchungen über Phytophthora infestans de By. als Ursache der Kartoffelkrankeit. Sep. Journal für Landwirtschaft. Pag. 71-73 und Pag. 79-142. Mit 2 Tafeln.

and Clinton[1] are inclined to believe that the primary infection is widely caused by the perennial mycelium. In addition to this Jensen states that the repeated infection of the potato from year to year is not traceable to the presence of conidia in the soil. Many knowing these facts have thought it impossible for the conidia to grow on subteranean organs. But in experiments and observations on sunflower seedlings conidia were found growing both on roots and on underground stems. The conidiophores emerge from the stomata of the underground stem and break through the middle lamella of the epidermal cells of the root portion. The cell walls of the root epidermis are decidedly thinner and softer than those of the leaves, lacking the cuticular layer.

Caroline G. Howe[2] has studied the root hairs of various plants. She has noticed that there is no cellulose in the root hairs and that hairs grown in both loam and sand have a layer of pectic material on the outside and a layer of callose on the inside.

A morphological study of the underground and leaf conidia showed that conidia formed on the underground organs are slightly larger in size than those formed above ground. The conidia produced underground measure from $33\mu \times 48\mu$ to $56\mu \times 60\mu$, and those produced on leaves from $30\mu \times 33\mu$ to $36\mu \times 57\mu$.

The factors causing this enormous enlargement of the subterranean conidia are probably in the supply of nourishment and also in such environmental conditions as: humidity of air, lack of light and a comparatively constant temperature. These factors all tend to lesson the transpiration and to maintain constantly the turgor of the conidia.

As the number of conidia formed under such conditions is always remarkably less than the number formed on ordinary aerial spores, the nourishment which each conidium has to share is proportionately abundant. The number of zoospores formed in such a huge conidium is sometimes more than 40. Compared with the ordinary aerial conidia which are known generally to produce about 8 zoospores, such subterranean spores are gigantic.

In addition to observations on the various phases of conidiophores and conidia the affected roots, leaves and stems of matured sunflower

1) Clinton 1906, Downy Mildew, or Blight, Phytophthora infestans (Mont.) De By., of Potatoes. II. Report of the Connecticut Agricultural Experiment Station Part V. Report of the Station Botanist.

2) Howe, C. G. 1921, Pectic Material in Root Hairs, Bot. Gaz. 72: 313-320.

plants, and the seedlings in infected soil were studied. Special attention was paid to the roots and cotyledons. Conidia were found growing better on the roots and cotyledons of very young plants. It was observed that while conidia are produced in the sunflower garden from April to September, in the spring season (April and May) they grow better than in summer and autmn.

The conidia formed in the tissue of the stem or leaves were especially good materials for cytological studies. Such material was fixed with a weak Flemming's solution for 24 hours using about 50 parts of the solution to one of the material. The material was cut into pieces 7–10 mm. in length and fixed and imbedded in the usual way. Sections were cut 5 μ—7 μ in thickness. In most cases Flemming's triple stain, gentian violet, safranin and orange G. were used. Some sections were stained with iron haematoxyline. The mode of cleavage, the development of cilia, centrosomes and the appearance of cytoplasm were thus well demonstrated.

In studying haustoria ruthenium-red and methyl-blue are especially suited for differentiating the cell wall of the host from the haustorium.

III. Development of the Oosphere Membrane.

a. On the Free Cell Formation in Oogenesis.

The fertilization process and oospore formation of *Peronosporales* have been studied in some detail by Davis on *Albugo candida*,[1] by Stevens on *Albugo Bliti*[2] and on *Albugo*,[3] by Wager on *Peronospora parasitica*,[4] and by others. But the formation of the boundary between the periplasm and ooplasm, and especially the origin of the accumulation of kinoplasmic substance which forms the membrane, have not been satisfactorily explained.

The oospore formation of *Peronosporales* has been considered as one case of free cell formation. This formation is characterized by the fact that the whole of the cytoplasm of the mother cell is

1) Davis, B. M. 1900, The Fertilization of Albugo candida. Bot. Gaz. 29: 297.

2) Stevens, F. L. 1899, The Compound Oosphere of Albugo Bliti. Bot. Gaz., Vol. 28: 149-176.

3) Stevens, F. L. 1901, Gametogenesis and Fertilization in Albugo. Bot. Gaz., Vol. 32: 77-98, and 238-261.

4) Wager, H. 1900, On the Fertilization of Peronospora parasitica. Ann. Bot. 14: 263-278.

not used up. A nucleus usually becomes the center around which cytoplasm is accumulated and separated from the rest of the cell contents. Thus the new cell lies free in the protoplasm of the mother cell. Hitherto the examples of this free cell formation have been limited to a very few instances, namely, the formation of ascospores, the embryo cell of *Ephedra*, and probably of other *Gymnosperms*. Free cell formation is also exemplified in the conditions presented by the egg and synergids and the antipodals of an embryo sac.

As the researches of Harper[1] have shown, in the final divisions in the *Ascus* the nuclei lie in the cytoplasm, each with an aster at its side. A delicate prolongation carries the aster with its centrosphere away from the main body of the nucleus. The rays of the aster now bend over and grow around the nucleus, presenting an umbrella-like figure. They finally meet on the opposite side. Thus is formed the peripheral layer. The substance of the aster fibers forms the basis of a kinoplasmic film which becomes the plasma membrane of the ascospore and develops the spore wall externally after the usual method.

In the developing embryo of the *Gymnosperms*, free cell formation takes place during the differentiation of the embryo cells. The cytoplasm collects around each nucleus, forming a sphere, and a wall is developed on the outer side of this body. Details of the process are not known, and it is not clear whether the position of the membrane is determined by the vacuoles which are generally conspicuous in the region or whether there are fibers radiating from the nucleus which might lay down a cell membrane around the denser protoplasm; but the evidence favors the former possibility.

In the egg apparatus of many embryo sacs slightly similar conditions may be found. The egg nucleus and synergids, in some forms, are surrounded by a dense mass of radiating fibers. Possibly the position of the plasma membrane forming the cell wall is defined by these fibers plus the cell membrane. However, in the egg apparatus of many other embryo sacs fibers are not conspicuous. The protoplasm, in these cases, gathers about the nuclei appearing as dense areas bounded by vacuolar cytoplasm. Possibly the vacuoles fuse with each other to delimit the respective regions and thus to

1) Harper, R. A. 1897, Kerntheilung und freie Zellbildung im Ascus. Jahrb. f. wiss. Bot. 30: 249.

determine the plasma membranes of the egg and synergids.

Oogenesis in *Peronosporales* has been described in some detail by others, but the process has not generally been called free cell formation. Yet at the end of the process the oospore, enveloped by periplasm, lies free in the oogonium. From the cytological point of view, in the process of wall formation of oospores a study of zonation is necessary for a full understanding of the free cell wall formation in oogonia.

In 1899 Stevens[1] described the differentiation of the compound oosphere in *Albugo Bliti*. Earlier studies had carried the history of the sex organs up to the penetration of the oogonium to 1/4 or 1/5 its diameter by the antheridial tube. As the tube develops further the periplasm and oosphere are differentiated and the nuclei are thrust out from the center of the oogonium. This process is essentially a centripetal movement of the cytoplasm. The cytoplasm concentrates in the center of the oogonium forcing the vacuoles and nuclei to the outer edge of the more dense central portion. As a knowledge of this complex process is essential to an understanding of the further development of the oospore a detailed description must be given.

The first indication of a centripetal aggregation is discovered when the cytoplasm shows a tendency to collect in masses in the interior, departing from the uniform distribution seen in young oogonia. As these denser portions run together, several conspicuous bodies of cytoplasm are formed which are separated from each other and from the cell wall by vacuoles of various sizes. These denser portions are of homogeneous structure. They contain minute vacuoles, uniform in size, distributed throughout a matrix of granule-free cytoplasm. They do not contain any nuclei as these have been extruded from the cytoplasm to a position on its outer edge. Next in the process, the denser portions coalesce forcing the vacuoles out further. This coalescence frequently proceeds slowly and irregularly. As it continues the last gap narrows until only a few vacuoles remain to mark its location. These soon float outward leaving a solid mass of cytoplasm which is the rudimentary oosphere. As the vacuoles pass outward captive nuclei are often left behind, but these soon follow. Along the boundary between these two regions most of the nuclei are gathered, though a few may be scattered through the

1) Stevens, F. L. op. cit.

periplasm or occasionally in the oosphere.

The next stage in the development of the oosphere ends in producing a complete differentiation between the oosphere and the periplasm. This stage is distinguished by the coming together of the nuclei, which were situated at the rudimentary boundary of the two regions, into an oval or irregular hollow sphere. While the nuclei are thus arranging themselves, important changes occur in the cytoplasm. In contrast to the dense central cytoplasm the region soon to become periplasm is coarsely vacuolated but the two regions gradually blend where they meet. As the hollow sphere of nuclei becomes more regular, dense granular cytoplasm is formed around and between the nuclei. The inner border of the rudimentary periplasm develops a film more densely granulated than any other part of the oogonium. This finally delimits the oosphere, but it is not yet an organized wall for a careful examination reveals nothing more than a dense film of protoplasm.

Thus the stages of development in the process of differentiation seem to be complete. In this sequence of events the position of the zonation stage is clear. With this stage comes a characteristic sharp demarcation between the oosphere and the periplasm which is maintained until maturity. Before zonation this demarcation did not exist.

Stevens[1] states that in *Albugo portulacae* the gathering together of the cytoplasm into several regions of greater density is the first indication of the development of the oosphere. These regions coalesce to form one large mass of fine uniform cytoplasm. There is a difference not only in the structure of the dense alveolar center and the vacuolated periphery but also in their stain reactions. The denser, fine-grained cytoplasm refuses the gentian violet, but takes the orange G. lightly, while the vacuolated peripheral cytoplasm takes the gentian violet readily. The uniformly dense alveolar region is the rudimentary oosphere. It is in the center of the oogonium.

Throughout this differentiating process the nuclei, which are now in mitosis, are crowded out of the denser mass and come to lie near the larger vacuoles. Therefore, after the denser mass has coalesced and the larger vacuoles are forced into the periplasm, the nuclei are to be found near the rather indefinite boundary between

1) Stevens, F. L. 1901, Gametogenesis and Fertilization in Albugo. Bot. Gaz., Vol. 32: 77-98, 157-169 and 238-261.

the periplasm and the oospere. This boundary, however, becomes distinct and sharp immediately after the nuclei have passed to the periphery. Meanwhile the mitosis has advanced from prophase to metaphase. In this condition the oogonium contains a region of uniformly, finely vacuolated cytoplasm devoid of nuclei, near which is a zone of cytoplasm bearing large vacuoles and containing nuclei in metaphase.

It has been reported by several scientists that certain species present the first clear differentiation of the oosphere from the periplasm at a time previous to the existence of any wall between these parts, and they have found that the oosphere was nearly devoid of nuclei.

Zonation is very definitely and clearly marked in *Albugo Bliti* and *Albugo portulacae*, the periplasm and oosphere being as sharply separated as though an actual wall existed between them. It is much less conspicuous in *Albugo tragopogonis* and *Albugo candida*, thus rendering these species more difficult to understand. Stevens[1] found that the mode of zonation in *Sclerospora* is not that of *Saprolegnia* which was studied by Trow[2] and Miyake,[3] nor that of primitive *Albuginaceae.*[4] It does agree closely, however, with *Peronosporaceae.* While the zonation of *Albugo candida* agrees with that of *Peronosporaceae*, *Sclerospora* shows by the nature of its coenocentrum, which in structure, history, and function is clearly like that found in *Peronospora*, that it has a closer relationship to *Peronosporaceae* than to any *Albugo.*

Davis[5] suggests the probability of an accumulation of kinoplasm, derived possibly from the plasma membrane of the numerous vacuoles which form a delicate layer between the two regions of the oogonium. This idea arose from the fact that a primary wall is certainly formed between two plasma membranes, because, on both sides of it, secondary layers are added. He also suggests a probable split in this kinoplasmic layer along the line of vacuoles found between the oosphere

1) Stevens, F. L. 1902, Studies in the Fertilization of Phycomycetes. Bot. Gaz. 34: 420–425 pl. 17.

2) Trow, 1901, Biology and Cytology of Pythium ultimum. Ann. of Bot., Vol. 15: 269.

3) Miyake, K. 1901, The Fertilization of pythium de Baryanum. Ann. Bot., Vol. 15: 653–667.

4) Stevens, F. L. 1899, The Compound Oosphere of Albugo Bliti, Bot. Gaz., Vol. 28: 149–176.

5) Davis, 1904, Studies on the Plant cell. The American Naturalist. Vol. XXXVIII. No. 450: 462–464.

and the periplasm. Frequently nuclei in the division stage are found very near the boundary of the oosphere but there appears to be no relation between the kinoplasmic membrane and these mitotic figures. In other words, no fibriallae are found which might contribute substance to the membrane. Hence it must be that its development is concerned with the vacuoles only. There is an analogy between this and the part taken by the vacuoles in the plasmodium and in certain of the sporangia during the process of cleavage by constriction.

The species above mentioned have a few points in common, namely: the aggregation of protoplasm, the migration of nuclei, in some species the appearance of a coenocentrum, and the development of a monocyst (receptive papilla). These interesting phenomena should be studied more fully together with the wall formation of the oosphere.

The wall of the oosphere is developed without any relation to the cell wall, and thus the shape of the mother cell apparently is not changed by cell division.

In an earlier stage of the development of the oogonium the cytoplasm was vacuolated uniformly, and there was no difference in the arrangement of nuclei in the periphery or the central region. This is true in all species of *Peronosporales*. In *Plasmopara Halstedii* the cytoplasm was distinct in the earlier stages, and it was noted that the vacuolar membrane (tonoplast) absorbed orange G; however, in the inside solution, the cell sap appeared as a hyaline liquid.

The most prominent changes occur to the vacuoles in more advanced stages. The first sign of differentiation of the cytoplasm is the aggregation of vacuoles which usually occurs in the central region in one, two or three groups. This change in the cytoplasm is very essential in the process of free cell formation and may be caused chiefly by changes of surface tension. The viscosity of the cytoplasm gradually lessening in the later stages, and the surface tension constantly tending to form the minimal square measure of the vacuoles, causes them to come in contact with each other. This action goes on in all directions in the vacuoles. In the early stages the viscosity of the cytoplasm being stronger the vacuoles keep their number and their size, but in the later stages the vacuoles fuse, decreasing in number but increasing in size. This must be the natural result of protoplasmic degeneration.

b. Noticable Factors in the Wall Formation at about the Zonation Stage.

The important relations between the zonation stage in oogonia and the wall formation of oospores has already been mentioned.

For a clear understanding of the early development of the wall attention must be called to the following points: the nature of the coenocentrum, the formation and disintegration of the vacuoles, the activity of muli-nuclei and the development of the monocyst.

1. The Nature of the Coenocentrum.

The coenocentrum was found first by Wager[1] in the oosphere of *Albugo* (*Albugo candida*), and later in other fungi. Its characteristic features were investigated by Stevens[2] on *Albugo Bliti*, and later Davis[3] studied it more closely on *Albugo candida*. In 1907 Wager studied it on *Peronospora parasitica*, and noticed that a re-entering female nucleus was attracted by the coenocentrum. Stevens[4] recognized similar phenomena on *Albugo tragopogonis*. He pointed out that the phenomenon most strikingly demonstrated on *Albugo candida* is the several daughter nuclei which remain anchored to the coenocentrum. He stated that "whether there is an organic attachment or merely an imbedding of a projection it is impossible to say, but certainly these daughter nuclei protrude a long pseudopodium-like extension which penetrates the coenocentrum to a considerable depth. In a later stage, as the nuclei pass to the periplasm, fewer are found attached to the coenocentrum, with the result that eventually, only one remains. This nucleus enlarges greatly, and is often found lying in the cytoplasm in such a position as to suggest that it had been fixed while swaying to and fro on the stalk-like pseudopodium which connects it to the coenocentrum. The migration and attachment of the nuclei to the coenocentrum seem inexplicable on any basis save that of chemotactic influence."

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1) Wager, H. 1890, On the Structure and Reproduction of Cystopus candidus Lev., Ann. of Bot. 10: 295.

2) Stevens, F. L. 1899, The Compound Oosphere of Albugo Bliti., Bot. Gaz., 28: 149-176.

3) Davis, 1900, The Fertilization of Albugo Candida. Bot. Gaz., 29: 297.

4) Stevens, F. L. 1901, Gametogemesis and Fertilization in Albugo. Bot. Gaz., 32: 77.

Phenomena similar to those mentioned above are also found in *Plasmopara Halstedii*. One nucleus is attached to the coenocentrum and often shows an irregular, amoeboidal shape. It appears that the coenocentrum has the power to attract the nucleus. This attractive power is recognized by several scientists. Davis[1] describes the entire development of the coenocentrum, and shows that in a later stage, large globules appear at its center. This is very similar to the case of *Albugo tragopogonis* with the exception that the coenocentrum is very granular. These granules show a stain reaction. Their size is similar to that of the nucleoli. In some cases they appear to enter the coenocentrum from the ooplasm in very early stages and may be seen in great numbers in the adjacent region. The nuclei at this period are in mitosis, and it is quite common to see two, three or even more spindles with one apex imbedded in the coenocentrum. Their form is elongated before their attachment, and it is evident that the coenocentrum possesses an attracting influence. Similar attraction for resting nuclei has been observed by Wager[2] in *Peronospora parasitica*, and by Stevens in *Albugo tragopogonis*; but in *Albugo candida* the phenomenon of attraction is exhibited only during mitosis. Attachment often results in a spindle nearly twice the normal length and proportionately narrower.

Stevens is intensely interested in the fact that the coenocentrum is the source of nutrition for the nuclei, so he introduces cases from other forms of life, which may be summarized brifly as follows: Evidence of light specialization in chloroplasts is given in the observations of Oltmanns on *Coleochaete* (1898), and of Davis on *Authocerous* (1899), where the chloroplasts divide in advance of the nuclei, so as to provide daughter structures equal in number to the nuclei which are to be formed. The development of the pseudopodium-like structure is analogous to the animal cell, where the nucleus becomes amoeboid or protrudes many pseudopodia in order to enlarge its absorptive surface (Korschelt 1889). In the nucleus under consideration the nature of the nutriment may render it more advantageous to penetrate by means of a sharp projection. Many plants possess cells having very irregularly shaped nuclei, e. g., endosperm cells of *Zea*, epidermal cells of *Allium* and *Hyacinthus* (Zimmerman 1896).

1) Davis, 1900 The Fertilization of Albugo candida. Bot. Gaz., 29: 297.

2) Wager, H. 1900, On the fertilization of Peronospora porastica. Ann. of Bot., 14: 263.

Kohl (1897), using *Asparagin*, has incited such movements in the nuclei of the cell of *Flodea* and *Tradescantia*, and considers them comparable with the phenomena observed by Korschelt (1889). This also throws light on the behavior of the nuclei in *Albugo*. Kohl agrees with Korschelt that the amoeboid movement stands in direct relation to the increased exchange between the nucleus and cytoplasm. The coenocentrum may in a sense be likened to some of the so-called yolk nuclei or Dotterkern of animal eggs. In *Albugo Bliti* (Stevens 1899, Fig. 69) and in *Albugo candida* (Davis 1900 Fig. 2) there is a slight indication of a radiating structure which somewhat resembles the figures in Munson's (1898) illustration of the yolk nucleus of *Limulus*. The structures agree in having nutritive functions. In certain cases, as in *Limulus* (Jordon 1893), the yolk nuclei seem to develop directly from the cytoplasm, thus further resembling the coenocetrum. A comparison of these structures emphasizes the fact that protoplasm, in diverse organisms under certain conditions, may become similarly differentiated for the performance of particular functions. Stevens' observations and consequent conclusion as to the coenocentrum being the source of nutrition for the nuclei are excellent.

The relation between the coenocentrum and zonation must be emphasized especially. A study of the nature of the coenocentrum in *Plasmopara Halstedii* shows that the dense and deeply stainable mass of granules, probably of a chromidial nature is present at the zonation stage. Presumbly this mass is nutritive, and possibly it is concerned in producing oily material in the oosphere. It seems that the function of the coenocentrum in the oosphere formation is very significant.

In this connection it is necessary to describe the protoplasmic activity in the oogonium before the coenocentrum formation. In *Plasmopara Halstedii* it was noticed that the changes of protoplasm are caused by both external and internal stimuli. The mechanical pressure caused by the contact of the antheridium with the oogonium is the external stimulus. The internal stimulus takes place later after the antheridial tube has penetrated the oogonium and finally discharged its contents into the interior. These chemical and physical stimuli induce metabolism in the oogonium and there commences various morphological changes, such as the migration of nuclei, the aggregation of central vacuoles, etc. It has been demonstrated in *Plasmopara Halstedii* that the coenocentrum consists of two or more

small granules and is surrounded by a region of denser cytoplasm. The cytoplasm is connected with the coenocentrum and takes the form of radiating strands.

This especially advanced and extraordinarily vacuolated cytoplasmic mass develops as the oosphere matures and disappears before fertilization. There is a tendency to aggregate around the coenocentrum as a center of adhesion, so it evidently does more than act merely as a source of nutriment for the nucleus. Its position and cytoplasmic connections would seem to justify Stevens opinion that it is the dynamic center of the compound oosphere.

2. The Formation and Disintergration of the Vacuoles.

The changes in the vacuoles of the oogonium were especially noticeable, before and during the wall formation period. The problem to be considered is whether they directly or indirectly aid in this wall formation. It was noticed that the vacuoles surrounded the oospore separating it from the periplasm; thus it would seem that the plasma membrane of the vacuoles forms the primary membrane of the free cell wall in the oogonium.

Davis[1] favors this vacuolar theory. According to him, "The spore wall develops at the boundary of the ooplasm, so that it lies close to the large vacuoles in the periplasm. There must be an accumulation of kinoplasm, perhaps from the plasma membranes of numerous vacuoles, to form a delicate layer between the two regions of the oogonium. This layer of kinoplasm probably splits along the line of vacuoles between the oosphere and periplasm, for the primary walls are certainly established between two plasma membranes, because the secondary layers are added to it from both sides. Nuclei in division frequently lie very close to the boundary of the oosphere, but there is no evidence that the kinoplasmic membrane has any relation to these mitotic figures. That is to say, there are no fibrillae to contribute substance to the membrane, and its development must be concerned with vacuoles alone." However, this statement is not entirely satisfactory, because in some cases even where the number of nuclei in the oogonium is small, a wall is dimly discernable, but where there are a large number of nuclei the wall is more clearly defined and its development progresses more

1) Davis, B. M. 1904, Studies on the Plant cell. The American Naturalist. Vol. XXXVIII. No. 450: 462-464.

more rapidly. This indicates that the nuclei play an important part in the wall formation, and their location near the boundary of the central mass becomes reasonable. Another very important fact is that in all cases, whether the number of nuclei be great or small, the outer vacuoles together with the above mentioned nuclei connect the oosphere with the vacuoles of the periplasm.

The fusion of the vacuoles takes place both in the periplasm and oosphere, but the speed of that fusion is far greater in the periplasm. The number of vacuoles in the periplasm gradually decreases as their size increases. Sometimes the vacuoles in one part are cleared up while in another part they are still connected with the outer vacuoles of the oosphere; in the former case the development at the wall of the free cell is much advanced.

This development recalls the part played by vacuoles in the plasmodium and in certain sporangia during cleavage by constriction. Such multi-nucleate masses of protoplasm as the plasmodium and portions of coenocytes usually divide to an extensive degree at reproductive periods. This is always accomplished by means of cleavage by constriction, in which the vacuoles frequently assist by helping to cut the protoplasm in the same manner as the cleavage furrows. The fusion of the vacuoles is noticeable but in addition to this attention must also be called to the fact that about the time of nuclear division a kinoplasmic substance collects near the nuclei and also near the surrounding membrane where the wall is forming. It would seem that this kinoplasmic substance comes not only from the nuclei which have degenerated but also from the tonoplast of the vacuoles. During the degeneration of the nuclei and the clearing up by fusion of the vacuoles in the periplasm, the wall formation process continues, as is clearly shown by an increasingly distinct staining.

A somewhat similar phenomena take place in other cases, and the following may give some further light upon this method of wall formation by the connecting of minute granules. Farmer and Williams[1] and Strasburger[2] observed and described in detail the cell division in the oogonium of *Fucus*. First, three successive divisions result in the formation of eight nuclei. Then, each individual

1) Farmer and Williams, 1896, On the Fertilization and Segmentation of the Spores in Fucus. Ann. of Bot., 10: 479.

2) Strasburger, 1897, Kerntheilung und Befruchtung bei Fucus. Jahrb. F. Wiss. Bot., 30: 351.

nucleus becomes elongated and radiating fibers appear at the poles. At the same time the granules begin to gather at the dividing surface, and the spindle fibers which are related to the division, disappear. Then all the granules migrate towards the dividing surface. These connect with one another thus forming the layer-like cell plate.

According to Yabe and Yasui[1] the wall of the spore in Ceratopteris thalictoroides Brongn is formed as followed: Four groups of chromosomes move towards the poles of the spindles and having arrived form a nuclear membrane. During the resting stage that follows the spindles fibers still connect the four nuclei. Then gradually contraction towards the center takes place and granuoles of kinoplasmic substance appear. These granuoles are deposited between the nuclei and out of them the wall is formed.

In *Plasmopara Halstedii*, however, most of the nuclei and daughter nuclei arrange themselves along a faint film which forms a line of demarcation but some daughter nuclei lie across this film opposite each other and show the line between them. It cannot be concluded that the film between the daughter nuclei forms the wall, as was demonstrated in the *Fucus*, but it must be recognized that the minute granules which collect close to the forming wall must be derived from the nuclei and vacuoles which have degenerated, because the phenomenon is found only during this particular stage.

Close observation leads to the conclusion that the activity of the vacuoles in the oogonium is as follows: In the early stage of the oogonium the cytoplasm gradually becomes finely vacuolated in the central region. These numerous vacuoles temporarily keep their form and volume without alteration. But in a later stage some of the minute vacuoles become larger by fusion while some of the larger vacuoles give off their cell sap and shrink. These vacuoles gradually become flattened and thus press upon neighboring vacuoles. As the pressure increases adjacent vacuoles fuse more rapidly.

This fusion results in a rapid decrease in the number of vacuoles which lie in the periplasm and takes place most actively in or about the zonation stage.

The changes of the protoplasmic nature may be judged by the following symptoms: first, the oil drops which are few in the early stage become more abundant in the later stage; second, the cytoplasm

1) Yabe, Y. and Yasui, K. 1923, On the Life-history of Ceratopteris thalictoroides Brongn. Bot. Mag., Tokyo, 27: 317.

takes the stain clearly in the early stage but gradually takes it less distinctly; and third, the material which absorbed safranin red and gential violet increases in the later stage.

The minute granules, which may come from the tonoplast and nuclei, are attracted where the surface tension is strongest, which indicates that the surface tension is greatest at the region of wall formation.

It is difficult to define exactly the relation of the nuclei to the wall formation of the oospore. There is fairly good evidence of an accumulation of kinoplasm for the wall formation. But this kinoplasm is mostly derived from the tonoplasts of the numerous vacuoles. There form the delicate wall between the oosphere and periplasm which gradually becomes visible as the fusion of vacuoles described above progresses. It was noticed that a primary wall was established between two plasma membranes; at that stage, however, there was no evidence of the presence of the kinoplasmic membranes of the mitotic figures of the nuclei. In short, there are no indications of an accretion to the membranous oosphere wall from the nuclei. On the other hand, in cases where for some reason the arrangement of nuclei described above fails to take place, the formation of the oosphere wall seems to cease.

3. The Activity of Multi-nuclei.

The development of the oosphere and its wall formation in the oogonium have no relation to the oogonial wall. But it is possible that they may have some relation to the cytoplasmic activity and the nuclei.

The nuclear migration and the appearance of the coenocentrum in the various species of *Peronosporales* and *Albuginaceae* take place in the zonation stage. These interesting phenomena seem to be very important in the free cell wall formation.

Stevens[1] maintains that in the zonation stage in *Albugo Bliti* the nuclei, usually in metaphase, are lined up around the oosphere and some of the spindles lie across a definite boundary that separates the oosphere from the periplasm.

In *Cladophoraceae*, Carter[2] found that usually the nuclei are at least partially imbedded in the chloroplast. This is true even in cells which possess a large amount of colorless cytoplasm. The

1) Stevens, F. L. 1899, The Compound Oosphere of Albugo Bliti. Bot. Gaz. Vol. 28: 149-176.

2) Carter, N 1919, The Cytology of the Cladophoraceae Ann. Bot. 33: 467-468. pl. 27 Figs. 2.

position of the nucleus is related to the cell functions. Generally it lies in that region characterized by the greatest metabolic activity. For example, in the cases of pollen tubes and growing root hairs the nucleus is usually found where elongation is in process. In the case of the thickening epidermal cells, frequently but not always, the nucleus is found near the wall where the thickening material is being deposited. This relation between the function and position of the nucleus was emphasized by Haberlandt[1] and Gerassimow.[2] Harper[3] in his studies of *Didymnium malanospermum* (*Pere*) *Macor* noticed many nuclei in the outer region of the oosphere, but he could not prove that this phenomenon had any relation to the wall formation of the oosphere.

In *Plasmopara Halstedii* the migration of nuclei, the zonation stage and the accumulation of nuclei about the coenocentrum have their physical causes in such things as surface tension and the development of vacuoles.

In the coenocentrum three small globules evidently fusing and surrounded by a region of denser protoplasm were visible. The resultant sphere had attractive power, and was also a source of nutrition. The phenomenon of chemotaxis, which affects the position of the nuclei, is quite parallel to that exhibited so abundantly in the animal cell, where the nuclei wander towards the source of nutritive supply (Korschelt 1889). There would seem to be a reciprocal relation between the migration of the nuclei towards the outer region of the oosphere and the dense accumulation of cytoplasm around the coenocentrum. This later accumulation certainly seems to indicate strong cohesive tendency in the central region.

The accumulation of abundant cytoplasm around the oosphere is a very important phenomenon in the formation of the wall of the free cell. After formation a secondary growth occurs from both sides. The question then naturally arises as to how the cytoplasm accumulated around the oosphere. It was noticed that in the

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1) Haberlandt G. 1887, Ueber die Beziehungen zwischen Funktion und Lage des Zellkerns bei den Pflanzen. Jena.

2) Gerassimow, J. J. 1890, Einige Bemerkungen über die Funktion des Zellkerns. Bull. Soc. Sci. Nat. Moscow: 548-554.

Gerassimow, J. J. 1899, Ueber die Lage und die Funktion des Zellkerns. Ibid. 220-267.

Gerassimow, J. J. 1901, Ueber den Einfluss des Kerns auf des Wachsthum der Zellen. Ibid: 185-220.

3) Harper, R. A. 1914, Cleavage in Didymium Melanospermum (Pers) Macor. The American Journal of Botany, Vol. 1. No. 3: 127-144.

oogonium before the zonation stage three aggregations of cytoplasm appear in the center.[1] Most of the nuclei divide around these. Later the aggregation combines into one spherical mass in the oogonium with the nuclei more distinctly arranged in a circle around the sphere.[2] In this stage the cytoplasm around the periphery condensed. In one of Stevens' figures[3] which shows the condensation of the peripheral cytoplasm most clearly, the nuclei are beginning to degenerate. These nuclear changes may have a very important relation to the formation of the oosphere wall, because in *Plasmopara Halstedii* the wall develops very rapidly at the period of nuclear degeneration. The nuclei apparently aid the wall formation by supplying material for it. This fact was suggested by the stain reaction, because after many nuclei had degenerated the wall absorbed the gential violet and became clearly and brilliantly defined.

The cell wall formation at the division of the somatic cell of *Cutleria* is here recalled. Yamanouchi's[4] description is briefiy as follows: Soon after nuclear division the spindle fibers between the daughter nuclei disappear, then the cytoplasm gradually forms a coarse, irregular network-like structure between the daughter nuclei. This structure becomes connected and perhaps its nature changes so as to form the cell wall. On the whole this method of cell wall formation is similar to that which takes place in *Sphacelaria* and *Dictyota*. On *Cutleria* after the spindle fibers disappear, the network which later becomes the wall, forms with apparently no relation to the spindle fibers. This fact sheds light on the free cell wall formation which takes place in *Plasmopara Halstedii*, namely, that there is little if any relation between the cell wall formation and the spindle fibers connecting the daughter nuclei. However, it must be kept in mind that after nuclear division the free cell wall becomes distinctly visible.

It has been suggested that in *Plasmopara Halstedii* the nuclei control the aggregation of the cytoplasm. In some cases the cytoplasm collects more densely at the place where the nuclei are arranged in

1) Stevens, F. L. 1899, The Compound Oosphere of Albugo Blitii. Bot. Gaz. Vol. 28: 149-176. Fig. 60.
2) Ibid, Fig. 65.
3) Ibid, Fig. 69.
4) Yamanouchi, S. 1909, Mitosis in Fucus. Bot. Gaz., 47: 173-197. pls. 8-11.
Yamanouchi, S. 1912, The life history of Cutleria. Bot. Goz., 54: 441-502. pls. 26-35, fig. 15.

a circle around the periphery of the oosphere particularly in the zonation stage. Even between the nuclei cytoplasm collects somewhat densely to form a cement. This would indicate that the free cell wall may be formed in a like manner.

In short the nuclei, together with the vacuoles, control the cytoplasmic aggregation and guide the cytoplasmic changes before the wall formation in oogonium. Some students believe that the wall formation of the oosphere is brought about by the activity of vacuoles alone. But in *Plasmopara Halstedii* the nuclei work, indirectly at least, for the wall formation. Some oogonia which have a comparatively small number of nuclei show a very thin line of demarcation by vacuoles between oosphere and periplasm. But in other oogonia containing many nuclei the line of demarcation develops into a distinct wall. Hence, the conclusion that vacuoles and nuclei cooperate.

4. The Development of the Monocyst.

The monocyst, also called the receptive papilla, does not appear in all the species of *Peronosporaceae*. Stevens[1] found no sign of it in *Sclerospora*, and it was thought to be lacking in this genus. But it has been found by others in many of the species of this family. Its nature and structure differ specifically in different species. A consideration of it is very important for an understanding of the fertilization process. And special attention must be called to its relation to the wall formation of the free cell in oogonium.

Just as the antherozoids enter the oosphere at a certain point in ferns and other species, in *Plasmopara Halstedii* first a papilla-like structure appears on the oosphere. It is formed during or just at the completion of the nuclear division in the oogonium and is always to be seen on the side where the oogonium comes into contact with the antheridium. It gradually develops and finally penetrates through the periplasm into the antheridium. When this papilla withdraws, a short fertilization tube goes into the oosphere, delivers one male nucleus and the greater part of the antheridial contents, and thus the fertilization process begins.

The interest here is in the relation between the formation of the receptive papilla and the wall formation of the free cell in oogonium. This phenomenon does not appear in the oogonium which is not in

1) Stevens, F. L. 1902, Studies in the Fertilization of Phycomycetes, Sclerospora Bot. Gaz., 34: 420-425. Pl. 17.

contact with an antheridium. Observation showed oogonia which plainly developed zonation without ever forming monocysts. It is to be concluded that the formation of monocysts is caused by the contact stimuli of antheridia, and also by physical changes in the oogonia, such as surface tension, and the relative density of the cytoplasm in different portions of the oogonia. It is very important to note that in cases when a monocyst developed, the wall formation of the oosphere was usually visible very early, and that the wall formation of the oosphere commenced in the region where the monocyst developed.

In an earlier study[1] the construction of the cytoplasm in the oogonium was discussed. The central mass is vacuolated and many nuclei are dividing on its periphery. The monocyst is beginning to form and has an extremely thin wall. The contents are watery, staining homogeneously with the gentian violet. Sometimes the contents are highly vacuolated. The wall itself is also stained by gentian violet. The film of the oogonium is just commencing at the region of the monocyst. In a further stage[2] an accumulation of a denser cytoplasm is to be seen at the tip of the monocyst. This denser cytoplasm represented in black, was stained a deep red by the safranin. Tracing the oogonial wall, below and a little to the right of the monocyst, the film is clearly visible. In one case[3] a large papilla has entered the antheridium, and in the oosphere the spherical surface commencing at the monocyst plainly shows that the delimitation of the wall is not yet complete. Here the denser cytoplasm is stained a deep red by safranin. In the part opposite the monocyst the wall of the oosphere is not visible in the oogonium, but the cytoplasm is uniformly distributed. If this tendency is true in other species, then the development of the monocyst has an important significance for the wall formation.

Accordingly, the following conclusions can be drawn. At the point of demarcation of the monocyst the oosphere wall is developed earlier than in other parts and, because no nuclei are here visible, nuclear division seems already to have taken place. In other parts of the oosphere the nuclear division continues actively. It must

1) Nishimura, M. 1922, Studies in Plasmopara Halstedii. Jour. Col. Agric., Hokkaido Imp. Univ., Vol. XI, Part 3. Plate II, Fig. 4.

2) Ibid, Fig. 5.

3) Ibid, Plate III. Fig. 6.

be kept in mind that the wall formation goes on more rapidly where the activity is greater and where the nuclear division has taken place earlier. At the place where the receptive papilla in the oogonium develops the most active metabolism takes place during this stage. This evidence sheds great light on the free cell wall formation.

c. The Closing of the Opening in the Oosphere Membrane Caused by the Entrance of the Fertilizing Tube.

It is generally recognized that the opening in the oosphere wall is caused by an antheridial tube. In a later stage, this opening is closed. The question naturally occurs as to how this closing is accomplished. As a rule, at the beginning of the fertilization period, the primary wall is clearly established between the oosphere and the periplasm and the secondary layers are about to be added to it from both sides. In *Plasmopara Halstedii* after the fertilizing tube has discharged its contents it remains in the oogonium; its normal outline is visible in the oosphere, but its basal part in the periplasm is degenerating (Plate I, Fig. 7). In some other species the exact opposite is true. For example, in *Phythum de Baryanum,*[1] the basal part of the fertilizing tube remains intact in the periplasm while the apical portion in the oosphere degenerates. In either case there remains an opening at the point of contact between the tube and the oosphere membrane (Plate I, Fig. 7).

It was noticed that at the opening, the wall adheres closely to the tube, so that no intercourse between periplasm and oosphere can occur. This phenomenon is very important.

In *Plasmopara Halstedii* the diameter of the oospere is only seven or nine times larger than the diameter of the opening made by the antheridial tube, so this opening is comparatively large. If this large opening can not retain vapour, then such volatile substances as oil and water etc., may escape and the spore dies. But if the wall adheres to the fertilizing tube, such vapors are retained, and then normal development takes place. In the oogonium the contact of the antheridium with the wall results in a similar protection. This is all due to the fact that in haustoria the mycelium, antheridial tube and the wall of the oosphere have strong adhesive power, and the ability to fuse. These conditions are very important in the fertiliza-

(1) Miyake, K. 1901, The Fertilization of Pythium de Baryanum. Ann. Bot., Vol. 15: 653-667. (see Figure.)

tion process and the spore maturation.

After the discharge of the contents of the antheridial tube the cytoplasmic conditions in the periplasm and oosphere are much changed. For example, the former is coarsely vacuolated and contains large oil drops, while the latter is finely vacuolated and contains only minute oil drops. These differences become more distinct in later stages.

When the nuclei in the oogonium degenerate the vacuoles disappear from around the opening in the oosphere wall. Thus factors which are important in the wall formation are not concerned in the closing up of the opening. It was observed, however, that the portion of the antheridial tube remaining in the ooplasm contains one large vacuole. This vacuole fills the tube so as to prevent any movement of cytoplasm between the oosphere and the periplasm (Plate I, Fig. 7). Close observation showed that the opening is closed by the tonoplast of this large vacuole together with the cytoplasm, which had accumulated on the tonoplast. In a further stage of the replacement process the vacuolar membrane aided by the cytoplasm which contains the degenerated or degenerating nuclei aids the work still further. All of this material is from the antheridial tube.

It was noted that the degeneration of the antheridial tube commences soon after the male nucleus has been discharged. As above mentioned this degeneration first occurs sometimes in the ooplasm, sometimes in the periplasm. In either case the tube loses its general form, but at the place where it penetrated the oosphere it remains intact and continues to adhere to the wall. This adherence induces the degenerated nuclei and cytoplasm from the tube to accumulate in the opening. This point is of significance in explaining the gradual rebuilding of the wall.

Account must be taken of the fact that the periplasm and oosphere also assist in the reconstruction by adding the secondary growth.

It has been shown that the organic union of the two cells is completed by the closing of the opening in the oophere wall. In this process since the cytoplasm and the tonoplast in the antheridial tube perform the same function of wall formation as the protoplasm in the oogonium it is clear that they must be of a similar biological nature. This interesting phenomenon is related to the general phenomenon of cell fusion and also to grafting in both animal and plant tissue.

The cytological studies in cell fusion have been carried on in many different cases; as for instance in the fusion of centrosomes,

gametes, nuclei, sporocytes, and in the fusion of the triple nuclei of endosperms. A fusion of laticiferous vessels is found in fungal hyphae. The fusion occurs at that part of the wall where the two branches come into contact and their protoplasmic contents unite. As is well known, a striking example of this is found in the amoeba stage of the *Myxomycetes* before the formation of a plasmodium. Many amoeboidal bodies assume irregular, constantly changing shape, and are finally capable of performing only amoeboid creeping movements. A number of amoeba eventually collect and fuse.

Grafting is a process in which the scion is inserted in the stalk, in such a way that the former through the use of food materials derived from the latter continues to grow and becomes organically united to the stalk. Experiments heretofore have been somewhat limited. G. Schöne[1] and W. Schultz[2] suggest that the ordinary alternating Mendelian theory of heredity may perhaps hold good in these cases; and that the homoio-character may be dominated over by the auto-character. Schultz points out the significance of tissue transplantation for the analysis of the biochemical nature of the protoplasm. This would seem to show that the biochemical nature of the cells is even more important than the structure for judging the natural system. And that grafting can take place only where the natural systems are closely related.

It is a well known fact that there is a greater possibility for the grafting of embryonic tissue than of adult tissue. Experiments have shown that this is probably due to the similarity in the biochemical and biophysical nature of the protoplasm in the embryonic stage. This applies not only to grafting in the same species but it is also often true for different species. The modern theory is that the transplantibility of the two tissues is chiefly affected by the degree of the reaction. If the special nature of the protoplasm is similar the reaction is so slight that no disturbance is caused by grafting.

The replacement of the opening in the oosphere wall led to the conclusion that the cytoplasm of the oogonium and antheridium is of a similar nature. Additional evidence is found in the fact that no distinct reactions occur. Morerver, this is also proved by the develop-

1) George Schöne, 1912, Die Heteroplastische und homoeplastische Transplantion. Berlin.

2) Walther Schultz. Archiv f. Entwicklungsmechanik, 1910, XXIX, 79. 1912, XXXV, 484; 1913, XXXVI, 353; 1913, XXXVII, 265 und 285.

ment of the oogonium and antheridium. For instance, in *Pytheum* both organs develop on the same hypha, whilé in *Plasmopara Halstedii* some of the organs develop on one hypha and some on a different hypha; in either case the protoplasmic appearance is cytologically the same. When both organs develop on the same hypha the cytoplasm of both antheridium and oogonium is of course of a similar biological nature. In *Plasmopara Halstedii* the formation of the oosphere wall from the antheridial cytoplasm corresponds to the grafting which may take place between different species.

d. Discussion.

The wall formation of the oosphere has been considered under five phases; namely, (1) the coenocentrum, (2) the vacuoles, (3) the nuclear activity, (4) the effect of the monocyst (receptive papilla) and (5) the manner of closing the opening caused by the intrusion of the fertilizing tube. The observations made on these five phases call for a brief discussion.

Before the zonation phenomenon in the oogonium there is no indication of a boundary membrane between the oosphere and the periplasm. The zonation stage is characterized by the cytoplasmic accumulation which forces the vacuoles with most of the nuclei to the periphery. This is believed to have a direct and fundamental connection to the wall formation of the free cell. The cytoplasmic activity orginates in the contact stimulation of the antheridium, and it continues to be affected by such sexual acts as, the penetration of the fertilizing tube and the discharging of the male nucleus and other protoplasmic contents into the oosphere, etc.

When the aggregation commences in the central region of the young oogonium, the protoplasmic metabolism proceeds very actively. The most prominent event which takes place at this stage is the formation of the coenocentrum. Before fertilization this dense, deeply stainable mass of granules is thought to have the nature of chromidia which it later loses. It is presumably nutritive, and possibly concerned in producing oily reserves in the oosphere. It is held in the center by the denser cytoplasm from which radiates fine, cytoplasmic strands (Plate I, Fig. 5). Since the whole structure of the oosphere is connected to it by these strands Stevens is probably right in saying that the dynamic center of the compound oosphere is the coeno-

centrum.

Davis[1] states that in the beginning, the ooplasm gathers in the center of the oogonium as a denser alveolar region around that peculiar protoplasmic body (generally present) the coenocentrum. This accumulation forces the vacuoles, together with most of the nuclei, to the periphery, where they lie as a sort of protoplasmic force next to the cell wall and constitute the periplasm. The spore wall develops close to the inner boundary of these large vacuoles in the periplasm. There must be an accumulation of kinoplasm, perhaps from the plasma membranes of numerous vacuoles, in order to form a delicate layer between the two regions of the oogonium. This layer of kinoplasm probably splits along the line of vacuoles between the oosphere and the periplasm, for the primary wall is certainly established between two plasma membranes because the secondary layers are added to it from both sides. Nuclei in division frequently lie very close to the boundary of the oosphere, but there is no evidence that the kinoplasmic membrane has any relation to these mitotic figures. That is to say, there are no fibrillae to contribute substance to the membrane, and its development must be the part played by vacuoles in the plasmodium and in certain sporangia during cleavage by constriction.

Stevens[2] in *Albugo Bliti* recognized that at the time of complete zonation the nuclei are near the inner edge of the periplasm. Spindles are frequently found lying at right angles across this line, so that one pole lies in the ooplasm and the other in the periplasm. The mitosis that takes place at this period marks an important and characteristic phase in the history of the oogonium. Those dividing nuclei that lie tangential to or wholly outside of the boundary line between the oosphere and the periplasm leave their daughter nuclei in the periplasm. Each of the spindles which cross the line (Stevens Figs. 66) gives one daughter nuclei to the oosphere and the other to the peripasm. The line of differentiation is sharply defined and unmistakable.

In *Plasmopara Halstedii* the zonation stage is completed just before the wall formation begins. In one case,[3] nuclei in mitosis were found

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1) Davis, op. cit.

2) Stevens, F. L. 1899, The Compound Oosphere of Albugo Bliti, Bot. Gaz., Vol. 28: 149-176. (Figs. 65 and 66.)

3) Nishimura, M. 1922, Studies in Plasmopara Halstedii. Jour. Col. Agric. Hokkaido. Imp. Univ., Vol. XI. Part 3. 135-210 (Plate III. Fig. 1).

arranged in a circle, about half of which corresponded exactly with the surface of the oosphere, and the remainder passed inside the finely vacuolated region. Close observation showed that minute granules were collected together between the nuclei along the line of the circle but a distinct wall had not yet been formed. This phenomenon seems to indicate that the nuclei probably work toward some particular form of arrangement which aids in the formation of the wall.

In discussing the development of the oosphere membrane it was concluded that the primary wall is formed by the plasma membrane of the outer-most vacuoles of the oosphere. The vacuoles are first present and are arranged so as to indicate clearly the location of the primitive wall (Plate I, Figs. 4 and 6) and the vacular membrane (tonoplast) becomes the most fundamental substance in the formation of that wall. As the development proceeds the metabolic activity becomes more rapid, and the nuclei approach the periphery of the oosphere where the wall is forming. This nuclear migration is probably explained by the Haberlandt[1] theory that the nuclear position is related to the function of the cell and that, in general, the region where the nuclei lie is characterized by the most active metabolism. Both in the division stage and later also in the disintegration stage these nuclei seem to be working in some way for the wall formation.

As before stated, especially in oogonia which have a smaller number of nuclei, the outer-most vacuoles form the line of demarcation between the periplasm and oosphere, but it is uncertain as to whether or not this very line is the wall because usually in such oogonia the wall is not developed as much as in oogonia having a greater number of nuclei. Over and above these factors still further evidence of nuclear relation to the wall formation is found in the fact that the monocyst (receptive papilla) begins its development at the period when many nuclei are dividing on the margin of the oosphere, and that the wall first develops in the region where the monocyst is formed. Where the monocyst is discernible the wall is already formed, and no nuclei are to be found in this region, indicating that nuclear division has already been completed, which is in distinct contrast to other parts along the line of demarcation where many nuclei are still to be found actively dividing.

Finally, emphasis must be placed on the fact that there is no

1) Haberlandt, G. 1887, Ueber die Beziehungen zwischen Function und Lage des Zellkerns bei den Pflanzen. Jena.

distinction, morphologically or micro-chemically, between the original wall and the new layer formed to close up the opening caused by the intrusion of the fertilizing tube. This indicates that for the wall formation of the oosphere the cytoplasm in the antheridium has the same value as that in the oogonium. This is of interest in connection with the protoplasm of the different sexes, and also in connection with the problem of the union of different individuals by apposition (grafting). For Strasburger[1] proved that in grafting a fusion of the protoplasm of the stock and scion occurs.

Heretofore it has not been known whether the antheridium and the oogonium in *Plasmopara Halstedii* develop from the same hypha or from different hyphae. This distinction is very important in determining the nature of the protoplasm. In *Achyla*[2] and *Pythium*[3] it has been clearly demonstrated that both the antheridium and the oogonium develop on the same hypha. And now it is definitely known that the antheridium and the oogonium in *Plasmopara Halstedii* are uni-sexual on different hyphae (Plate I, Fig. 5).

The cytoplasm which is contained in the antheridial tube has the same value as that employed in the formation of the oosphere wall. This was demonstrated in the closing of the wall opening which was formed by the intrusion of the fertilizing tube. The reconstruction of the cell wall appears to have been done principally by the tonoplast of the large vacuole in the fertilizing tube together with the cytoplasm and degenerating nuclei.

In summarizing, it is evident that the active cooperation of the nuclei and vacuoles, is a factor towards the wall formation. The tonoplast is fundamental in the earliest film stage, but without the aid of the nuclear activity this film would never develop. It is clear that the nuclei are related to the accumulation of the minute particles and that this accumulation contributes to the development of the first demarcation of the wall.

Moreover, because nuclei are always found at the outer region of each aggregation of cytoplasm even before the zonation stage, it is evident that the nuclei have power to control the cytoplasmic

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1) Strasburger, E. 1901, Jahrb. f. Wiss. Bot. 36: 493.

2) Trow, A. H. 1899, Observation on the Biology and Cytology of a new variety of Achlya amevicana. Annales of Botany, Vol. XIII. No. XLIX. pl. VIII, Figs. 3-9.

3) Trow. 1901, Biology and Cytology of Pythium ultimum. Ann. of Bot. 15: 269.

aggregation before the wall formation commences. It is possible that the degeneration of the nuclei may also contribute towards this wall development.

IV. A Cytological study of Zoospore Formation in Conidia.

a. Historical.

The conidia of the genus *Plasmopara* has been studied both morphologically and physiologically by many scientists. Recently, the germination of conidia, and the nature and development of zoospores have become subjects of much interest.

Many have been the experiments conducted to determine the relation of temperature to germination. Patrigeon[1] states that a temperature of from 25° to 30°C. is best suited for the purpose. This is confirmed by Viola[2] who affirms that the germination of zoospores occurs within the space of from thirty minutes to one hour at a temperature of from 28° to 30°C. At a temperature of from 10° to 17°C. germination occurs only after two or three days, and at from 2° to 5°C. germination is entirely prevented. Istvanffi,[3] on the other hand, states that the optimum temperature for germination is from 20° to 22°C., while at from 28° to 30°C. germination practically ceases, being feeble at the end of from six to ten hours. He found that after 2 or 3 hours at a temperature of 14° or 15°C. germination is profuse and that at 8°C. it becomes slight. Melhus[4] states that a temperature of 10°C. is most favorable to conidial germination. Gregory[5] states that the principal factors influencing the germination of conidia, aside from the presence of moisture, are the temperature and the proper age of the conidium. He attempted to germinate conidia at room temperature of from 21° to 27°C. and often as high as 32°C. In some cases there was absolutely no germination. From

1) Patrigeon G. 1881, Le Mildiou: 16-25. Fig. 1-10.

2) Viola, P. 1893, Les Maladies de la vigne: 57-155.

3) Istvanffi, G. and Palinkas, G. 1913a—Etudes sur le mildiou de la vigne. Inst, Centr. Ampel. Roy. Hong. Ann. 4: 1-122. 1913b—Etudes sur le mildiou de la vigne. Rev. Vit. 40: 481-484, 509-513, 540-543.

4) Melhus, I. E. 1911, Experiments on Spore Germination and Infection in certain Species of Oomycetes. Wisconsin Agr. Exp. Sta. Research. Bul. 15: 46-71.

5) Gregory, C. T. 1913, Spore germination and infection with P. viticola phytopath. 2: 235-249.

these statements it can be seen that the divergence of opinion on this subject is very great.

Farlow[1] investigated mildew on the American grape vine and observed a mass of swarm spores pausing at the mouth of the conidium and later dividing into individuals. The number of swarm spores in one conidium differed according to circumstances, varying in general from two to seventeen. He noticed that germination is quite regular though a longer period is required by some. Usually an hour and a quarter is taken. After germination the swarm spores swim about actively and constantly change their shape. This may continue for about fifteen or twenty minutes. Then coming to rest, they become spherical and drop their cilia. In about a quarter of an hour a germination tube is produced. These facts are fundamental to what is known of this group. Those scientists who followed Farlow either confirmed his findings or added something to his work.

Patrigeon[2] in 1881 evolved a new theory of conidia germination. He said that the entire protoplasmic mass escapes out of the conidium and then sends out a germ-tube, but that in the case of *Plasmopara* this is rather rare. Wüthrich[3] never observed the *plasmatoparous* and so never found a germ-tube. However, he made a physiological study of conidia and believed that simply water and damp air are necessary for germination with the optimum temperature of from 35° to 30°C.

In 1887 Cuboni[4] stated that intense light prevents the germination of the conidia.

Würthrich[5] showed by experiment that the conidia germinate in one or two hours. Then fifteen or twenty hours after germination the zoospores swim about for a period of from three to five hours.

To these theories should be added still another reported by Gregory[6] who claims that several amoeba-like bodies emerge from

1) Farlow, W. G. 1876, The American Grape Mildew. Bussy. Inst. Bul. 1: 423.

2) Patrigeon, G. 1881, op. cit.

3) Wüthrich, Ernst. 1892, Ueber die Einwirkung von Metalsalzen und Säueren auf die Kaumfähigkeit der Sporen einiger parasitischer Pilze. Zeitsch. Pflanzenkr. 2: 16-31, 81-94.

4) Cuboni, J. 1887, La Peronospora dei grappoli. Atti Congr. Mazion. Bot. Gritt. in Parma: 91-108. French translation by A. Picaud. 1889.

5) Würthrich, Ernst. 1892, op. cit.

6) Gregory, C. T. 1915, Studies on Plasmopara viticola, the session of the International Congress of Viticulture, P. P. I. E. San Francisco. California. July 12-13: 126-150.

the apical end of the conidium. After a short period of moving about outside the conidium, they become globose and then come to rest. He[1] claims that in some cases all the zoospores do not escape from the conidium at one time, but that one or two remain swimming within the conidium. These experience considerable difficulty, so to speak, in escaping, seeming to be unable to squeeze their nuclei through the opening.

In 1913 Gregory figured the various stages in the germination of the condia of *Plasmopara viticola*. Two years later he rewrote the subject, changing some of the details. He claimed that the germination of conidia may be distinguished and divided into several stages. (1) The protoplasm is finely granular, but about an hour after being placed in the water there appear lighter hyalin spots in it. (2) At the same time it becomes a little denser and more granular. When stained with methylen blue it will readily be seen that the "hyaline spots" are the nuclei. (3) This continues until there is a dense granular mass with distinct spots arranged at regular intervals. (4) In a short time there appear in the protoplasm dark lines which mark out portions around each nucleus. (5) These lines become more and more distinct and finally there are slight indentations along the surface of the previously smooth protoplasm. The contents of the conidium are now very rough and irregular. By close observation, the individual zoospores can be made out. In a few minutes the spores break apart and become distinct bodies. Then after a pause they burst forth. Gregory recognized that the opening is made at the papilla, and is not caused by a breaking of the conidium wall.

Istvanffi[2] observed that the papilla is a cap-like growth which is pushed off with the emergence of the zoospores. Gregory[3] watched this process of emergence very closely. He fixed and stained conidia in all stages of germination. Yet he saw nothing to agree with Istvanffi's opinion.

Farlow[4] says that conidia swell out a little, perhaps due to the suction of water, during the early stages of germination. This increase in size has been measured as about 1 μ in both length and

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1) Gregory, C. T. 1913, op. cit.
2) Istvanffi, G. 1913, op. cit.
3) Gregory, C. T. 1915. op. cit.
4) Farlow, W. G. 1876, op. cit.

width. After the evacuation of the zoospores the conidial wall becomes wrinkled and shrinks. The conidium itself decreases 2 μ in size becoming smaller than it was originally. To describe Farlow's observations in detail:—The zoospores pass out singly and slowly, remain for an instant in front of the mouth of the conidium and then separate themselves from the common mass. Their movements are very active at this stage. Soon a fully developed zoospore with two cilia darts off very rapidly.

Gregory thinks it probable that in *Plasmopara viticola* the pulling apart of the spores causes the formation of the cilia. He observed a considerable jerking and wrenching previous to their separation. He sometimes found two or more spores remaining attached for a long period and noted that not until after much pulling were they free and able to swim away. This made him think the cilia to be slender strands of protoplasm pulled from the spores as they were pulling apart.

Clinton[1] made a similar observation in *Phytopathora phaseoli ;* namely, that the motion of the zoospores is due to a slender thread or cilium drawn out by the pulling apart of the narrow zone joining the two adjacent bodies.

Istvanffi holds a different opinion. He maintains that the flagella are present on the spores previous to their emergence from the conidium. He states that they do not hesitate even an instant at the mouth. Gregory, on the contrary, has never seen cilia on newly formed zoospores within a conidium and he maintains that if there were free and matured zoospores that were unable to emerge from a conidium, the cilia could be stained. In this connection he also calls attention to the fact that, as stated above, not all the zoospores escape at the same time. Some are found swimming about within the conidium walls. These spores have much difficulty in squeezing out through the opening, which accounts for their dumbbell shape.

b. Development of Zoospore.

Cytological studies on the development of zoospores have thus far failed to give such important details as: the division of the nuclei in conidia, the relation between the blepharoplast and

1) Clinton, C. P. 1906, Downy Mildew, Phytophthora Phaseoli. Thaxt. of lima beans. Connecticut Agr. Exp. Sta. Rept. 288.

cilia, and the cleavage of individual spores by means of furrows in the protoplasmic mass. The most important contribution to the knowledge of this subject has been made by Gregory, who has described in detail the spore germination in *Plasmopara viticola*.[1] In most cases he used living conidia, staining them with methylen blue and carbol fuchsin. Evidently he was not successful in sectioning them for he makes no mention of it. For the present study, however, conidia were sectioned and stained, and a comparison was made with nonsectioned material. As a result conclusions, differing at many points from those of Gregory, are drawn.

Gregory says that at first the protoplasm is finely granular but that after being placed in water for about an hour the hyaline spots in the protoplasm become lighter and at the same time a little denser and more granular. He adds that if stained with methylen blue it may be seen readily that these hyaline spots are the nuclei which finally arrange themselves at regular intervals.

Many scientists have written on this period of maturity and germination and have observed in particular the plasmic changes. They have recognized that the protoplasm of an immature conidium appears to be finely granular, denser and to have a very greenish colour. This is also true of *Plasmopara Halstedii* but in addition it was noted that later, just before germination, the cytoplasm becomes more hyaline and less dense. This is probably due to the appearance of vacuoles. Hence anyone who has studied and carefully observed conidial germination should be able to determine with a fair degree of certainty the conidia which will germinate.

Gregory and Istvanffi agree in their observations that the full sized conidia which do not germinate are immature, and also that conidia, complete in karyokiness, are not necessarily mature. Gregory has observed that conidia undergo a certain transformation if they are maintained in a moist atmosphere. Within a period of three or four hours the plasma becomes reticulated in structure and the vacuoles gradually grow larger. In this stage the plasma in the living conidia of *Plasmopara Halstedii* was stained and examined under water and it was found to be granular.

In order to understand the critical changes of nuclear division conidia should be studied from the very beginning. Gregory stained conidia and found a number of hyaline spots which he recongnized

1) Gregory, C. T. 1913 and 1915, op. cit.

as nuclei but unfortunately he did not illustrate them. He maintains that these hyaline spots (nuclei) are formed in the cytoplasm but he fails to explain how. The important question to answer is just this: Were all these spots contained in the conidia from the very beginning or was there only one spot? The conidiophore shows an apical mode of growth and its unprotected growing points are described as naked. It is upon these naked points that the conidia first appear as very small pro tuberances each containing finely granular cytoplasm. Observation showed that in this primordial stage a nucleus enters a conidium from the conidiophore. It multiplies by repeated division up to a certain degree and then ceases. The daughter nuclei are smaller in size than the mother nucleus. From each daughter nucleus a zoospore developes.

Conidia were fixed and stained by Flemming's method. The succesfully sectioned material showed in the first stage a spherical nucleus with a nucleolus in the nuclear cavity. The nucleolus was more deeply stained. The blepharoplast was not detected. In the next stage a well stained granule was seen attached to the apical end and the nucleus was generally egg-shape. In the third stage the nucleus took the form of a pear with the nucleolus situated at the broader end, while the deeply stained granule was found at the narrow end. This granule afterward developed into the blepharoplast. In the following stage the narrow end of the nucleus prolonged itself into the shape of a beak.

It was noticed that by degrees all the nuclei approach the outer region of the protoplasmic mass. The narrower ends of the nuclei point outward and the broader ends point toward the center. Thus, the apical region of the nuclei reach the surface of the protoplasmic mass in the conidium. As they develop the beak ends undergo their maximum growth. A side view reveals the fact that the nucleoli have bulged a little. In addition to this red spots are seen at the center of the beak ends. These are the blepharoplasts. In a short time dark lines appear in the cytoplasm around each nucleus. These lines become more and more distinct and finally slight indentations are to be found along the surface of the previously smooth protoplasmic mass. These indentations become more visible owing to the growth of the primordial zoospores. This phenomenon extensively increases when the conidia absorb water. The delimitation of the spores from

the cytoplasm gradually follows nuclear division. The appearance of dark lines around each nucleus indicates that the first step towards the zoospore formation has ended. In a young conidium the cytoplasm is dense and the vacuoles are small. As the conidium develops the original vacuoles increase in size. Those adjacent fuse and enlarge, and thus decrease in number. The dark lines which are found around the nuclei are nothing but a dense packing of cytoplasm around the zoospores.

In connection with this the cleavage of the cytoplasm was observed. First a vacuole joins with its neighbouring vacuole producing a short cleavage furrow. The resulting vacuole in connection with a third vacuole makes the cleavage furrow deeper, (Plate III, Figs. 33, 34 and 35) so that in a relatively short period indentations are produced in the entire surface. (Plate III, Figs. 31, 32 and 36). This phenomenon of cleavage immediately increases when the conidia swell, the outer region of the cytoplasmic mass becoming filled with a liquid, which is mainly the water obtained by absorption. The absorption of water not only increases the size of the conidial outline but also swells the protoplasmic mass of the primordial zoospores, and this swelling in effect helps to form the cleavage furrows. Thus the cleavage furrows develop from the exterior.

Farlow in 1876 claimed that the conidia swell a little during the early stage of development. He measured this increase in size and noted it to be about 1μ in length and width. The conidial wall wrinkles and shrinks about 2μ after the evacuation of the zoospore so that it is much smaller than originally. He supposed the above increase in size to be due to the absorption of water. A similar increase in size was also noticed in the conidia of *Plasmopara Halstedii*. The cytoplasmic condition in the conidia evidently becomes much changed in the later stages, especially where the cleavage of the cytoplasmic mass into individual spores takes place. These changes are caused by the osmotic condition of the conidial contents and result in the suction of water and the consequent swelling.

The extensive fusion of vacuoles determines the planes of segmentation which occur in the sporangium of the *Mucoroles* and in the *Hydrodictyons*. The cell walls are not formed from the fibrillae but from the walls of vacuoles as is very clearly described by Harper[1]

1) Harper, 1899, Cell Division in Sporangia and Asci. Ann. of Bot. 13: 467.

and Timberkake.[1] The fusion of the vacuoles and the formation of a cleavage furrow may be brought about by the balance between the movement in the protoplasm and the surface tension in the conidia. In this connection Gray's[2] statement is very important for a complete understanding. He demonstrated the surface tension and cell division by experiments.

In the beginning the nucleated protoplasmic mass in a conidium is never separated, but segmentation proceeds in such a way that the final products each contain only one nucleus. It is probable that the nuclei are the ultimate centers controlling the segmentation.

Gregory states that the contents of conidia are very rough and irregular in the preliminary stage of germination. He says that in a few minutes the spores break apart and become distinct bodies but he does not describe the process. As already pointed out, the indentations occur on the surface of the central protoplasmic mass. At these points cleavage proceeds around the zoospores and the individuals become distinct bodies within a definite period. After a brief pause it seems that all the spores burst simultaneously. A slight movement may be discerned among them. Suddenly, through the tip of the conidium, there appears a bit of protoplasm from one of the zoospores which slowly forces its way out until the opening becomes full-sized. Then the entire mass of spores jerkingly, but rapidly, slips out. Gregory is certain that the opening is at the papilla. By observing the remnants of the walls after the evacuation he concludes that the zoospores do not break the walls, but that the walls themselves give way by dissolution as they reach maturity. Istvanffi[3] claims that the papilla is a cap-like structure which is pushed off when the zoospores emerge. The papillae on the condia were examined. Even in very early stages when many nuclei are dividing the apical region is stainable, demarking it from other parts at the point where later the papilla appears and opens to give passage to the zoospores.

After carefully watching the development of zoospores and after

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1) Timberlake, 1902, Development and Structure of the Swarm Spores of Hydrodictyon. Trans. Wiss. Acad. of Sci., Arts and Let. 13: 486.

2) Gray, J. 1922, Surface tension and cell division. Q. J. M. S., Vol. 66, pt. 11.

3) Istvanffi, G. and Palinkas, G. 1913 a.—Etudes sur le mildiou de la vigne. Inst. Centr. Ampel. Roy. Hong. Ann. 4: 1-122. 1913 b.—Etudes sur le mildiou de la vigne. Rev. Vit. 40: 481-484, 509-513, 540-543.

having fixed and stained conidia in all stages of germination it is impossible to agree with Gregory's explanation regarding the appearance of the cilia. Varying opinions are held about it, hence careful and detailed study is required. Gregory says that the zoospores pause for a time at the end of the conidium and then, pulling apart, swim away. In this connection Farlow says that the zoospores pass out slowly, usually one at a time, and remain for an instant in front of the opening as if not yet free from one another. After a while they free themselves and swim away, each zoospore possessing two cilia. Observing the jerking and wrenching, he supposed the cilia to have been formed at the time of pulling apart. To support his statement he claims that at times two spores remain attached for a long time and finally by dint of much pulling they snap apart and swim away. He has seen at other times as many as four or five spores apparently joined together by their cilia. Gregory, following Farlow's concludes that the cilia are slender threads of protoplasm pulled from the spores as they split apart.

Clinton[1] has made a similar statment in the case of *Phytophathora phaseoli*. He says that the motion is due to a slender thread or cilium drawn out by the pulling apart of the narrow zone connecting two adjacent zoospores. Istvanffi[2] claims that the cilia are present on the spores before they emerge from the conidium and that there is no hesitation after emergence. Gregory has never seen cilia on the newly formed zoospores before evacuation from the conidium. He says that they are clearly stained on free spores and upon mature zoospores which are unable to escape. So he infers that the cilia are produced during the period of emergence or while the zoospores are hesitating at the apex. He seeks to convince us by saying that he has stained them with methylen blue and carbol fuchsin and has found that they are not connected by their cilia, but by minute strands of protoplasm. But the evidence strongly favors Istvanffi's (1913) contention that the cilia are formed in the zoospores before emergence. Observation showed that cilia formation begins as soon as the hyaline spots appear. But at this stage the cilia are not as well developed as in the adult stage.

The cilia sprout from the beak-like apex of the nucleus at the

1) Clinton, G. P. 1906, Downy mildew, phytophthora Phaseoil. Thaxt., of lima beans, Connecticut Agr. Exp. Sta. Rept. 288.

2) Istvanffi, G. and Palinkas, G. 1913, op. cit.

spot where a well stained granule can be distinguished. The spot is believed it to be the blepharoplast. The cilia can be detected clearly when the primitive spores are produced by the indentations of the surface. Often the cilia of two adjacent spores are connected, but rarely more than two. When developed to this stage the apical points of the nuclei reach the surface of the plasma membrane. The surface of the zoospores being sticky the cilia can be seen adhering to their sides (Plate II, Figs. 20 and Plate III, Figs. 30, 31 and 32). In this study it was found to be impossible to satisfactorily stain the living conidia with methylen blue and carbol fuchsin. Accordingly it may be inferred that Gregory failed to observe the cilia because of his defective method of staining and because he evidently did not section his material. When Flemming's triple stain was used on sectioned conidia the cilia were plainly visible. Therefore it seems reasonable to maintain that the cilia are formed inside the conidium before germination. They adhere to one another. And naturally after germination the zoospores appear to be connected and to be pulling agart as Greogry and others observed. Moreover the fact that sometimes a single zoospore with two well developed cilia not connected with other zoospores emerged from a conidium at germination, affords conclusive evidence that cilia are not formed by the pulling apart of zoospores after germination.

The relation between the blepharoplast and cilia formation will be taken up later.

V. Development of Haustoria.

Haustoria can be observed in the mesophyll of the leaf, and the parenchyma of the stem and root of the host plant and can be studied in either cross or longitudinal sections.

The younger haustoria in *Plasmopara Halstedii* are more active than the older ones. Often the older and larger haustoria lose their contents and collapse (Plate IV, Fig. 53 and 54). Sometimes the cytoplasm of the host cell collects around the haustoria making them easier to observe. The younger haustoria are full of cytoplasm and nuclear division takes place actively (Plate IV, Figs. 47, 48 and 49).

In *Plasmopara Halstedii* a well developed mycelium spreads out in all directions in the intercellular space of the affected tissue and often forms an intricate network (Plate V, Fig. 64). In *Plasmopara* although the mycelia usually develop in a similar manner hitherto

nobody seems to have noticed them growing in the scalariform vessels. However, a few cases in which hyphae grew in scalariform vessels were observed.[1] In these cases no haustoria were formed on the mycelium, which showed an abnormal growth and presented irregularly thickened hypertrophy. Such an absence of haustoria was also observed in mycelia growing in a lysigenous cavity formed by nematodes or some injurious insects. In this case the mycelia were weak and slender. This condition was apparently due to a scarcity of nutriment for the fungus, because the cells surrounding the cavity were either badly injured or dying.

The abnormal growth of the mycelium in the scalariform vessel and the lysigenous cavities may be explained by the lack of proper nourishment or by the presence of some substances which stimulate such development.

Concerning the number of haustoria on the mycelia in different tissues of the host some interesting facts were observed. In a section 1 mm. long sometimes more than 20 haustoria were developed, at other times only from 5 to 7, and in a few cases none.

A comparison of the average number of haustoria in the leaf, stem, and root of inffected young seedling was made. This is given in the following table which shows the average number in thirty typical regions of mycelium, each 1 mm. in length.

Regions of growth.	Average number.
Leaf	
Epidermis	0
Spongy parenchyma	8
Palisade layer	5
Cotyledon	10
Stem	
Pith	14
Cortex	18
Epidermis	0
Medullary ray	12
Phloem	0
Vessel	0
Cambium	4

1) Nishimura, M. 1922, Studies in Plasmopara Halstedii. Jour. Col. Agri. Hokkaido Imp. Univ., Vol. XI. Part 3: 185-210.

Cork	0
Root	
Main root	7
Lateral root	5

From this table it will be seen that a larger number of haustoria are formed in cells which have thin walls and are full of nutriment for the fungi. This indicates that the development of haustoria depends in part upon external stimulus.

However, the fact that the haustoria may develop anywhere on a mycelium seems to show that the mycelium itself has the power to form haustoria. In some cases a number of haustoria developed at about the same time in a host cell rich in nutriment. In other cases a few haustoria were beginning to develop near one or two already well developed haustoria. This would seem to indicate that the absorption of nutriment from the host cell by the active haustoria increases the activity of the mycelium and stimulates the development of haustoria. Thus the development of haustoria depends on both internal and external causes.

The formation of haustoria may be due to the chemotropism of the mycelia. This stimulus is induced by some chemical substances in the host cell. It is found more abundantly where there is more nourishment; and wherever the irritating power of the host cell is greater, increased development in that cell naturally results.

Miyoshi[1] has already made many interesting experiments on the chemotrotropism of various fungi. He used *Mucor stolonifer*, *Saprolegnia ferax*, etc., and observed that the mycelia and especially the germ-tubes have marked chemotropism. For instance, he observed that the apical end of a mycelium and the germ-tube show a decided response to the stimulus of a nutritive substance by at once turning the direction of its growth toward that substance. Smith[2] in his study of *Erysipheae* noticed the development of the absorbing organs of *Phyllactinia Corylea*. He found that the development in certain regions, which were abundantly supplied with available food, such as the parenchyma sheath of the bundles, indicated a selective chemotropism in the fungus.

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1) Miyoshi, M. Die Durchborung von Membrane durch Pilzfâden. Jahrb. f. wiss. Bot. 30: 280. 1895.

Miyoshi, M. Uber Chemotropismus der Pilze. Bot. Ztg. 1894.

2) Smith, G. 1900, The Haustoria of the Erysipheae. Botanical Gazette. Vol. XXIX: 153-180.

The progress and development of the mycelium in the host tissue is facilitated by the formation of new haustoria which absorb the nutriment from the host cells. In such development the forming of additional haustoria in a new region is a great advantage, since in spreading through the host tissue, the mycelium finds new cells which contain enough nutriment to cause the further development of new haustoria.

The mode of haustorial formation was first well studied by Grant Smith on *Erysipheae*, using the microtechnique. He found in *Erysiphae communis* that the haustorium after it had escaped from the cellulose thickening developed a long thin neck without a plasmic membrane.[1] In general after the haustorium has escaped from the cellulose thickening the plasmic membrane of the host cell gradually forms a bounding menbrane of sheath. Gregory[2] notes a similar phenomenon in his study of *Plasmopara viticola*. He says: "At first a very small tube is produced laterally from the mycelium into the host cell wall which becomes greatly thickened at this point.···The haustorium continues to grow inward still enclosed in its sheath of host wall. After reaching a variable length the end of the tube gradually swells into a globose sac accompanied to a certain point by the swelling of its sheath. After attaining about one-half of its mature size the terminal portion bursts or dissolves the sheath. Thus in many mature haustoria there may be seen about the base a goblet-shaped collar pierced by the greatly attenuated stem of a haustorium. After penetrating the cell wall, the terminal portion continues its growth for a certain period, never, however, completely filling the cell as is sometimes the case in certain of the *Erysiphaceae*. It is this terminal, globose portion which constitutes the absorbing sac of the haustorium."

In the study of the process of haustorial formation in *Plasmopara Halstedii*, it was observed that the haustorium of this fungus has a rather short thick neck as opposed to the long thin neck common in the haustoria of *Erysipheae*. And although the cell wall shows a decided thickening at the place of penetration, the same as is shown in *Erysipheae*, the neck remains shorter and somewhat thicker in form. This difference is striking (Plate IV, Fig. 52). It was noticed also that a plasmic sheath was already formed around the cellulose

1) Smith, G. 1900, op. cit: (Plate XI. Figs. 6, 8.

2) Gregory, C. T. 1915, op. cit: 5-6.

thickening even before the penetration of the haustorium tube, and that sometimes this sheath remained even when the haustorium had just escaped from the cellulose thickening (Plate IV, Figs. 40, 41). Generally there is no plastic sheath around the well developed haustorium. In cases where the sheath was found it was noted that the haustorium was either at the stage just before its escape from the cellulose thickening or else at the stage soon after the escape. This would indicate that the forming of the sheath is a protective measure on the part of the host cell.

In a young haustorium, which is at first devoid of nuclei a nucleus is usually seen at the entrance of the neck, (Plate IV, Figs. 42, 43, 52). The surface tention, vacuoles, relative density of cytoplasm in various portions of the cell, and other such physical conditions have great influence upon the position of this nucleus. A study of these points gives an excellent idea of the relation between the nucleus and the haustorium. In the case of a non-vacuolated cell the nucleus usually lies in the center of the mass of cytoplasm; while in vacuolated cells the nucleus is imbedded in some other position in the cytoplasm. The function of the cell also influences the location of the nucleus. Usually it lies in the region of greatest metabolic activity. Haberlandt[1] has shown that in elongating young roots the nuclei are to be seen near the point where the cells are constantly growing, and also near the thickening material. In *Plasmopara Halstedii* the nuclei in haustoria multiply by mitotical division; the younger haustoria containing only a small number of nuclei, which increase as the haustoria develop (Plate IV, Figs. 46, 47, 48 and 49). Observation on other species has revealed a similar increase in the number of nuclei during vigorous metabolic activity. This theory of the relationship between nuclear position and function has been emphasized by both Haberlandt and Gerassimow.[2]

The exact means by which the nucleus enters the haustorium is not fully understood. It approaches a developing haustorium and shows a tendency to enter at the mouth (Plate IV, Figs. 42, 43 and 44). It was often noticed that while in this position a thick strand

1) Heberlandt, G. 1887, Uber die Beziehungen Zwischen Funktion und lage des Zellkerns bei den Pflanzen. Jena.

2) Gerassimow, J. J. 1890, Einige Bemerkungen über die Funktion des Zellkerns. Bull. Soc. Sci. Nat. Moscow: 584-554.

Gerassimow, J. J. 1901, Uber den Einfluss des Kerns auf des Wachsthum der Zellen. Ibid: 185-220.

developed from the nucleus and extended into the cavity of the haustorium. At the end of this strand a centrosome appeared from which radiated a few very fine fibrils. The contraction of this strand probably caused the nucleus to enter into the haustorium. Levine[1] has pointed out that in the development of the basidiospores in *Boletus castaneus*, the strands which pass through the sterimata connecting the centrosomes with the nuclei in the basidia are thicker than the fibrils which radiate from the controsomes, and that "simultaneously with the develepment of the spores the nuclei begin to move towards the sterigmata." His theory being that the "migration is probably the result of the contraction of the kinoplasmic fibrils as claimed by Maire."[2]

An abnormal thickening of the host cell wall was usually observed at the point of the haustorial attack (Plate IV, Figs. 45, 50 and 59). There are two different explanations of this thickening. Cuboni[3] says that the thickening is caused by some enzymes producing gelatinization of the wall. Gregory[4] suggests that it is an indication of an attempt of the host to exclude the haustorium; in which case the thickening results from an additional deposition at this point by the protoplasm of the irritated cell. After observing the characteristics of *Plasmopara Halstedii*, both views seem correct, because this gelatinized substance of irregular thickness was found around the attacking haustorium (Plate IV, Figs. 45, 50 and 59), and often it was observed that when haustoria developed side by side these thickening layers adhered closely (Plate IV, Fig. 59).

In all probability this same adhesive power is also in operation between the walls of the haustoria and the host cell wall. The well stained cytoplasm is more densely accumulated on the outer side of the thickening wall of the haustoria. The inner layer of the wall, however, takes the stain faintly. It must be added that the metabolism of the host cell was increased by the contact stimulus of the haustorium and that the host nucleus often showed a tendency to approach the neighbourhood of the haustorium, indicating that the

1) Levine, M. 1913, Studies in the Cytology of the Hymenomycetes, especially the Boleti. The Bulletin of the Torry Botanical Club 40: 159, Pl. 7.

2) Maire, R. 1902, Recherches cytologiques et taxonomique sur les Basidiomycites. These présentées a la Faculté des Science de Paris.

3) Cuboni, J. 1889, Peronospora des grappes. French translation, 7-46. pl. 2.

4) Gregory, C. T. 1915, Studies on Plasmopara viticola. The sessions of the International Congress of vititculture, P. P. I. E. San Francisco, California.

nucleus lies in the region of the most active metabolic change (Plate IV, Fig. 55).

In the case of *Plasmopara Halstedii* when the haustoria attack the host cells, two ways of absorbing nourishment are to be observed. One way has already been described by Smith[1] in his study of *Erysipheae* and also by Gregory[2] in his study of *Plasmopara viticola*. A haustorium penetrates the host cell wall and obtains nourishment as a result of direct contact with the cytoplasm of the host cell. In an early stage of the penetration of the host cell wall a slight thickening on the inner surface of the wall develops at the point of attack. This thickening elongates as the penetrating tube of the haustorium grows. Signs of disintergration begin to show at the distal end of this cellulose thickening. As the distal end of the penetrating tube begins to enlarge the disorganization of the cellulose increases until an opening is made in the cellulose thickening and the young haustorium appears.

Then the disintegration ceases, but the thickened cellulose remains close about the neck of the haustorium like a collar, indicating adhesive power (Plate IV. Figs. 40, 41, 42, 52 and 55).

The other way of absorbing nourishment begins with the thickening and elongation of the cell wall just as is described above, but in the later stage there is no noticeable disintegration of the cell wall. A growth is to be seen around the primordial haustorium as it continues its advance in a globular form into the host cell.

Then the cellulose around the young haustorium gradually becomes gelatinized and the outer surface becomes irregular, showing a denser cytoplasm. The haustorium absorbs nourishment through this mucilaginous layer. (Plate IV, Figs. 45, 49)

It is believed that in the second case the host cell in order to protects itself prevents the penetration of the haustorium. But even in this case a slight disintergration sometimes takes place (Plate IV, Fig. 50).

A living nucleus often can be seen near the haustorium (Plate IV, Fig. 55). Concerning this two explanations are possible. First, the haustorium may enter at the best place to absorb nutriment from the host cell. When the cellular contents are homogenous, entrance

1) Smith, G. The Haustoria of the Erysipheae. Botanical Gazette. Vol. XXIX. 153-180. 1900.

2) Gregory, C. T. 1915, Op. cit.

is made at any point. But if nutriment is plentiful only near the nucleus it goes there. Second, the host cell may be working for its existence. In order to protect itself great metabolic change takes place in the region of attack. The nucleus then naturally moves toward the point of greatest activity, which in near the haustorium.

Istvanffi[1] has reported as many as four nuclei in one haustorium, while Gregory[2] has found in certain cases that a haustorium may contain only two nuclei. They recognized in these observations that the nuclei are produced by the division of a single nucleus which entered the haustorium during an early stage of its development.

In *Plasmopara Halstedii* the nuclei in haustoria increase in number by division; thus the younger haustoria usually contain a small number of nuclei, which number increases as the haustoria develop. However, it is not always true that the older haustoria contain more nuclei, because nuclear division depends on protoplasmic activity, so that often fewer nuclei are found in the older, but less active, haustoria.

These organs of absorption vary greatly in size. According to Istvanffi's measurement they are from 4μ to 10μ (rarely from 15μ to 25μ) in diameter, and from 4μ to 12μ (rarely from 20μ to 25μ) in length. Gregory's measurements correspond very closely with the above. In addition he gives the measurements for the stems. They may reach nearly one half the total length of the haustorium being from 1.5μ to 5.5μ long. The diameter of the stem is from 0.4μ to 1.0μ. Observation was made on fifty typical haustoria in *Plasmopara Halstedii* to determine the relation between the size of the haustoria and the number of nuclei. The specimens were divided into two groups according to their size. In the smaller group, with a diameter of from 5μ to 9μ and a length of from 8μ to 9μ, the number of the nuclei was usually 1 or 2. In the larger group, with a diameter of from 9μ to 10μ and a length of from 10μ to 12μ, the number of the nuclei was 5 to 7.

It is evident from the above that there is a great variation to the number of nuclei in haustoria. To illustrate this further, in one case where two haustoria developed side by side in the same host cell

1) Istvanffi, G. and Palinkas, G. 1913, Etudes sur le mildiou de la vigne, Inst. Centr. Ampel. Roy. Hong. Ann. 4: 1-122.

2) Gregory, C. T. 1915, Studies on Plasmopara viticola. The session of the International Congress of Viticulture. P. P. I. E. San Francisco, California.

one contained only one nucleus and the other contained two (Plate IV, Fig. 46.) A similar two nucleated case was also observed in which a single, though larger, haustoria was in the host cell (Plate IV, Fig. 55). In still another and more advanced case, where two haustoria developed side by side in the host cell, one contained three nuclei and the other four (Plate IV, Fig. 59). All of these nuclei were in the resting stage. The division stage was also observed. Two elongated nuclei in anaphase were seen with kinoplasmic strands connecting with astral rays (Plate IV, Fig. 47). Of three daughter nuclei in late telophase two of them still showed astral rays (Plate IV, Fig. 48). Of four nuclei, which had completed the second division, two still showed astral rays.

Haustoria occasionally become cup-shaped. This is evidently due to the pressure of the host cell upon the upper portion of it (Plate IV, Figs. 53 and 54). The cell contents of the host plant in such a case exert turgor at the place of depression and the haustoria take this shape naturally. This shape helps the host cell to keep its contents.

The wall of the haustorium is thin. But because the host cell wall is about the partially swollen tip and because of the space between the plasma menbrane and the haustorium the wall seems thick. Istvanffi (1913) states that the wall is sometimes thin and sometimes very thick. In the first instance he has probably observed a condition in which the plasma membrane is closely pressed to the haustorium, and in the second he may have confused the wall with the encompassing sheath of host cell wall. Others have held the same opinion.

The haustorium penetrates the wall of the host cell for the purpose of continuously absorbing its contents. In order to do this it must adhere closely to the wall of the host cell at the point of penetration. The power of adhesion which it manifests is indeed remarkable (Plate IV, Figs. 52 and 55). A similar case of adhesion was mentioned before in connection with the penetration of the antheridial tube into the wall of the oogonium. This power of adhesion is essential because it enables the parasites to draw nourisment from the host cell without destroying it. *Plasmopara Halstedii* attack *Helianthus annuns L.* and especially because each adheres to the other absorption takes place naturally. If there were no adhesion the host cell would be damaged and die, which would result in the death of the parasite itself. Thus in the selection of its host plants this

phenomenon of adhesion is very important and may be the decisive factor.

VI. Nucleus and Centrosome.

Nuclear activity in the oogonium is more pronounced before the oospore formation. After the degeneration of many nuclei in the oosphere, one nucleus remains in the center of the oosphere for fertilization. At this stage one or more male nuclei are discharged from the antheridial tube into the oosphere and then fertilization takes place, After the male and female nuclei fuse much time elapses before the division stage begins. It was observed by Stevens[1] that in *Albugo Bliti* the fused nuclei pass the winter. in the resting condition without further perceptible change. But in *Plasmopara Halstedii* two different stages in the oospore are recognized during late summer, namely, metaphase and anaphase (Plate I, Figs. 12 and 13).

The resting stage is well illustrated in the mycelium of *Plasmopara Halstedii.* The nucleolus is prominent. The resting nuclei are almost spherical. Such spherical nuclei on entering the oogonium show a rather elongated shape, and centrosomes as well as nucleoli are found upon them. In the antheridium and oogonium a faint nuclear membrane was observed during the spirem stage.

In prophase the nucleus gradually becomes more elongated into a spindle shape, and a thin faint linear network with granules on it becomes visible. Later when the centrosomes become noticeable the net work gradually disappears and chromosomes collect at the equatorial plate (Plate I, Fig. 13). In anaphase separating chromosomes, centrosomes and astral rays are well represented (Plate I, Fig. 2). In this stage a nuclear membrane was observed in haustoria (Plate IV, Fig. 57). At the beginning of anaphase the chromosomes usually appear individually and then later migrate to the poles (Plate I, Fig. 3). During late anaphase the chromosomes which have migrated to the poles often appear as masses connected by a fiber (Plate I, Fig. 12).

In telophase when daughter nuclei are forming, astral rays still show. This condition is especially well illustrated in mycelia and haustoria (Plate II, Fig. 23 and Plate IV, Figs. 47, 48 and 49).

1) Stevens, F. L. 1899, The Compound Oosphere of Albugo Bliti. Botanical Gazette, Vol. XXVIII: 149-176.

The behavior of centrosomes can be observed in conidia, haustoria, mycelia, oogonia and antheridia. This behaviour through the succesive stages of zoospore formation has been studied, and the fact that the nuclei of the conidium increase by division with the successive stages of development is worthy of note. Observation as to the relation of size to number reveals the fact that in general the number of nuclei varies in direct proportion to the size of the conidia, the smaller conidia especially in the early stages, having a comparatively small number. Later the conidia gradually increase in size and in the number of nuclei. In some abnormal cases small mature conidia contain few nuclei. These smaller conidia usually develop in a group on dwarfish conidiophores. But they are also often found developing at the terminal regions of normal conidiophores. It may be that the dwarfed condition is due to insufficient nutrition. The zoospores normally form in number equal to the number of nuclei in the conidia and almost all of the cytoplasm is used up by this formation, as was observed by Gregory[1] on *Plasmopara viticola.* It is believed that the irregularity of the number of zoospores is dependent upon the condition of nourishment.

Concerning the first nucleus in a conidium Istvanffi[2] states that a single nucleus passes into each spore at the beginning and that the division of this nucleus produces the multi-nucleated condition obtained in the mature conidium. Gregory[3] observed that before the formation of the conidia the conidiophore is filled with protoplasm, which is abundantly provided with nuclei. The conidia are formed by the swelling of the end of the sterigmata, into which a portion of the protoplasm passes. After mature size is attained a septum is laid down separating the conidium from the sterigmata. The entrance of the first nucleus into the primordial conidium is by the contraction of the kinoplasmic strand of the centrosome. This process is described later in connection with the entrance of the first nucleus into a haustorium. After the entrance of the nucleus into the primordial conidium it divides repeatedly until the full number is reached (Plate

1) Gregory C. T. 1913, Spore Germination and Infection with Plasmopara viticola. Phytopathology 2: 235-249.

2) Istvanffi, G. and Palinkas, G. 1913a.—Etudes sur le Mildiou de la vigne. Inst. Centr. Ampel. Roy Hong. Ann. 4: 1-22. pls. 1-9. 1913b.—Etudes sur le Mildiou de la vigne Rev. Vit. 40: 481-484, 509-513, 540-543.

3) Gregory D. T. 1915, Studies on Plasmopara viticola. The Session of the International Congress of Viticulture, P. P. I. E. San Francisco, California: 3-27.

II, Figs. 16, 17 and 18). This is followed by a resting stage. In this stage each nucleus is pear shape with a distinct nucleolus in the broader portion, and a deeply stained granule in the narrow tip (Plate II, Fig. 19). This well stained granule is the centrosome. These nuclei gradually move outward and as they approach the periphery with their narrowed parts facing outwards, become more elongated at the neck (Plate II, Figs. 19 and 20). In a later stage the centrosomes reach the surface of the plasma membrane. In the final stage cilia formation begins (Plate II, Figs. 20 and 21).

Gregory[1] claimed that the cilia of zoospores in *Plasmopara viticola* develop from two points on each side of the nucleus and are of unequal length; but he paid no attention to the fact that a relationship exists between the centrosomes and cilia formation. In *Plasmopara Halstedii*, however, the cilia plainly develop from the centrosome in each spore (Plate III, Figs. 28, 29, 30 and 38).

Similar observations of cilia formation have been made on *Polytoma* by Dangeard[2] and on *Hydrodictyon* by Timberlake.[3] In these species the cilia grow out from centrosome-like bodies. These small bodies were described under the name of "blepharoplasts" (Plate III, Fig. 37). The connection between the cilia and the blepharoplasts is shown in Plate II, Figs. 20 and 21, and Plate III, Figs. 28 and 29. The views of the tangential section of the conidia especially help to a clear understanding of this connection. The side view shows the way two cilia growing out of of a blepharoplast divide and develop (Plate III, Figs. 28, 29 and 30). In the polar view two cilia connect into one wavy line (Plate III, Fig. 31).

The length of the cilia varies in different stages. At the beginning the cilia often seem to be just a point (Plate III, Fig. 37), and in later stage they elongate and become more visible (Plate II, Fig. 21). Then each spore begins to separate itself from the mass and due to the pressure of one spore upon another takes on a polygonal shape (Plate III, Fig. 32). Finally, the whole protoplasmic mass in the conidium forms a cluster (Plate III, Fig. 36).

In the cluster stage each mass of primordial spores becomes distinct. The cilia develop rapidly along the surface and adhere to

1) Gregory, C. T. 1915, op. cit.

2) Dangeard. 1901, Etude sur la structure de la cellule et ses fonctions, Le Polytoma uvella. Le Bot. VIII: 5-58.

3) Timberlake. 1902, Development and structure of the swarm spores of Hydroctyon. Trans. Wis. Acad. Sci. Arts and Letters 13: 486-522.

one another (Plate III, Figs. 30 and 32). This development was studied in various stages from the polar view. Plate III, Fig. 31 already suggests that the cilia connect in a later stage by adhesion. A side view of this is shown in Plate III, Fig. 30.

The cleavage furrows on the protoplasmic mass develop from the periphery. They occur at the stage of cluster formation. The very small vacuoles fuse with adjacent vacuoles and the wall is laid down. This proces has been well demonstrated (Plate III, Figs. 33, 34, 35 and 37).

The cilia of each spore adhere closely at first. Later they separate from the surface at the basal portion, near the centrosomes (Plate III, Figs. 28 and 29). Gregory[1] on *Plasmopara viticola* observed that sometimes two spores remain attached for a long period but finally, by dint of much pulling, they snap and swim away. At other times as many as four or five spores are joined together by their cilia. The same phenomena are true in *Plasmopara Halstedii*.[2] Gregory was of the opinion that the cilia are slender threads of protoplasm pulled out from the spores as they split apart. However, cilia have been found on the protoplasmic mass before germination. They adhere only to the surface of the cluster, and most of them are joined together. This condition continues for a time even outside of the conidium. In some cases all of the spores do not escape from the conidium at the same time. For instance, in one case where six spores remained in a conidium only the upper three had visible cilia, while in one of the lower spores astral rays could still be seen. This suggests an earlier stage of ctlia formation (Plate III, Fig. 38).

The mature zoospores, when treated with damp air or water, continue their jerking motion for a time, then lose their cilia, take a more perfect spherical shape and enter a resting stage. Under favorable conditions they will germinate (Plate III, Fig. 39).

In haustoria centrosomes are first recognized when the nucleus enters. For example, in Plate IV, Fig. 43, which shows a nucleus just at the entrance of a haustorium, the broader portion of the nucleus is still in the mycelium while the narrow end has already entered the haustorium. Tracing this end, astral rays were only faintly recognizable while the kinoplasmic strand connecting with the

1) Gregory, C. T. 1913, Spore Germination and Infection with Plasmopara viticola. Phytopathology, Vol. II: 235-249. With Seven Figs. in the Text.

2) Nishimura, M. 1922, Studies in Plasmopara Halstedii. Jour. Col. Agric., Hokkaido Imp. Univ., Vol. XI, part 3: 185-210.

nucleus was prominent. This figure calls to mind one of Levine's[1] made from a study on *Boletus castaneus.* (In the spore of *Boletus castáneus* a number of fibers radiate outward from the centrosomes through the cytoplasm, but the strand which runs to the nucleus is thicker than the others). It is Maire's[2] theory that in *Basydiomycetes* the migration of the nuclei is caused by the contraction of the kinoplasmic fibers. Levine[3] in his study of *Hymenomycetes* agrees with this theory, namely: that simultaneously with the development of the spores, the nuclei begin to move towards the sterigmata; this migration probably being caused by the contraction of the kinoplasmic fibers. An interesting confirmation of this theory is found in the fact that the entrance of the nucleus into the haustorium is affected by the contraction of a kinoplasmic strand (Plate IV Fig. 43). In haustoria also the centrosomes and astral rays are distinctly visible.

Nuclei, each with a spot, were well stained. This spot—the centrosome—is not spherical, but is of a rather oval shape with its flattened face towards the nucleus (Plate IV, Fig. 58). In anaphase (the first division of the nuclei in haustoria) a nuclear membrane, well marked centrosomes, astral rays, and separating chromosomes are visible (Plate IV, Fig. 57). In the second nuclear division the strands at both poles which connect the astral rays with the nucleus are much elongated (Plate IV, Fig. 47). The astral rays still show after the second division of the nuclei (Plate IV, Figs. 48 and 49). Sometimes only three nuclei show, the fourth one being in an adjacent section (Plate IV, Figs. 48, 49, the latter shows all four nuclei). The typical nucleus shows astral rays and a nucleolus (Plate IV, Fig. 56).

The various phases of centrosomes in mycelia are also easily demonstrated. These phases are drawn in detail in Plate II, Figs. 22–27. First there is a resting nucleus with a centrosome at the apex and then a dividing nucleus in late metaphase. (Fig. 25). After this there are two daughter nuclei, moving in opposite directions but still connected by two fibers; these have astral rays at the free, pointed end (Fig. 27). In some cases the daughter nuclei are connected by fibers and arranged at an angle of about 90° (Fig. 26). In a daughter nuclei at about this stage two or three chromosomes may be seen

1) Levine, M. 1913, Studies in the Cytology of the Hymenomycetes, especially the Boleti. The Bulletin of the Torrey Botanical Club: 40: 137–181. pl. 4–8.

2) Maire, R. 1902, Recherches cytologiques et taxonomiques sur les Basidiomycetes. Théses présentées a la Faculté des Sciences de Paris.

3) Levine, M. 1913, op. cit.

(Fig. 24). After division when the daughter nuclei have been completely separated, astral rays are still visible (Fig. 23). At times one daughter nucleus may retain a part of the connecting fiber, but this soon disappears (see lower part of Fig. 23). When the daughter nuclei have formed but are still connected, a slightly chromatic substance has been found upon the connecting fiber (Fig. 22). It is significant that a figure drawn by Minchin[1] showing the nuclear division of *Coccidium schubergi* reveals a very similar phenomenon. Minchin's figure was drawn to show late anaphase when some chromatic substance remained at the place of connection of the daughter nuclei.

Trow[2] in *Saprolegniae* observed two daughter nuclei, one of which had turned through an angle of nearly 90°. A similar case in *Plasmopara Halstedii* has been mentioned above (Plate II, Fig. 26). In some cases elongated nuclei appeared. These dissimilar elongated nuclei were seen in late metaphase and anaphase and were no doubt derived from the earlier metaphase by the loss of the nuclear membrane and the elongation of the spindle. Harper[3] recognized a similar elongation in the nuclear division of *Ascus* of *Lachnea.*

The oogonium and the antheridium also show centrosomes but the oogonium has been more closely studied. When division occurs in these nuclei spindles with centrosomes forming astral rays appear. They are especially well marked in anaphase. The daughter nuclei move to their respective centrosomes. The astral rays are still present in late telophase. In some cases of division the astral sphere develops at opposite ends and often a long kinoplasmic fiber is connected to the astral rays. In anaphase of the oogonium one nucleus is present (Plate I, Fig. 2). In the next stage the daughter nuclei are forming yet one connecting fiber extends between them (Plate I, Fig. 9). A polar view of a similar stage shows two daughter nuclei forming. The astral rays are still visible (Plate I, Fig. 10). After the division of the nuclei a gradual migration towards the periphery occurs, some nuclei still showing astral rays (Plate I, Fig. 14). In the next stage the astral rays have almost disappeared (Plate I, Fig. 11).

Nuclear division in the antheridium is practically the same as in the oogonium.

1) Minchin, E. A. 1912, An Introduction to the study of the Protozoa. London.

2) Trow, A. H. 1904, On Fertilization in the Saprolegniae. Annals of Botany. Vol. XVIII. No. LXXII: 531-569. pls. XXXIV-XXXVI.

3) Harper, R. A. 1899, Cell division in Sporangia and Asci. Ann. of Bot. 13: 467.

Summary.

1. The coenocentrum appears in the center of the oogonium and the female nucleus is attached to it before fertilization. There must be some attractive power between the coenocentrum and the female nucleus.

2. The coenocentrum is composed of small granules surrounded by a region of denser cytoplasm. The radiating cytoplasmic strands together with the coenocentrum may be the dynamic center of the activity which results in zonation.

3. The vacuoles which surround the oosphere play a very important part in the free cell wall formation. The vacuoles in the periplasm which are in contact with these outer vacuoles gradually fuse and thus decrease their number by increasing their size. At the same time cytoplasm and kinoplasm accumulate around the outer vacuoles.

4. In the zonation stage many nuclei arrange and divide in the oosphere near where the wall is to be formed. Some daughter nuclei lie across the line of demarcation between the oosphere and the periplasm. The wall first becomes visible after nucelar division is completed.

5. The nuclei are attracted toward the points of greatest metabolic activity. For example, at the beginning of zonation the nuclei always appear wherever there is protoplasmic aggregation. In the zonation stage the nuclei are arranged around the central mass of the oosphere.

6. At the region where a monocyst appears the oosphere wall is developed earlier than in other parts. It is very noticeable that at this point nuclear division has already taken place, because here no nuclei are visible, but elsewhere on the oosphere surface nuclear division is still active.

7. At the opening the wall adheres closely to the fertilizing tube, so that no intercourse between the periplasm and the oosphere can occur.

8. The apical portion of the fertilizing tube remains in the oosphere, the upper part continuing to adhere closely to the wall which surrounds the opening. A large vacuole closes the opening in the tube by means of its tonoplast together with the cytoplasm and degenerated nuclei of the fertilizing tube which had accumulated on it.

9. The opening in the oosphere wall is repaired with materials drawn entirely from the antheridial tube. There is no morphological or micro-chemical difference between the original wall and the new layer formed to close the opening. Hence the protoptasm of the oogonium and the antheridium are of a similar biological nature. The lack of any distinct reaction confirms this view.

10. The cooperation of nuclear and vacuolar activity is a factor in wall formation. The tonoplast is the foundation material in the primary film stage. If nuclear division does not take place near the film a complete wall never developes.

11. The nuclei in the conidia gradually migrate outward. Finally the apical ends reach the surface of the protoplasmic mass. These ends take on a beak-like appearance. They are called the blepharoplasts.

12. Before germination in the conidia slight indentations are found along the margin of the previously smooth protoplasmic mass. These indentations become more and more visible as the primordial zoospores develop in size.

13. The size of the conidia increases and the protoplasmic mass of the primordial zoospores swells as a result of the absorption of water. This swelling hastens the formation of cleavage furrows, which form from the exterior and work inward by means of the fusion of adjacent vacuoles.

14. The cilia of the zoospores in conidia develop before germination and they sprout from the blepharoplasts. Well developed cilia connect the zoospores in conidia. After germination these zoospores are still connected by the cilia.

15. Haustoria develop in any direction where nutriment is to be found. Under favorable conditions two or three haustoria may develop at about the same time in a single host cell. Usually new haustoria form only in the growing part of a mycelium. The neck of well developed haustoria is short and thick.

16. The nucleus of the host cell is near the haustorium. A number of observations showed that the nucleus of the host cell lies in the region of most active protoplasmic change.

17. Two ways of absorbing nourishment are to be observed. In one way a haustorium attacks the host cell, penetrates the abnormal thickening as it disintergrates, and obtains nourishment as a result of direct contact with the cytoplasm of the host cell. In the other

way a haustorium advances into the host cell in globular form but does not penetrate the wall. No disintergration takes place but instead the cellulose around the haustorium gradually becomes gelatinized. Nourishment is absorbed through this wall.

18. A plasmic sheath is often formed around the cellulose thickening before the penetration of the haustorium. This sheath sometimes remains for a short time even after the haustorium escapes from the thickening. It is evidently a protective measure of the host cell.

19. An abnormal thickening of the host cell wall takes place at the point of the haustorial attack. This thickening is probably caused by enzymes which produce a gelatinization of the wall, and also by an additional deposition by the protoplasm of the irritated cell, produced in its attempt to exclude the haustorium.

20. Haustoria average from 4μ to 10μ in diameter and from 4μ to 12μ in length, the maximum measurement in either case being 25μ. The number of nuclei in haustoria varies usually, but not always, directly with the size. In a smaller group with the average measurement of $7\mu \times 8\frac{1}{2}\mu$ the number of nuclei was usually 1 or 2, while in a larger group which averaged $9\frac{1}{2}\mu \times 11\mu$ the number was from 5 to 7.

21. At the inception of a haustorium a nucleus usually remains near its point of entrance. This nucleus seems to be in some way aiding the development of the haustorium. When the nucleus develops a long kinoplasmic strand reaching into the haustorium astral rays grow at the apical end. The entrance of the nucleus may be aided later by the contraction of this long strand.

22. Centrosomes are present in the nuclei. The astral rays are especially well represented at the stage of anaphase but even later in telophase they are still visible in each daughter nucleus.

The author wishes to acknowledge his indebtedness to Professor K. Miyabe and Professor K. Fujii for their many valuable suggestions and criticisms.

VIII. Explanation of Plates I—V.

All the figures were drawn with the aid of a camera-lucida at table level. Zeiss 1.8 mm. oil-immersion objective, 1.25 N.A., and oc. 4. The magnification is as indicated.

PLATE I.

Fig. 1. An oogonium showing the first division of nuclei in several stages (anaphase, metaphase and daughter nuclei already formed). Each daughter nucleus shows four chromosomes. The cytoplasm is homogeneously vacuolated (×1450).

Fig. 2. An oogonium being attacked by an antheridium. One nucleus is in anaphase. Astral rays are visible (×1630).

Fig. 3. Nuclei already accumulated in the oosphere. The chromosomes in some of the daughter nuclei are very clear (×1450).

Fig. 4. An oogonium showing the demarcation between the periplasm and ooplasm. The vacuoles in the oosphere are smaller. The larger vacuoles in the periplasm are adjacent to the line of demarcation (×1450).

Fig. 5. An oogonium in contact with an antheridium. Each is attached to a different mycelium. The oogonium is shown in the zonation stage. At the center is a nucleus attached to the coenocentrum from which cytoplasmic strands radiate. Many nuclei are arranged along the margin of the oosphere where the wall is forming (×1120).

Fig. 6. An oogonium showing the free cell wall in formation. Several nuclei in telophase are seen in the periplasm near the forming wall (×1120).

Fig. 7. The free cell wall of the oogonium already formed with denser cytoplasm accumulated on the outer side of it. A nucleus is shown in the oosphere. The free cell wall has been penetrated by a fertilizing tube. Its basal portion in the periplasm has already degenerated; its apical region in the oosphere is still visible. A large vacuole is visible in the tube. The wall is being rebuilt to fill up the opening caused by the penetration of the tube (×1120).

Fig. 8. The free cell wall already formed in the oogonium. Many nuclei show centrosomes (×1120).

Fig. 9. Daughter nuclei (telophase) from an oogonium connected by a fiber. Each shows astral rays (×1630).

Fig. 10. A stage similar to Fig. 9, but the daughter nuclei are arranged at an angle (viewed from the broader side) (×1630).

Fig. 11. A nucleus from an oogonium after division (late telophase) showing astral rays (×1630).

Fig. 12. An oospore nucleus in anaphase (×1800).

Fig. 13. A nucleus in metaphase at an early stage of the oospore (×1800).

Fig. 14. Nuclei in a conidium after division. Four of the nuclei migrating towards the surface still show clearly defined astral rays (×1400).

PLATE II.

Fig. 15. A nucleus in a conidium at the resting stage showing a centrosome and nucleolus (×1400).

Fig. 16. A young condium with nuclei dividing. Daughter nuclei still attached by connecting fibers. The cytoplasm is homogenous (×1000).

Fig. 17. Similar to Fig. 16, but observed a little later (×1400).

Fig. 18. A young conidium with many nuclei in different stages (metaphase, anaphase and telophase). The upper portion of the conidium stains markedly where the papilla will form in the mature stage (×1400).

Fig. 19. A part of a conidium showing many nuclei migrating towards the outside. Controsomes and nucleoli are visible (×1400).

Fig. 20. Similar to Fig. 19, but observed at a more advanced stage. Many nuclei have now reached the plasma membrane. Some have developed cilia. Two and sometimes three nuclei are connected by these cilia (×1400).

Fig. 21. A nucleus in a conidium with cilia developing from the blepharoplast (×1400).

Fig. 22. Nuclei in a mycelium, connected by fibers with a chromatic spot visible between them (×1400).

Fig. 23. A mycelium with nuclei, which shows astral rays. One nucleus still shows a connecting fiber. Two haustoria are seen on this mycelium (×1400).

Figs. 24 and 25. The nuclear division in mycelia. The elongated nuclei are in anaphase. Some show chromosomes. Astral rays are also visible (×1400).

Fig. 26. Division of nuclei in a mycelium with daughter nuclei at an angle of about 90°, still connected by fibers (×1400).

Fig. 27. Daughter nuclei in a line in a mycelium; still connected by two fibers (×1400).

PLATE III.

Fig. 28. A nucleus in a conidium with two cilia developing. The terminal region is attached to the plasma membrane. The basal parts are separating (×1400).

Fig. 29. Similar to Fig. 28, the separation of the cilia from the plasma membrane shown more distinctly (×1400).

Fig. 30. Two nuclei in a conidium connected by cilia, which are more developed than in Fig. 29 (×1400).

Fig. 31. Nuclei with cilia developed, some showing the upper and some the side view. Some cilia are connected together (×1400).

Fig. 32. Many nuclei developing cilia, some of which connect the nuclei with each other. The surface of the protoplasmic mass show indentations, and already cleavage furrows are visible, beginning at the points of indentation (×1400).

Figs. 33, 34 and 35. Successive stages in the development of cleavage furrows in the vacuolated cytoplasm of conidia (somewhat diagramatic).

Fig. 36. A more advanced stage of Fig. 32. Each individual zoospore has been distinctly set off by cleavage furrows extending from the surface indentations at almost right angles (×1400).

Fig. 37. A cleavage furrow extending inward from the surface (×1400).

Fig. 38. Six zoospores which have remained in a condium. The upper three show cilia clearly, but astral rays are still visible only on the lower one (×1800).

Fig. 39. Four zoospores; two of them germinating, the other two in the resting stage (×1800).

PLATE IV.

Figs. 40 and 41. A long narrow haustorium penetrating the abnormally thickened wall, a plasmic sheath is formed around the cellulose thickening (×1250).

Fig. 42. A nucleus just entering a haustorium. The wall of the host cell is already penetrated but clings tightly about the neck of the haustorium (×1250).

Fig. 43. A nucleus entering a haustorium. The kinoplasmic strand extends into the haustorium and shows astral rays (×1250).

Fig. 44. A long narrow haustorium penetrating the abnormally thickened host cell wall. There is no plasmic sheath such as is shown in Figs. 40 and 41. At the base of the haustorium a slight swelling has occured and a nucleus, which has already entered, is visible there (×1250).

Fig. 45. A haustorium in a host cell. An irregularly thickened layer of the host cell is marked by dense cytoplasm on the inner surface of the indentation. In the haustorium two nuclei may be seen (×1250).

Fig. 46. Two haustoria side by side, the smaller one with one nucleus and the larger one with two nuclei (×1250).

Fig. 47. A polar view of a haustorium with two elongated nuclei in anaphase. Centrosomes and astral rays are visible (×1800).

Fig. 48. A polar view showing three nuclei, two of which have astral rays. The nucleoli are prominent (×1800).

Fig. 49. A polar view showing four daughter nuclei, two of which still have astral rays (×1800).

Fig. 50. A haustorium attacking a host cell. The wall around the haustorium is abnormally thick towards the top. This part is gelatinized and one section of it is stained safranin. This stained portion was probably the point of penetration (×1250).

Fig. 51. A further stage of Fig. 43; a nucleus in haustorium with the centrosphere still faintly visible (×1250).

Fig. 52. A nucleus at the entrance of a haustorium which has already penetrated the host cell wall. The wall adheres closely to the haustorium, its broken ends curving outwards. The broken ends stained safranin (×1250).

Fig. 53. A cup-shaped haustorium incapable of absorbing any more nutriment. The host cell wall clings tightly so that no cell contents can escape (×1250).

Fig. 54. A haustorium similar to that in Fig. 53, but showing the top view (×1250).

Fig. 55. A host cell penetrated by a haustorium. The nucleus in the host cell is near the haustorium (×1250).

Fig. 56. A nucleus in a haustorium with astral rays, and a nucleolus prominent (×1800).

Fig. 57. A nucleus in a haustorium in anaphase. Astral rays are seen with the nuclear membrane present, but poorly stained (×1800).

Fig. 58. Two resting nuclei in a haustorium showing centrosomes clearly (×1800).

Fig. 59. Two penetrating haustoria side by side, enclosed with a gelatinous layer. The layer adheres closely. One haustorium has three nuclei and the other four nuclei (×1250).

PLATE V.

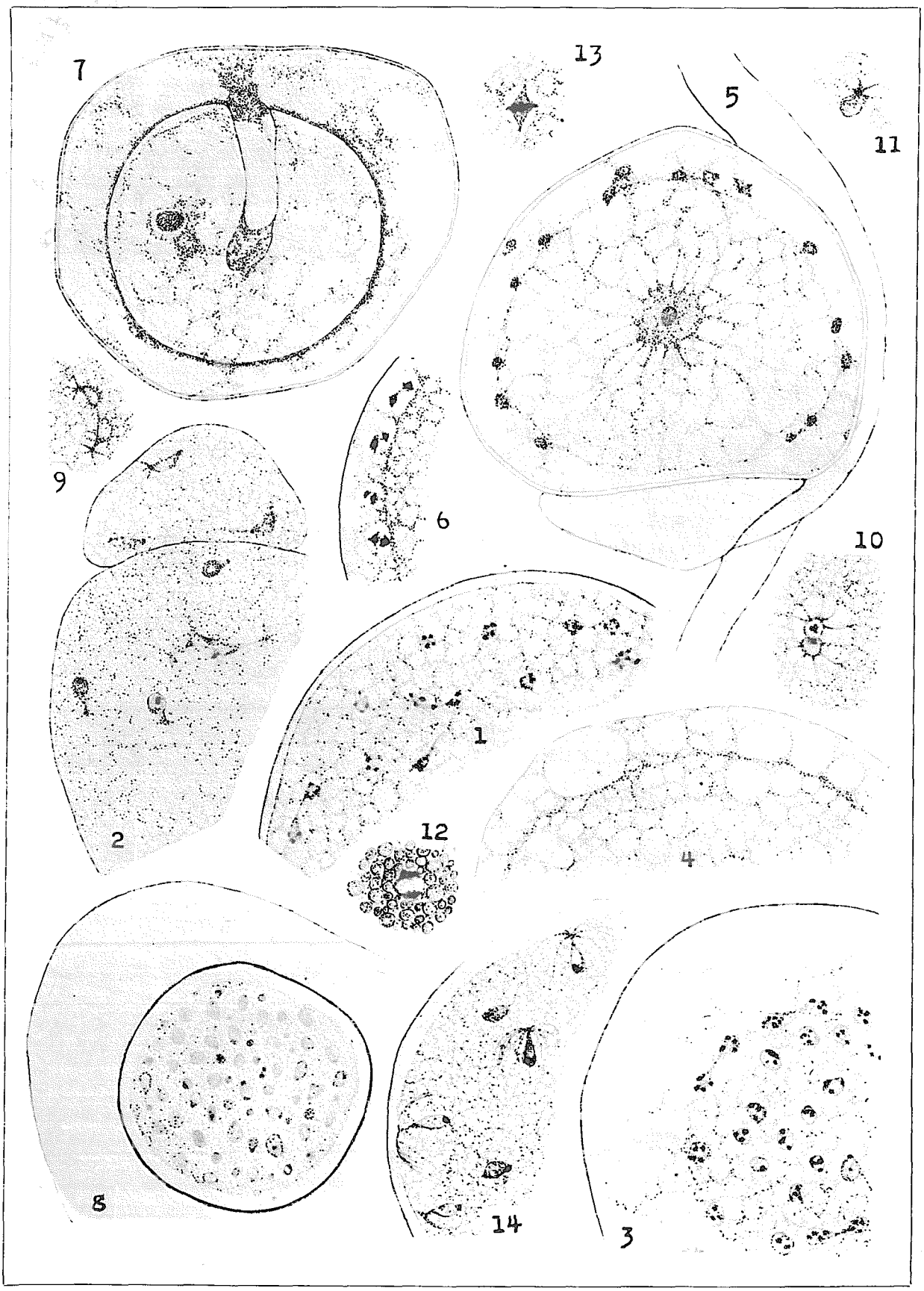
Fig. 60. The stem of an infected sunflower plant sectioned to show the conidiophore and conidia. (cp) Conidiophore, (c) Conidium, (×450).

Fig. 61. The cotyledon of a sunflower plant sectioned across a stoma. The conidiophore is emerging from the stoma (×450).

Fig. 62. The lateral root of a sunflower plant longitudinally sectioned to show the characteristic mycelia, conidiophores and conidia. (cp) Conidiophore, (c) Conidium (m) Mycelium (×450).

Fig. 63. Conidiophores developed on root of a sunflower plant (×450).

Fig. 64. A mycelium in the pith of a sunflower plant showing characteristic branching and haustoria developing in all directions (×370).



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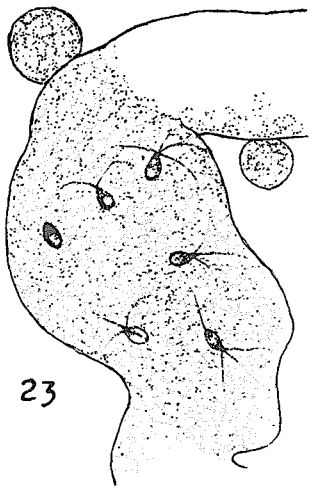
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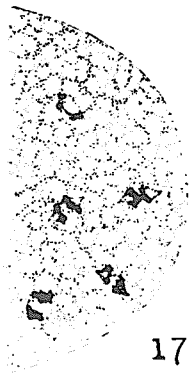
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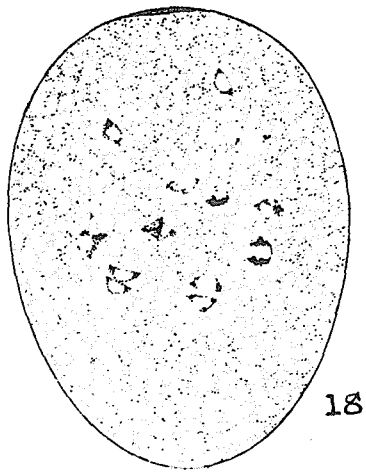
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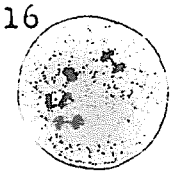
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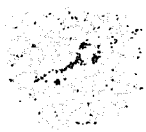
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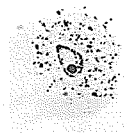
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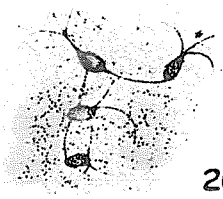
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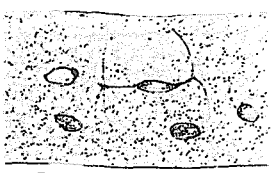
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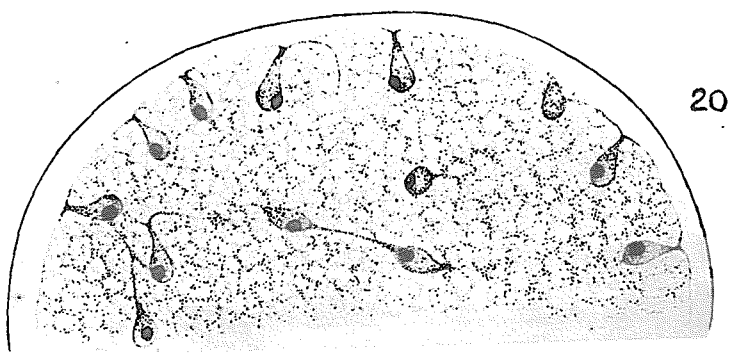
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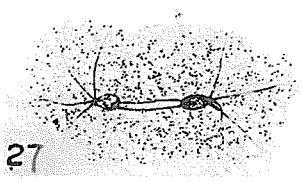
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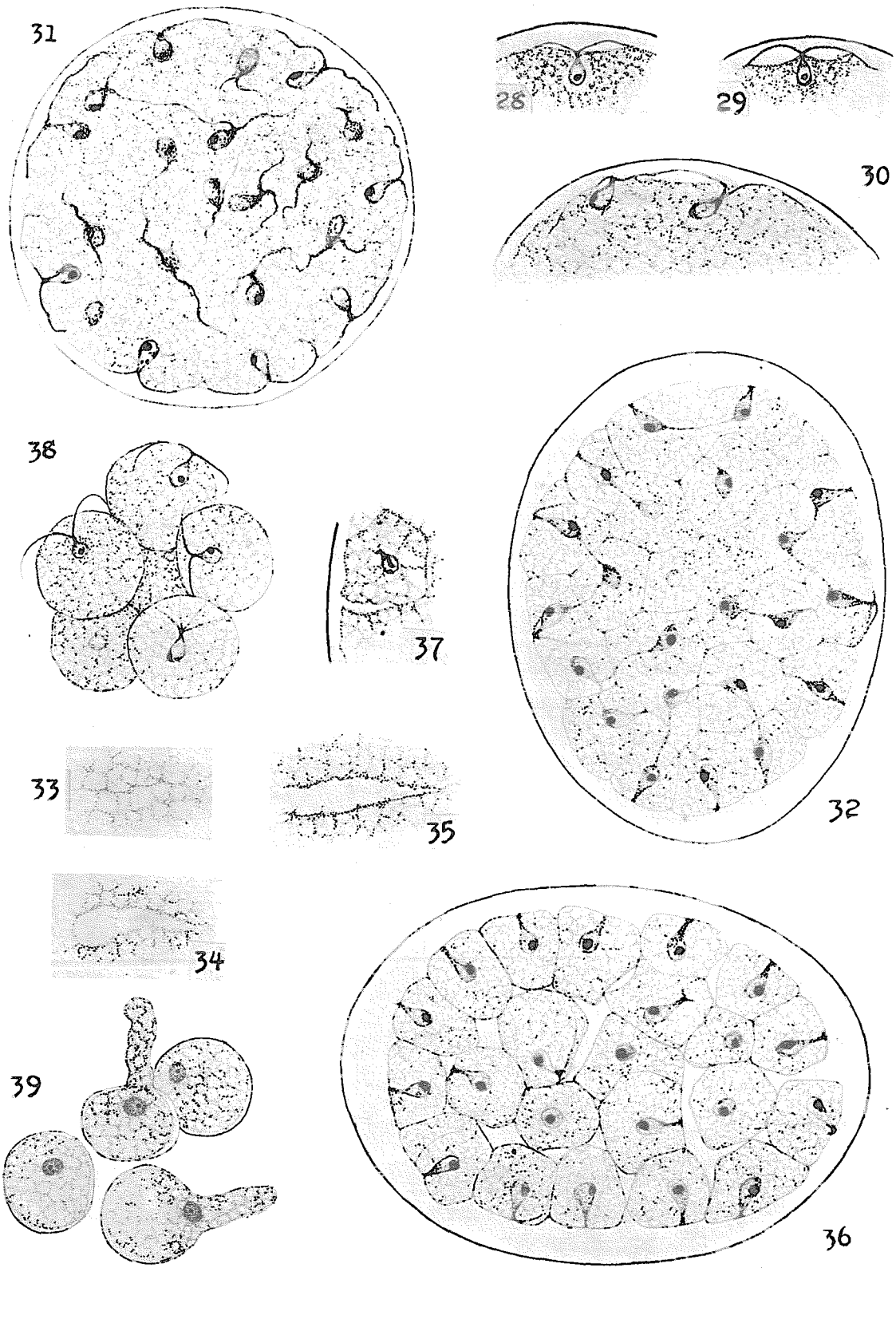

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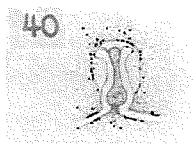

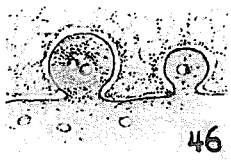

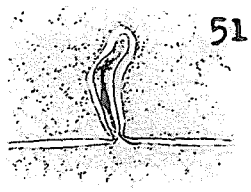

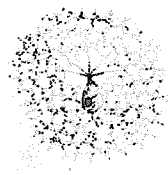

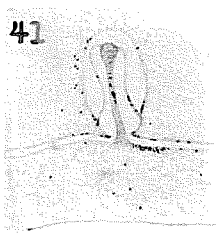

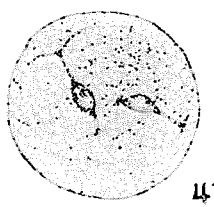

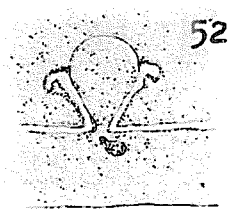

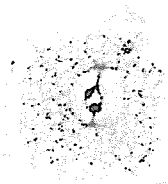

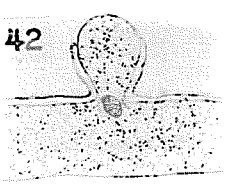

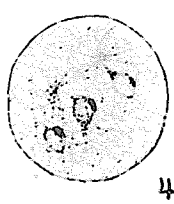

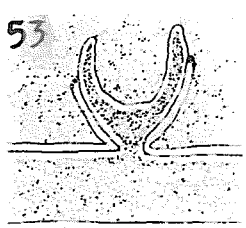

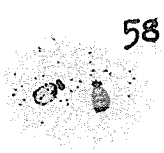

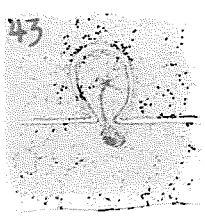

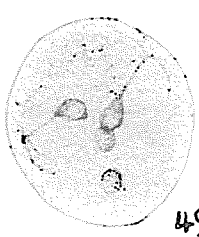

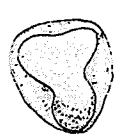

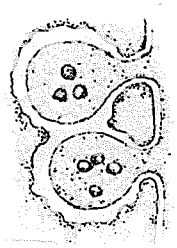

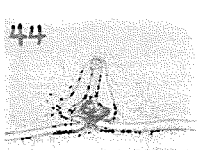

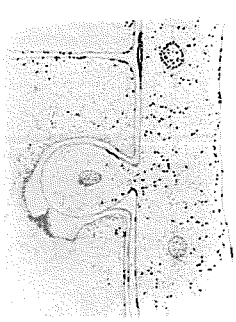

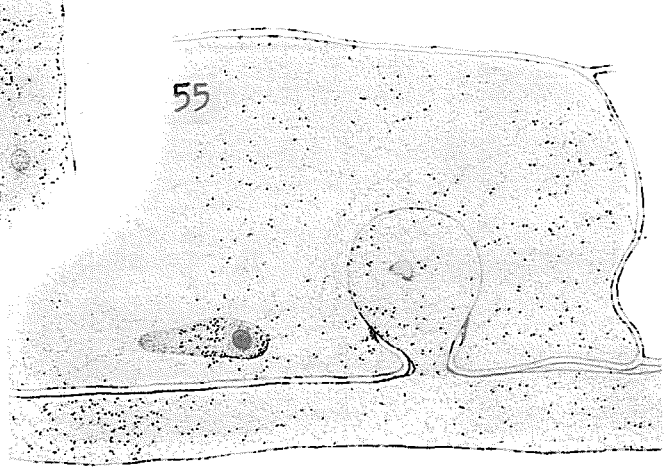

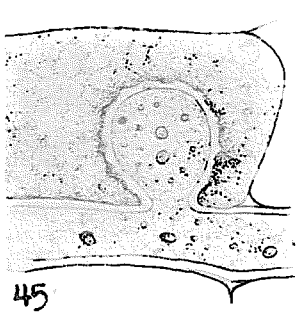

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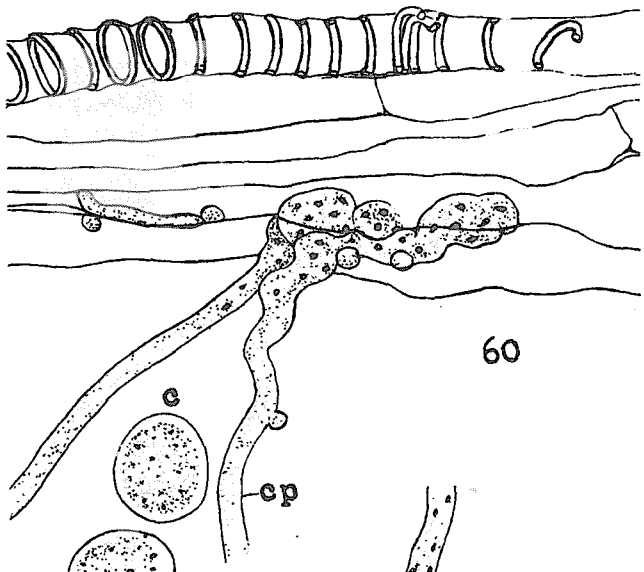

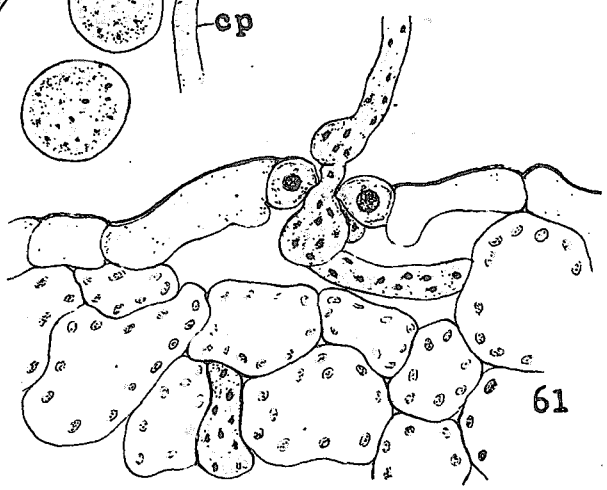

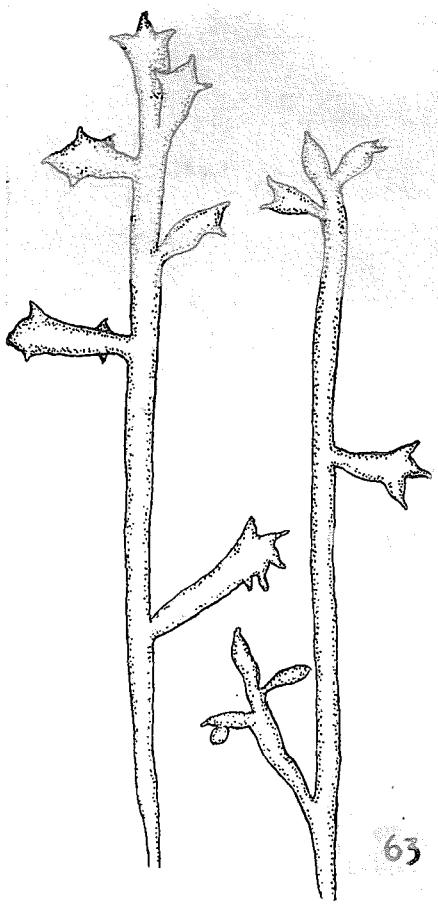

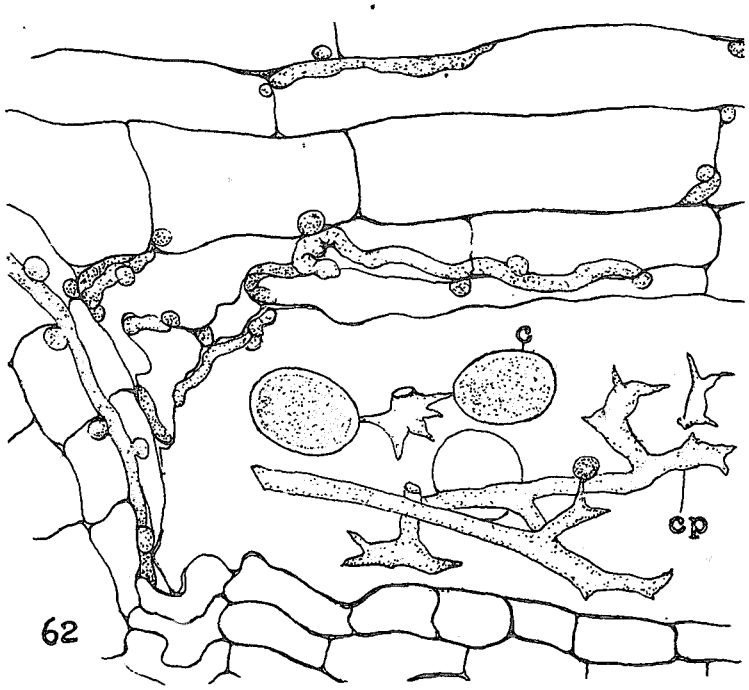

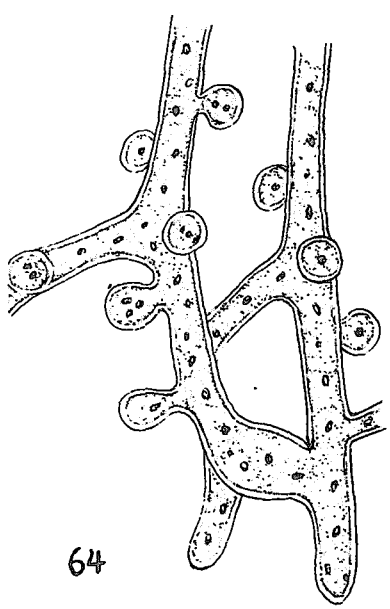

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