Studies in *Plasmopara Halstedii*

BY

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With Plates I-VI and Figures 1–7 in the Text.

I. THE INFECTION OF HELIANTHUS ANNUUS L. BY ZOOSPORES.

The writer has, during the last three years had an opportunity to study *Plasmopara Halstedii* as it occurs on *Helianthus annuus* in the garden at Columbia University. The results of the study will be reported under three heads: I. The study of the infection of *Helianthus annuus* by *Plasmopara Halstedii*; II. A study of the fertilization processes; III. A cytological study of zoospore formation in conidia.

In this paper it will be shown that a new type of infection for the *Peronosporaceæ* is found in *Plasmopara Halstedii*, namely, root infection by means of zoospores. A detailed study of fertilization processes of this species which has not been hitherto made will also be included. The cytological study of spore formation will be taken up in a later paper.

Historical.

Plasmopara Halstedii Farlow, was found by HALSTED first on Eupatorium purpureum near the Bussey Institution in May, 1876, and FARLOW[12] referred to it in 1882. Since then the fungus has been collected in numerous other localities and on several different hosts, and it may now be regarded as one of the most widely distributed and characteristically American species. It was considered that this fungus

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may be expected to occur on almost any composite.

WILSON[39] (1907) called this species *Rhysotheca Halstedii* (Farl.). STEVENS[36] (1913) says that *P. Halstedii* is quite variable in form and should perhaps be separated into several distinct species. Former studies of the infection of host plants by *P. Halstedii* have not been very complete. MELHUS[20] (1915) gave an account of his experiments, which showed that the mycelium of *P. Halstedii* may be present in the rhizome of *Helianthus divaricàtus*, and this strongly suggested that it is perennial in the rhizome, the mycelium passing the winter in the rhizome. The next spring when the new shoots develop, the mycelium also develops in the shoots. Similar statements regarding several other species of *Peronosporaceae* were also made: *Phytophthora infestans* on Irish potato, by de BARY[1] (1861); *Peronospora Dipsaci* on *Dipsacus fullonum*, by KÜHN[18], (1875); *Peronospora alsinearum* on *Stellaria media*; *Peronospora grisea* on *Veronica hederaefolia*; *Peronospora effusa* on *Spinacia oleracea*, by MAGNUS[19] (1888); *Plasmopara viticola* on *Vitis vinifera*, by ISTVANFII[16] (1904); *Plasmopara pygmaea* on *Hepatica acutiloba*, by STEWART[33] (1910); and *Phytophthora Cactorum* on *Panax quiquefolium*, by ROSENBAUM[27] (1914). These examples give a very important idea of the connection of the fungus to the plants in successive years, but this connection is only possible in plants which are winter annuals, biennials, or perennials. It is presumed that these plants were originally infected through their leaves.

In annual plants, like *Helianthus annuus*, there must be considered other methods of infection. Resting spores or oospores are produced by most of the species in the *Peronosporaceae*. Their function, as is well known, is to bridge the fungus over periods unfavorable for its growth and development. De BARY[4] (1884) gave four methods of germination of the oospores in the *Peronosporales*. HE[2] (1863) also studied Cystopus and showed the oospore in members of this genus germinated by zoospores. MILLARDET[21] (1883) had the same view as de Bary about the oospores of *Plasmopara viticola*. FRÉCHOU[15] (1885) claimed to have seen the germination of zoospores. FRÉCHOU[13] (1885) also saw the oospores produce long tubes which might be interpreted as conidiophores, but no conidia were ever produced upon them. CORNU[6] (1882) worked with the family *Saprolegniales* and evolved the theory that the oospores produce conidia. GREGORY[14] (1912) demonstrated germination of the oospores of *Plasmopara viticola*. The conidia resulting were borne aloft

on stalks or promycelia of varying lengths and could be observed to proceed, in nearly every case, from one of the brown oospores. He has never followed the germination of these conidia. Thus oospores produce zoospores directly or may form conidia, and these zoospores of the oospores or conidia when falling on leaves cause infection of the new host plants.

The method of infection by zoospores of mildews has been discussed by many students. MILLARDET[22] (1886) states that infection can be effected through the upper side of the leaves of the host. De BARY[3] (1884) found that in a number of the *Peronosporales* the germ-tube can penetrate the epidermis directly. FARLOW[11] (1876) says that the underside is better adapted for infection by swarm spores than the upper side. Ruhland and von FABER[31] (1908) state that the infection always takes place through the stomata on the under surface and that it never occurs on the upper surface. MÜLLER[24] (1911) also claimed that infection could come about only through the stomata. FAES[10] (1911) verified this conclusion by means of some infection experiments. ISTVANFFI[17] (1912) demonstrated the same thing. RAVZA and VERGE[26] (1911) showed that the zoospores usually enter through the stomata which are limited to the under surface. They suggested the possibility that the conidia may fall on the upper surface, where they germinate and the zoospores may then swim over the edge of the leaf to the lower surface. GREGORY[14] (1912) made an infection experiment with the swarm spores of *P. viticola* and agreed with Ravaz and VERGE[26].

Observation and Experiments.

Sunflower plants infected by *Plasmopara Halstedii* were found in the garden of Columbia University in June, 1918. In this garden were planted about a hundred sunflower plants, but at this time only five plants were diseased. The diseased plants were dwarfed and the leaves showed beautiful mosaic areas of light green and darker green. Such mosaic leaves mostly developed conidiophores along the light green areas. The conidiophores developed usually on the under surface of the leaves, but some developed on the upper surface also.

The most interesting phenomenon was the spreading of the light green area on the leaves. Chlorosis appeared first along the veins at

the region of the petiole and gradually spread in all directions (Plate I, Fig. 1.). In some instances the entire leaf became yellowish and was covered all over with conidiophores. Young leaves, when the chlorosis occured along the main veins, usually became curled. In these leaves the mycelium of the fungus spread parallel to the area of light green,

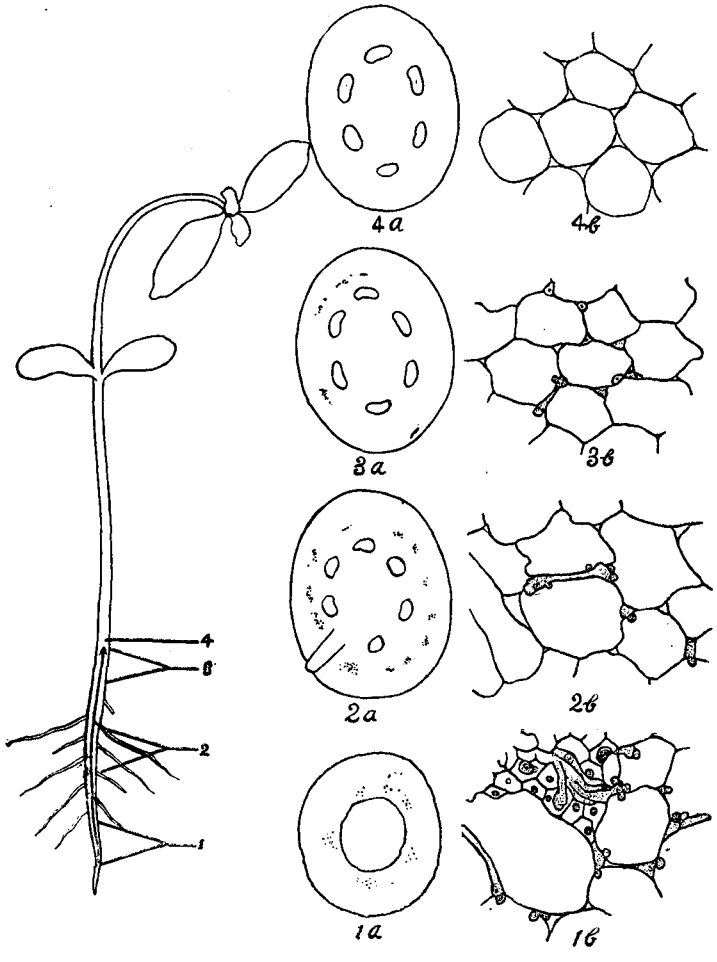

Fig. 1. Early stage of the infection, showing regions invaded by the mycelium. 1a, 2a, and 3a, diagrams of cross sections at 1, 2, and 3, the small groups of dots indicating the location of the hyphae. 1b, 2b, and 3b, represent the hyphae visible in one infected region of each of the sections 1, 2, and 3. Section at 4 showed no mycelium. 1b, 2b, 3b, 4b magnified 186 times.

showing that the chlorosis was due to the spread of mycelium developing from the stem to the leaf.

On examination of the diseased plants in young and old stages, it was noticed this fungus often developed from the underground roots and underground stems and spread into the aerial parts. For instance, some plants showed the mycelium just invading the rootlet, and others showed the mycelium in the main root and still other plants showed the main root and stem infected up to the cotyledons. This demonstrates the general and progressive character of the fungus.

The case of infection of *Helianthus divaricatus* takes place through the stomata first and travels down the stem and rhizome, but has never been reported to go down into the root nor has root infection by this fungus been reported. The above examples of diseased plants led the author to examine the root infection in the case of this sunflower plant.

He made an examination to see if any spores of this fungus could be found in these seeds. Seeds taken from diseased plants showed a low percentage of germination, but the seedlings did not show any symptom of the disease.

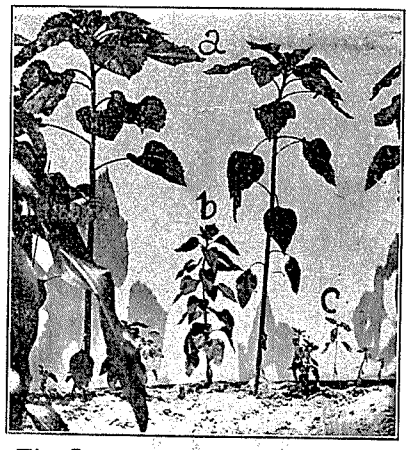

Fig. 2. Normal and diseased sunflower plants grown from seeds planted on April 15 and photographed on August 1. (a) Healthy plants. (b and c) Infected by P. Halstedii. Plant (b) few matured seeds.

The question that remains is, to prove that the fungus enters from the soil. The writer collected the soil where the diseased sunflowers had grown and placed it in a box in the greenhouse. Seeds were then sown in this soil. A similar box of soil was secured from a field in which no disease had occurred and seeds were sown in this soil as a control. In the examination of the plants in the two boxes, there was 70% infection by P. Halstedii in the former box, while in the latter box the plants kept in a healthy condition. Similar experiments were repeated four times and the results were similar, showing the following percentage of infection: 40%, 50%, 70%, and 72%. These percentages were based on 40, 20, 40, and 50, experimental plants, respective-

ly. No infection occurred when seeds were planted in steam sterilized soil from infected fields.

Sunflower seedlings (2-3 inches high, with cotyledons and some of them with the first two leaves) were planted in moist soil which had been inoculated with conidia of *P. Halstedii*. Other seedlings were planted in similar soil that had not been inoculated. The result showed infection by this fungus of the former plants to be 40% with the 70 experimental plants, while in the control plants no infection took place.

Similar experiments were tried using seeds of sunflower instead of the seedlings. The soil was inoculated with zoospores and seeds were then immediately planted in the soil. No infection resulted. This might be explained by the fact that zoospores which are formed from conidia live only a short time. In water cultures eight hours was the longest record of their activity. Therefore no infection by zoospores could take place.

Experiments with Sterilized Soil Inoculated with Oospores.

Sunflower plants were naturally infected by this fungus in the same fields in 1918, 1919, and 1920, successively. How did the fungus live over winter?

The following experiments were performed. Soil was inoculated (a) with conidia collected from the leaves; (b), with mycelium which developed in young seedlings; (c), with oospores collected from the tissue of the host plants. These three different soils were left exposed through the winter (1919-1920). Then sunflower seeds were planted the next spring (April 28, 1920). The seedlings developed very well in each soil. After a month's time these plants were examined and infection found in the plants which had grown in soil inoculated with oospores while the plants in the other soils showed no infection.

Infection Experiments with Zoospores from Germinated Conidia.

To observe the infection method of the zoospores, the writer collected numerous zoospores which had germinated from conidia and kept them in a small glass vessel, containing distilled water about 1/8 of an inch

in depth. Young healthy seedlings (2-2½ inches from the root tip to the cotyledons) were placed so that just the tip of the roots were exposed to the water and so that there was no chance of zoospores reaching the stem or leaves. They were kept this way for three hours and then planted in sterilized soil. This experiment was repeated several times, 28 seedlings in all were used and the results showed 9 infections.

To further prove root infection with zoospores of conidia, the following method was used: an area about 1 by 1/2 inch on an ordinary glass slide was enclosed with a paraffin wax wall, the height of the paraffin wall being about 1/16 of an inch, and as thin as it could be made easily. In this space a drop of sterilized water was placed to which were added active zoospores and then the root of a sunflower seedling was immersed in the drop and covered with a cover glass so that the behavior of the zoospores under the microscope could be followed. The zoospores, singly, or two or three held together by their

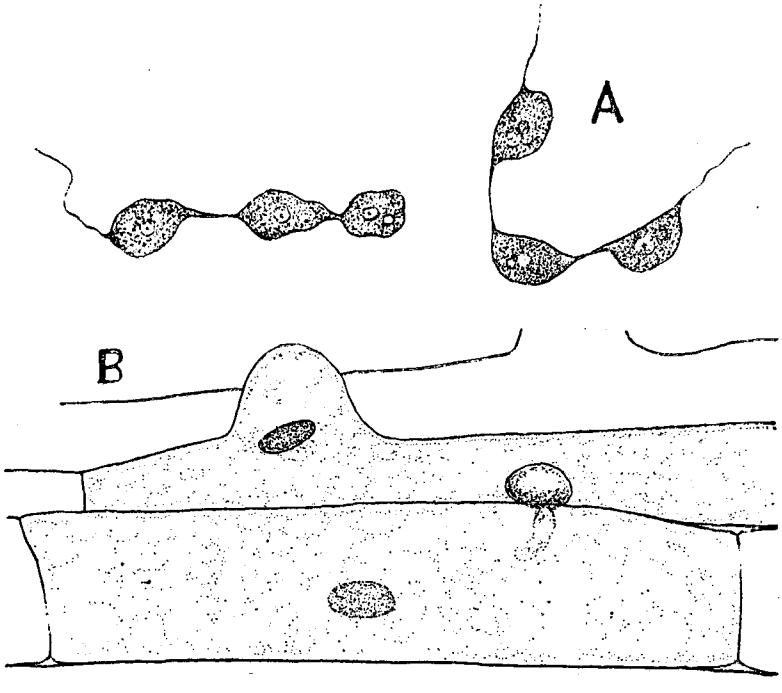

Fig. 3. Zoospores and the entrance of the germ-tubes into the roots (× 896). A. Zoospores still attached by cilia. B. Shows the entrance of the germ-tube of zoospore through the middle lamella of the epidermal cells.

cilia, approached the root by means of swimming and after one to two hours, approximately, had come to rest on the root and had lost their cilia. Thus they were scattered over the surface of the epidermis of the root. Some of them produced germ-tubes of varying lengths, which on reaching an intercellular space gradually developed in this region. After five hours the germ-tubes of the zoospores began to branch into the spaces between the cells. The place of infection was in some cases where the root hairs had broken off, so that the germ-tubes developed in the broken root hair.

It was noticed that staining the material with a weak solution of iodine was an aid in this particular study.

TABLE I.

Summary of Experiments

Methods	No. of plants used for experiments	Infected	No infection	Percentage of infected plants
Seeds sown in soil in which diseased sunflower had grown.	200	119	81	60 %
Seeds sown in disease free soil.	100	0	100	0 %
Seeds sown in sterilized soil in which diseased sunflowers had grown.	40	0	40	0 %
Young seedlings planted in sterilized soil inoculated with conidia.	70	28	42	40 %
Seeds sown in sterilized soil inoculated with conidia.	80	0	80	0 %
Inoculated soil with conidia left exposed through winter and seeds planted in the spring.	50	0	50	0 %
Same as preceding but mycelium used for inoculation.	50	0	50	0 %
Same as above but oospores used for inoculation.	50	10	40	20 %
Zoospores germinated from conidia kept in a glass vessel with distilled water. Young seedings were placed in it three hours, then planted in sterilized soil.	28	9	19	32 %

Host-parasite relationship.

General observations on this fungus in the tissue of the host plant were reported by MELHUS[20] and others. The mycelium of this fungus, according to these authors, develops in every portion of the host plant above ground, most so in the foliage where its presence is first indicated by the appearance of pale yellowish green patches on the leaves. The mycelium abounds in the spongy parenchyma. The conidiophores emerge th re through the stomata. The leaf then is covered with conidiophores on both its surfaces, though the under surface is more pronounced. The mycelium is developed entirely along the intercellular spaces. The mycelium has been found in all parts of the stem except the fibrovascular bundles of the host (*Helianthus divaricàtus*). Although in most cases in the writer's material the same distribution of mycelium in *Helianthus annuus* occurs, as Melhus found in his material, still the writer has found mycelium in the scalariform vessels, and also in the vessels of the root. Both cases, however, are from young seedlings which had not differentiated mature bundles as yet. This was not mycelium of some other fungus since the basal part is connected directly with hyphae producing characteristic haustoria.

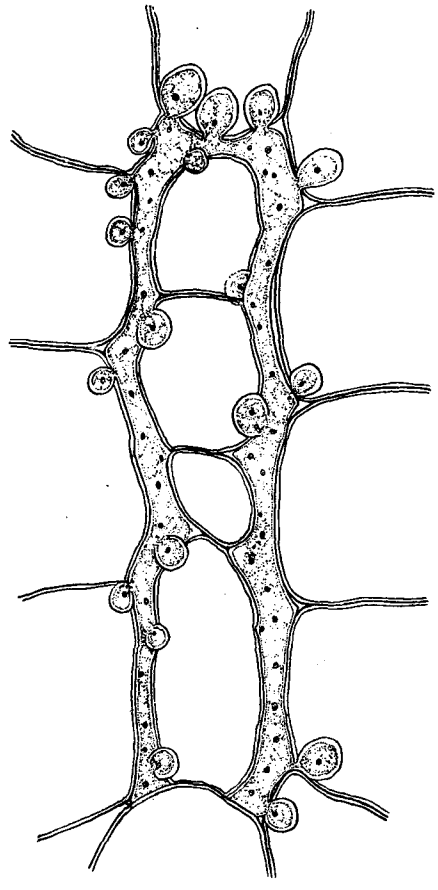

Fig. 4. Characteristic mycelium and haustoria in the pith region (× 370).

In the young seedling, therefore, it seems that the mycelium is easily developed in the vessels. The mycelium in the scalariform vessels has no well developed

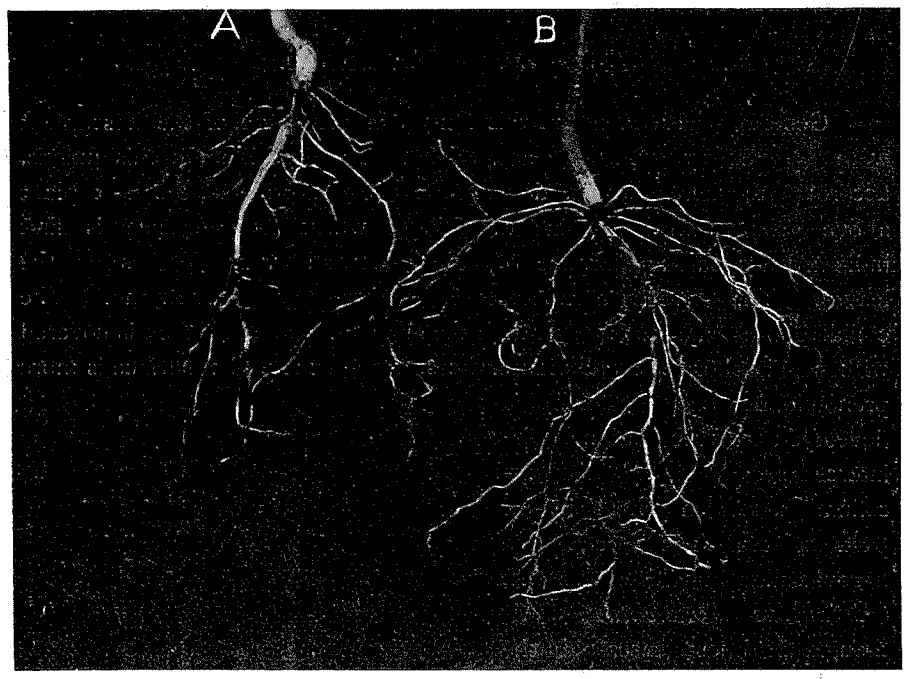

Fig 5. The root system of a diseased plant (A), and a healthy plant (B).

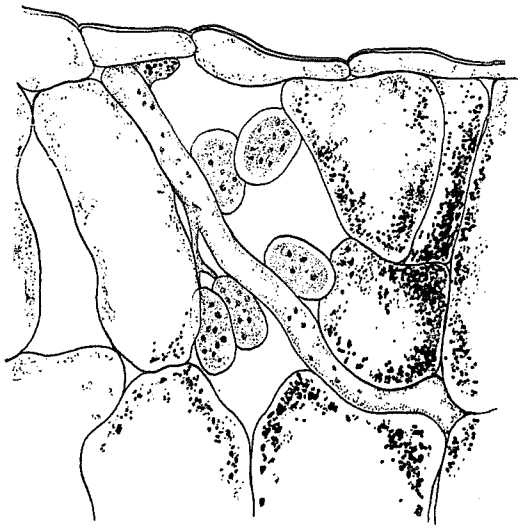

Fig. 6. Conidiophore and conidia in the substomatal cavity of a leaf (× 307).

haustoria but is joined to the rest of the mycelium which originates from the cortex of the root or stem and thus secures its nutriment.

Medullary rays are favorable places for the mycelium to pass from the cortical to the pith region. Some cases of well developed mycelium spreading out in every direction, growing up and down, and forming an intricate network have been observed.

In comparing the root system of a diseased plant

with that of the control it was found that there is a smaller number of secondary roots. Sections of the diseased roots showed especially, in many cases, that the mycelium invaded the secondary roots at their growing region, also roots which have not yet pushed out from the main root. (Plate I, fig. 3). It may be that such an active part of the tissue is rich in nourishment so that the fungus would have a tendency to attack that part as well as the medullary ray of the stem, and this is probably one of the reasons that diseased plants have poor root systems.

It was noticed in a number of cases that the conidia were found developed in the intercellular spaces of the spongy tissue and in the substomatal cavities of the leaves. Similar cases also were found in the tissues of the stem and roots, especially where small cavities have been caused by some injury, nematodes, insects, etc. In some cases conidiophores and conidia are formed on the roots in the soil. In addition to the formation of oospores in the leaves, they are also formed in the roots of the host plant.

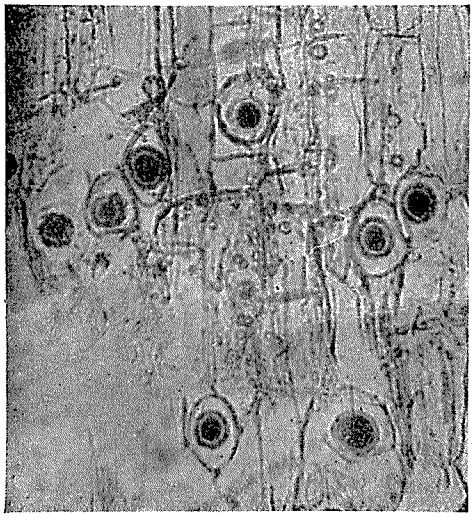

Fig. 7. Oospores in root of *Helianthus annuus L.*

In fresh material the oogonium and antheridium are scattered through the tissue of the roots. The oogonia are rather large, globose bodies about 30-48 μ in diameter. Some of the short branches of the mycelium swell at the end and as this swelling enlarges it is filled with a dense mass of protoplasm. The antheridium is rather irregular in form and smaller than the oogonium and about 12-30 μ in diameter.

Discussion.

A downy mildew is known to infect a host plant in two different ways: one, a perennial mycelium which may live in the tissue of the

host through the winter and renew its activity in the following spring when the fungus may sporulate and spread the disease. This has been reported by several authors on different species of mildew. These facts show clearly that infection takes place every year in the new shoots from the perennial mycelium in the subterranean stems, all of the host plants being winter annuals, biennials, or perennials. In the case of the ordinary annuals like *Helianthus annuus*, as the mycelium could not live over winter in dead plant parts or in the soil, infection could only take place through the resting oospores.

There recently have been given a number of reports of the entrance of conidial zoospores of mildew into the host plants. Many critical studies have been carried on to find out whether the germ-tube of the swarm spores penetrates the cuticularized wall of the epidermis of the host plant or can enter through the stomata only.

A similar question occurred to the writer; namely, can a germ-tube of a zoospore penetrate the epidermis of a root, in other words, can this fungus infect a root from the soil in nature. ROSENBAUM[25] (1915), working with the Phytophthora disease of ginseng, stated that the organism can infect the ginseng through an artificial injury in the root, but that no infections occurred in a normal root. But as the writer stated before, in the case of *Plasmopara halstedii* on the sunflower, infection through the root by means of condial zoospores does take place. This, therefore, leads to the question concerning the viability of zoospores in the soil. By means of infection experiments, through soil inoculated with conidia, it was found that they germinated, and the zoospores were able to infect the sunflower roots through the soil quite well. This could very well take place in nature, after a rain when the soil particles are surrounded by a film of water sufficient for zoospores to swim in.

It is quite interesting to compare ROSENBAUM'S[25] statement with what the writer has found to be the case in *Plasmopara halstedii*. It was observed that the entrance of zoospores into a root, takes place not only where the root hairs have broken off, but also in regions of the epidermis where there are no root hairs and no injuries of any kind.

This is not surprising since the epidermis of the root has no cuticle as is the case with a leaf or a stem, and such a penetration by a germ-tube would not be different from that by a haustorium through cell walls in the parenchyma tissue of the same plant by the same fungus.

It has been shown that only the oospores of *P. halstedii* are capable

of living over winter. Both the conidia and oospores germinate in the soil by zoospores. The zoospores can reach the roots of the host plant by swimming and having reached the root, produce germ-tubes that penetrate and infect the plant. There are, therefore, two paths of infection: one starting from the leaf and gradually progressing through the stem and root; and the other from the root up through the stem to the leaves. In both cases conidia and oospores may be produced during the growing season of the host plant, but over winter and in the following spring the oogonial zoospores produce the infection on the new plants.

The life history of this fungus may be demonstrated by the following chart for both annual and perennial plants:

Perennial Mycelium of *P. Halstedii* on *H. divaricàtus.*

Fungus	Mycelium → Conidia → Zoospores → Mycelium				
Host	Rhizome → Stem → Leaf		Leaf → Stem → Rhizome		
	Host		New Host		
Season	Winter	Spring	Summer	Autumn	Winter
Year	1918	1919			

Annual Mycelium of *P. Halstedii* on *H. annuus.*

Fungus	Oospore → Zoospore Mycelium Conidia → Zoospores Mycelium ↗ Antheridium ↘ Oogonium → Oospore				
Place	Soil	Leaf → Stem → Root → Root → Stem → Leaf (A Host)		Leaf → Stem → Root → Root → Stem → Leaf (B Host)	Soil
Season	Winter	Spring	Summer	Autumn	Winter
Year	1918	1919			

Summary.

1. The plants which are invaded by the mycelium of *Plasmopara halstedii* become dwarfed, and the infected leaves show chlorosis and are often curled.

2. The pale yellowish green areas occur along the main veins of the leaves. In these areas the conidiophores develop on both upper and lower surfaces of the leaf, although the growth on the underside is more pronounced.

3. The light green area of the leaves spreads along the regions invaded by the mycelium.

4. The fungus often spreads from the underground root and underground part of the stem and gradually develops into the upper aerial parts, so that in leaf chlorosis it commences in the basal portion of the the leaf first.

5. The mycelium does not develop in the seed of the infected sunflower.

6. The soil in which the diseased sunflowers were grown becomes inoculated with oospores and passes the infection to the roots of new plants grown in that soil.

7. The soil which was inoculated with conidia was capable of carrying infection to new plants for two days.

8. Conidia or mycelium can not live over winter in the soil.

9. Zoospores from conidia can infect the roots in the soil. Zoospores are the means of infection which occurs through the middle lamella of the epidermal cells or through the cell where root hairs have broken off.

10. Mycelium of this fungus develops in every portion of the host. In some cases the mycelium invades the scalariform vessels of the root and stem in young seedlings.

11. Medullary rays are particularly favorable for the growth of the mycelium as is also the growing region of the secondary roots which have not yet pushed out from the main root.

12. Conidia of this fungus develop in tissue where there are large spaces, for instance, in stomatal chambers, intercellular spaces of spongy tissue of the leaf, and hollow places of the root and the stem which may have been injured by nematodes, etc. Conidia also develop from mycelium in roots in the soil.

13. Oospores develop in the tissues of the leaf, the root, and the stem.

[After having finished this essay I received a copy of Caroline G. Howe's paper "Pectic Material in Root Hairs," Bot. Gaz. 72: 313–320 (1921). Caroline G. Howe has studied the root hairs of various species. She noticed that no cellulose is found

in the root hairs and that the root hairs grown in both loam and sand have a layer of pectic material on the outside and a layer of callose on the inside. This fact has a very important relation to the problem of root infection by the fungi.]

II. THE METHODS OF FERTILIZATION AND OOSPORE-FORMATION.

MURPHY[26] (1918) very clearly discusses cytological investigations on the fertilization and oospore-formation in *Peronosporales*. He says "It is clear that *Pythium, Phytophthora, Sclerospora* and *Plasmopara* are closely related in the organization of the ooplasm and periplasm. They agree also, both among themselves and with *Peronospora,* in having no coenocentram comparable to the granular mass in *Albugo candida*, in the delayed fusion of the sexual nuclei, and in the devision of the fusion nucleus being so retarded that they all rest in a nucleate condition. Morphological and biological evidence suggested strongly that these genera, including *Peronospora,* form a natural group and it would require strong cytological evidence to prove the contrary."

Only three species of *Plasmopara* have been studied, *Plasmopara nivea* DANGEARD[5] (1894), *P. densa.* DANGEARD[5] (1894) and RUHLAND[30] (1904), and *P. alpina* ROSENBERG[29] (1903). DANGEARD[5] in 1894 found that the antheridia and oogonia are multinucleated in *Plasmopara nivea* and *P. densa.* But he could not show any details of fertilization. In *Plasmopara densa,* however, he made the interesting observation that when the protoplasm concentrates towards the center of the oogonium for the purpose of forming the oosphere, two small nuclei are visible at the center quite close together; the other nuclei are found in the periplasm. He was unable to observe whether these two nuclei fuse together or not. Murphy thought that Rosenberg was mistaken in supposing that the oogonial nuclei divide a second time.

Cytological studies on the fertilization process in the species of *Plasmopara* made by these authors are all more or less incomplete and lack observations as compared with those other relates genera.

Material and Method.

The sexual organs of *P. Halstedii* are developed abundantly in roots and leaves and less commonly in the stem. They are most frequently

found in the roots. For example, a microtome section of the root demonstrated 16-18 oogonia and antheridia in a section 800 by 200 μ in size and 7 μ in thickness. I have studied these organs in the roots of *Helianthus annuus* especially, as their presence there has not heretofore been reported.

In the sunflower seedlings, which were infected with this fungus late in April, there could be found a number of oospores four to six weeks later, while in July and August the sexual stage is not so common. As a rule, in this fungus the oospores are best formed when the vitality of the host has declined. On April 22nd the writer sowed sunflower seeds in the field where the fungus had attacked the plants the year before. The sunflower seeds germinated after 8-10 days. Every third day the seedlings showing the diseased symptoms were taken up and examined. Six weeks later, examining the roots of these diseased dying plants under a microscope, oospores were found in immense number in both main and secondary roots.

The tissues in which the sexual organs were formed were nearer the periphery than the center of the root. Very often the matured spore was found just under the epidermis, which was bulged out, due to the pressure of the oospore. In such cases, the inner cell wall of the epidermis is broken and the matured spore lies apparently within the dead epidermal cell, though in its young stage, it is found in the intercellular spaces.

The material was fixed with a weak Flemming's solution for 24 hours, using about 50 parts of the solution to one part of the material. The material was cut into pieces 7-10 mm. in length and fixed and imbeded in the usual way. Sections were cut 5-7 μ in thickness; Flemming's triple stain was used.

Observation.

The oogonium originates in an expansion of a hypha. Two kinds of oogonial primordia were distinguished, according to the difference of their position on the hypha upon which they appear. If the expansion occurred at the end of the hypha, then "terminal oogonium" is formed. The single terminal oogonium is the most simple type and rather common in this fungus. Double oogonia are similar in size and form and are found in pairs at the end of the hypha. This is rather rare. Double oogonia were found also in *Phytophthora infestans* by CLINTON[6] (1909).

There has been some question as to the possibility of both oogonia developing normally, but it was found, not rarely, in the present case that oospores in close contact with each other developed to their maturity, showing by their relation on the hypha that they are really the outcome of double oogonia.

If the expansion occurs in the middle of the hypha, then "intercalary oogonium" is formed. This has been found in many species: *Albugo Bliti* STEVENS[32] (1899) *Pythium De Baryanum* MIYAKE[23] (1901) and others. Usually in this primordial stage the cytoplasm of the oogonium is characteristically vacuolated and appears to be streaming. The nuclei are somewhat distorted and irregular in outline (Plate II, Figs. 1, 2). Such irregular, angular nuclei are usually elongated in the direction of the streaming. This phenomenon is well known in Albugo, and was noticed by WAGER[37] (1896) and STEVENS[32] (1899). Also it is well known in *Peronospora parasitica* as noticed by ISTVANFFI[15] (1896) and WAGER[38] (1900). The cytoplasm is rapidly flowing from the mycelium to fill the enlarging primodium, affecting the nuclei so that they elongate in the direction of the flow of the cytoplasm (Plate II, Fig. 1). The similar irregular nuclei are also found in the hypha some distance back from the oogonial primodium (Plate II, Fig. I). The oogonium is soon cut off from the hypha by a septum, which is usually formed at a short distance back from the oogonium. The size of the oogonia is variable, but well developed ones may measure about $52 \times 44\mu$. The cytoplasm of the oogonium, now cut off from the hypha, becomes vacuolated and the nuclei recover their normal appearance, then the cytoplasm gradually forms a network structure (Plate II, Fig. 3).

The formation of the antheridium in the early stage is similar to that of the oogonium; and it is difficult to distinguish them at first, but a little later with the septum formed, one can distinguish them by the number of nuclei. For example, in this fungus there are about 6-10 nuclei in the antheridium, 8 being most common. This represents the final number after division, so that in a very early stage there are only three to five. In the oogonium there are as many as thirty or more in the early stage. Fig. 12 in Plate IV shows an inter-calary antheridium containing three nuclei. The antheridium is spherical at first, but becomes usually flattened by pressure against the oogonium.

After the oogonium has been cut off, the wall thickens and the nuclei lose their irregular shape and become normal, increase in size

(Plate II, Fig. 3), and collect toward the center, where the cytoplasm has become differentiated into inner and outer portions, the latter extremely vacuolated (Plate II, Fig. 4).

Division of nuclei precedes the early stages in the formation of the monocyst (receptive papilla). The various stages of the monocyst are shown in Plates II, III, Figs. 4, 5, 6.

The nature and structure of the monocyst may be specifically different in different species and a consideration of it is very important in an understanding of the fertilization process of this group. Stevens in 1902 found no sign of monocyst in *Sclerospora*, and it was thought that this structure was lacking in this genus. But in many other species of the family it has been found by other writers. The processes are similar in all these different species, that is, the monocyst appears soon after the antheridium comes in contact with the oogonium. This consists of a densely granular mass of cytoplasm protruding toward the spot where the antheridium is in touch with the oogonium, but those writers could not find any farther development of the monocyst. In *Phytophthora erythroseptica*, however, MURPHY[20] (1918) observed that a very large monocyst (receptive papilla) is found, just as the nuclear division is being completed, passing through the stalk of the oogonium into the antheridium, and, when the monocyst is withdrawn a short fertilizaition tube grows in at the same place and delivers one male nucleus and the greater part of the cytoplasm of the antheridium to the oosphere. It is very interesting to compare the similarity of the process of the monocyst of *P. Halstedii* studied by the writer with that of Murphy's description. In *P. Halstedii*, at first, as in the case of *Phytophthora erythroseptica*, a large monocyst growth protrudes into the antheridial cell from the oogonium; but soon it retracts and conducts the fertilizing tube from the antheridium into the center of the oosphere (Plate III, Fig. 9 and Plate IV, Figs. 10 and 13). It is considered that this development of monocyst may be the inducing power for the antheridial tube, and the fertilization processes of such prominent species as *P. erythroseptica*, and *P. Halstedii* may be found to be widely spread over other species of this family. While these changes are taking place, the nuclei, both of the oogonium and antheridium, increase considerably in size, and the network of cytoplasm becomes more distinct and takes a deep stain.

The degeneration of nuclei in the oogonium usually occurs in the beginning of the oosphere formation. Those remaining arrange themselves

at the periphery of the oosphere, leaving one central nucleus. This brings about a differentiation of the cytoplasm into ooplasm and periplasm. The central granular ooplasm becomes vacuolated and reticulate, while the external periplasm becomes rather homogeneous and hyaline in character (Plate II, Figs. 4, 5). Then the nuclei in the periphery uudergo mitosis (Plate III, Fig. 7).

During this nuclear division the ooplasm becomes more vacuolate and finally the whole of the cytoplasm exhibits a distinct form structure due to the large number of small vacuoles. These minute vacuoles gradually fuse, so that the number of them decreases (Plates II-III, Figs. 4-7). During this change of the ooplasm, a dense mass of cytoplasm becomes prominent in the center which stains deeper than the surrounding cytoplasm (Fig. 7). This is the first sign of the coenocentrum. In later stages the body increases in density and stains still more deeply. The behavior of the coenocentrum is similar to that in *Albugo tragopogonis*, *A. candida* STEVENS[33] (1901), *Peronospora parasitica* WAGER[35] (1900), *Sclerospora* STEVENS[34](1902) and *Plasmopara alpina* ROSENBERG[29](1903). The coenocentrum usually disappears during fertilization. The coenocentrum is considered to have an important relationship with the female nucleus, since the latter is usually in contact with it. Stevens suggested that it may have the nature of a dynamic center. Coenocentra, however, may be specifically different in different species, for example: in *A. Bliti* and *A. candida* the central globule is prominent, while in *Sclerospora*, this globule has not been demonstrated. In *Plasmopara Halstedii* the central globule is not distinctly visible as in *Albugo*. A more detailed study is necessary before the exact relationship can be determined. Division takes place in the central nucleus in the ooplasm at about the same time as in the peripheral nuclei (Plate III, Fig. 7). These two daughter nuclei appear about equal in size at first and smaller than the mother nucleus. One of these nuclei degenerates immediately but the other remains for fertilization. This phenomenon was also observed in *Phytophthora erythroseptica* MURPHY[25] (1918). The female nucleus that remains is usually attached to the coenocentrum (Plate IV, Fig. 11). The nuclei on the periphery of the ooplasm degenerate and disappear, but in some cases these degenerate nuclei remain for a long time even after the eggs are fully formed as in *Saprolegnia* DAVIS[9] (1903). The fertilization tube of the antheridium elongates and touches the coenocentrum. Then one male nucleus is discharged with cytoplasm from the antheridial tube. The

male nucleus usually is a little smaller than the female nucleus. These two nuclei come first into close contact, but do not fuse at once as is the case in *Peronospora parasitica* WAGER[35] (1900). Both nuclei increase in size gradually and finally they become about equal in size. At this stage the nuclei fuse with each other. The most prominent sign that fertilization has taken place in the oogonium is the formation of oil drops. In this stage the cytoplasm has a prominent vacuolated character, with oil drops contained in the vacuoles. The cytoplasm at this stage stains faintly, but the nuclei stain more deeply. Very soon the network gradually disappears (Plate VI, Fig. 20-21). This agrees with *Peronospora parasitica* WAGER[35] (1900). Wager stated that "at the time the nuclear fusion takes place, the cytoplasm of the oospore contains numerous oil drops. DANGEARD'S[7] (1890) statement and his figure of the oil globule which appeared in the center of the oosphere during the stages of coenocentrum formation is doubtful." The oil drops have a tendency to fuse together one by one so that in the later stages the oil drops become increased in size and form large, irregular masses, thus reducing the number of oil drops (Plates V-VI, Figs. 19-22). These oil drops are aggregated at the center. Finally there is formed by their fusion a single spherical oil drop, which sometimes contains vacuoles (Plate VI, Fig. 22). This oil drop is surrounded by a layer of cytoplasm containing many nuclei in the mature spore (Plate VI, Fig. 23). The oil drop has a weak refringent power. The appearance is very much different in the various stages of the development of this central oil drop (Plates V-VI, Figs. 17-22). During these stages the wall of the oogonium gradually increases in thickness and formes a thick walled exospore with irregular folds. In the mature spore the large oil drop is in the center, surrounding this is a layer of homogeneous cytoplasm containing numerous nuclei distributed irregularly in the cytoplasm, then outside these are the endospores, primary and secondary, and finally is the exospore which is folded irregularly.

Summary.

1. The nuclei in the oogonium and in the antheridium divide once only, mitotically and simultaneously.

2. The coenocentrum is prominent in the center of the ooplasm.

3. The female nucleus is attached to the coenocentrum before fertilization.

4. A large monocyst (receptive papilla) begins to be formed during the nuclear division stage.

5. The monocyst of an oosphere protrudes into the antheridial cell at first, as Murphy described, but soon it retracts and conducts the intruding fertilizing tube from the antheridium into the center of the oosphere.

6. The fertilizing tube develops after the division is complete, and grows into the center of the oosphere, discharging only one male nucleus into it with a part of the cytoplasm of the antheridium.

7. The oosphere is uninucleated; after nucleor fusion the oospore becomes multinucleated.

8. The spore wall consists of three layers, the primary and secondary endospores, and an irregularly folded thick exospore.

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EXPLANATION OF PLATES I-VI.

With the exception of figure 1 on Plate I, all the figures were drawn with the aid of a camera lucida at table level. E. Leitz Wetzlar oculars and objectives were used, the magnification being as indicated.

Plate I.

Fig. 1. A leaf of *Helianthus annuus* infected by *Plasmopara Halstedii* (× 1).

Fig. 2. Characteristic conidiophores and conidia (× 453).

Fig. 3. The mycelium invading the rudimentary secondary root, which is still embedded in the cortical tissue of the main root (× 187).

Fig. 4. Various stages of the entrance of the germ-tubes of the zoospores through the intervening cells of the epidermis of the root (× 597).

Plate II.

Figures 1–24 on Plates II ~~and III~~ VI are magnified × 1120.

Fig. 1. The nuclei are elongated in the direction of the cytoplasmic streaming.

Fig. 2. Further view of the same. Irregular shape of the nucleii still evident.

Fig. 3. A young oogonium and a young antheridium. The nucle have recovered their normal shape and the cytoplasm forms a network. Eight nuclei can be seen in the antheridium and twenty-three in the oogonium.

Fig. 4. Further stage of the same. The stalks of both the antheridium and oogonium are parallel and each shows the septum in it. The contraction of the cytoplasm is shown in the oogonium. (a) The central mass is vacuolated and many nuclei are degenerating. (b) Some nuclei are dividing on the periphery of the mass. (c) The monocyst (receptive papilla) is commencing on the under part of the antheridium. (d) Few strands of cytoplasm are visible connecting the central mass and the wall of the oogonium. In the antheridium some nuclei are dividing and others

are degenerating.

Fig. 5. Further stage in the development of the monocyst. An accumulation of a denser cytoplasm is to be seen at the tip of the monocyst.

Plate III.

Fig. 6. The conspicuous papilla put into the antheridium. In the oosphere the network of cytoplasm is more vacuolated. In the antheridium six nuclei are present.

Fig. 7. Many nuclei are dividing in the periphery of the central mass. In the center two nuclei are present attaching to the coenocentrum. One of these may remain as a female nucleus. In the antheridium are two nuclei, one in the act of dividing.

Fig. 8. The coenocentrum formed in the center, and on the periphery of the oogonium many nuclei are dividing. Eight nuclei can be seen in the antheridium.

Fig. 9. Formation of the fertilizing tube. A line of demarcation in the oogonium between the ooplasm and periplasm is visible. The female nucleus is remaining in the center.

Plate IV.

Fig. 10. Further stage in the development of the fertilizing tube. A male nucleus is in the apex of the tube.

Fig. 11. A female nucleus is in the cen er attaching to the coenocentrum. The ooplasm contains larger vacuoles.

Fig. 12. Intercalary antheridium, after delimination from the hypha, containing three nuclei.

Fig. 13. The fertilization tube is reaching the center. Male and female nuclei are present near the center.

Fig. 14. Male and female nuclei are near each other. The wall of the oosphere is seen distinctly. Three nuclei are degenerating in the antheridium.

Plate V.

Fig. 15. Male and female nuclei in close contact with one another. One is a little larger than the other.

Fig. 16. Male and female nuclei in close contact with one another. Both are now equal in size and they are much enlarged. The fertilizing tube still remains, containing a large vacuole in the apex. In the antheridium are no nuclei.

Fig. 17. The wall of the oosphere is distinct. The cytoplasm in the periplasm disappearing in the network, where many nuclei remain in a degenerating condition. In the oosphere, the cytoplasm is very much vacuolated and small oil drops are forming.

Figs. 18, 19. Further stage of the same. The endospore is thicker and the oily character is more increased. In Fig. 18 a larger nucleus and two smaller ones are seen in it.

Plate VI.

Figs. 20, 21. Further stages in the formation of oil drops. The oil drops increasing in size and decreasing in number by means of fusion in later stages. In Fig. 21 the vacuolated form of cytoplasm in the oosphere has disappeared, and three nuclei are seen in it.

Fig. 22. A large oil drop formed in the center containing three large vacuoles, around it being homogeneous cytoplasm containing a nucleus.

Fig. 23. A matured oospore showing a large oil drop in the center around which is a cytoplasm layer containing 20 nuclei. The primitive wall fold.

Fig. 24. The surface view of the oospore.

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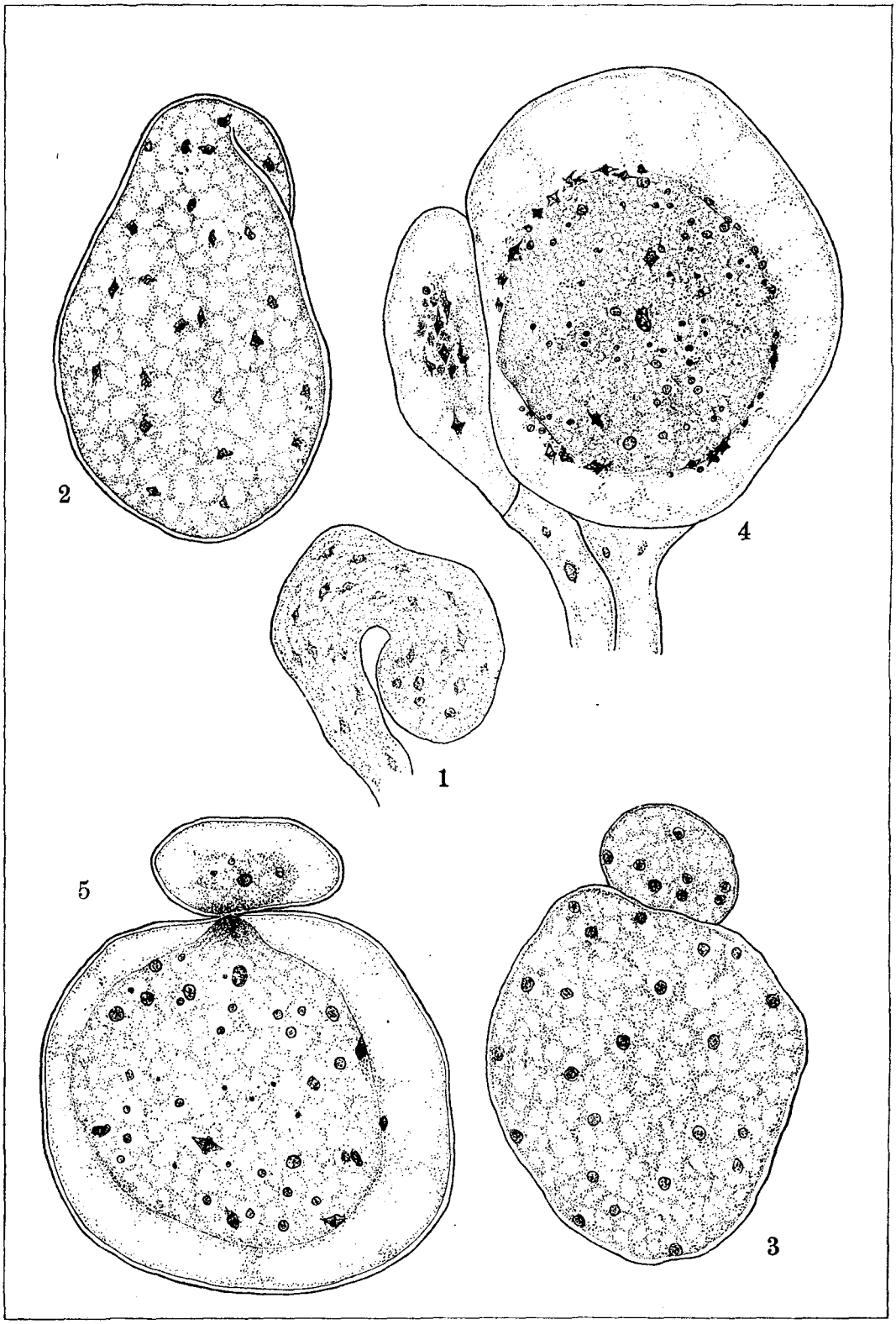

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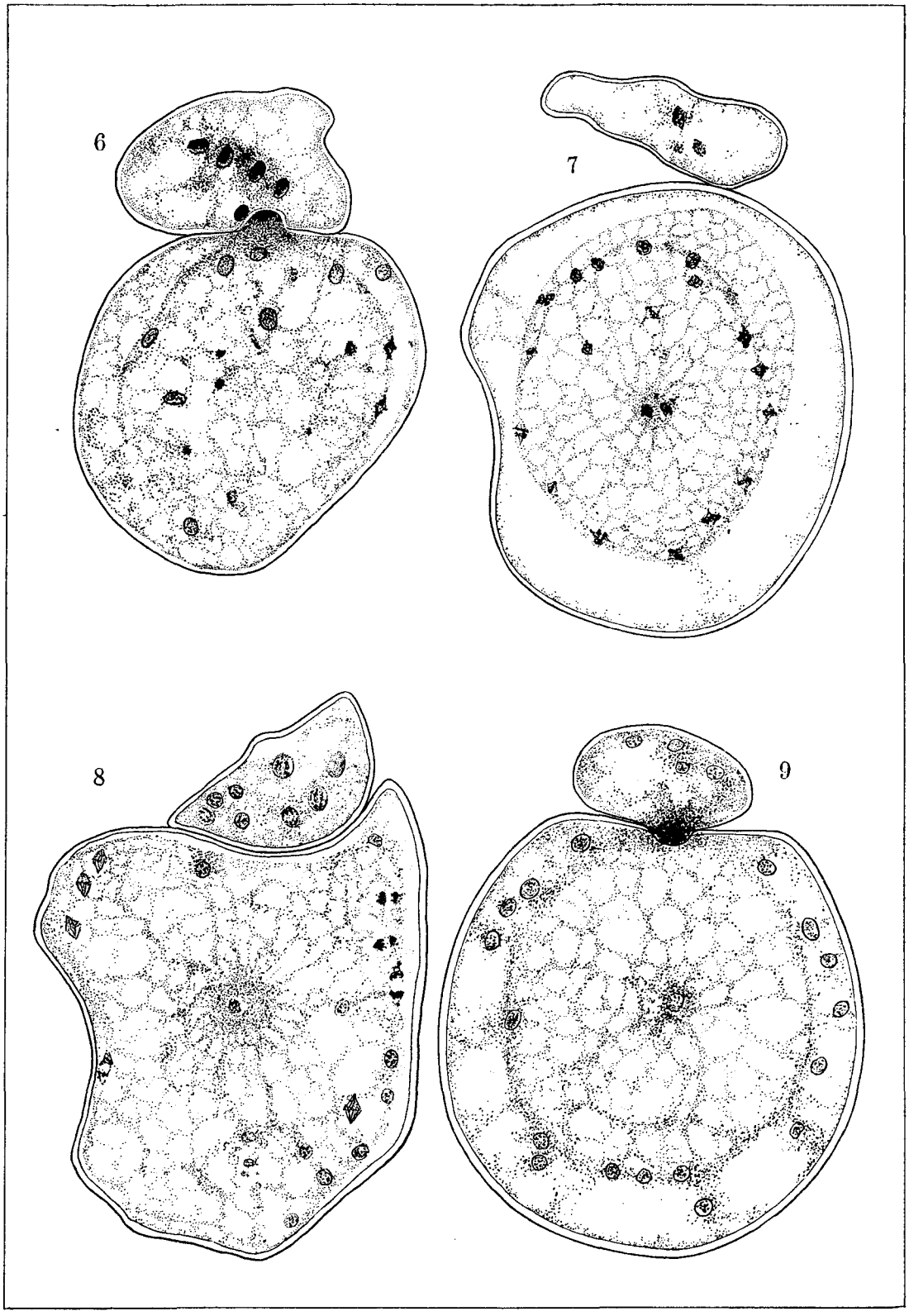

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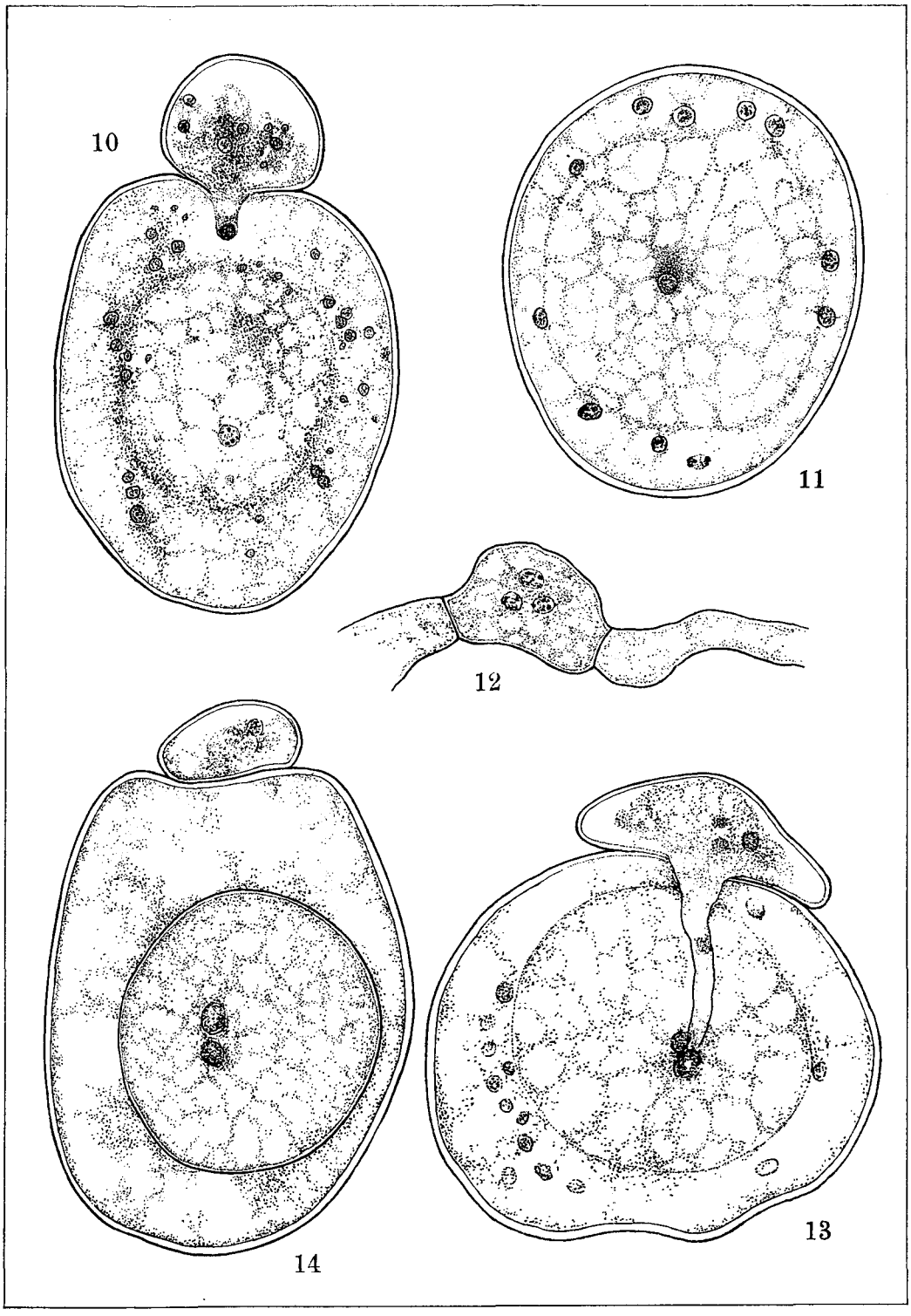

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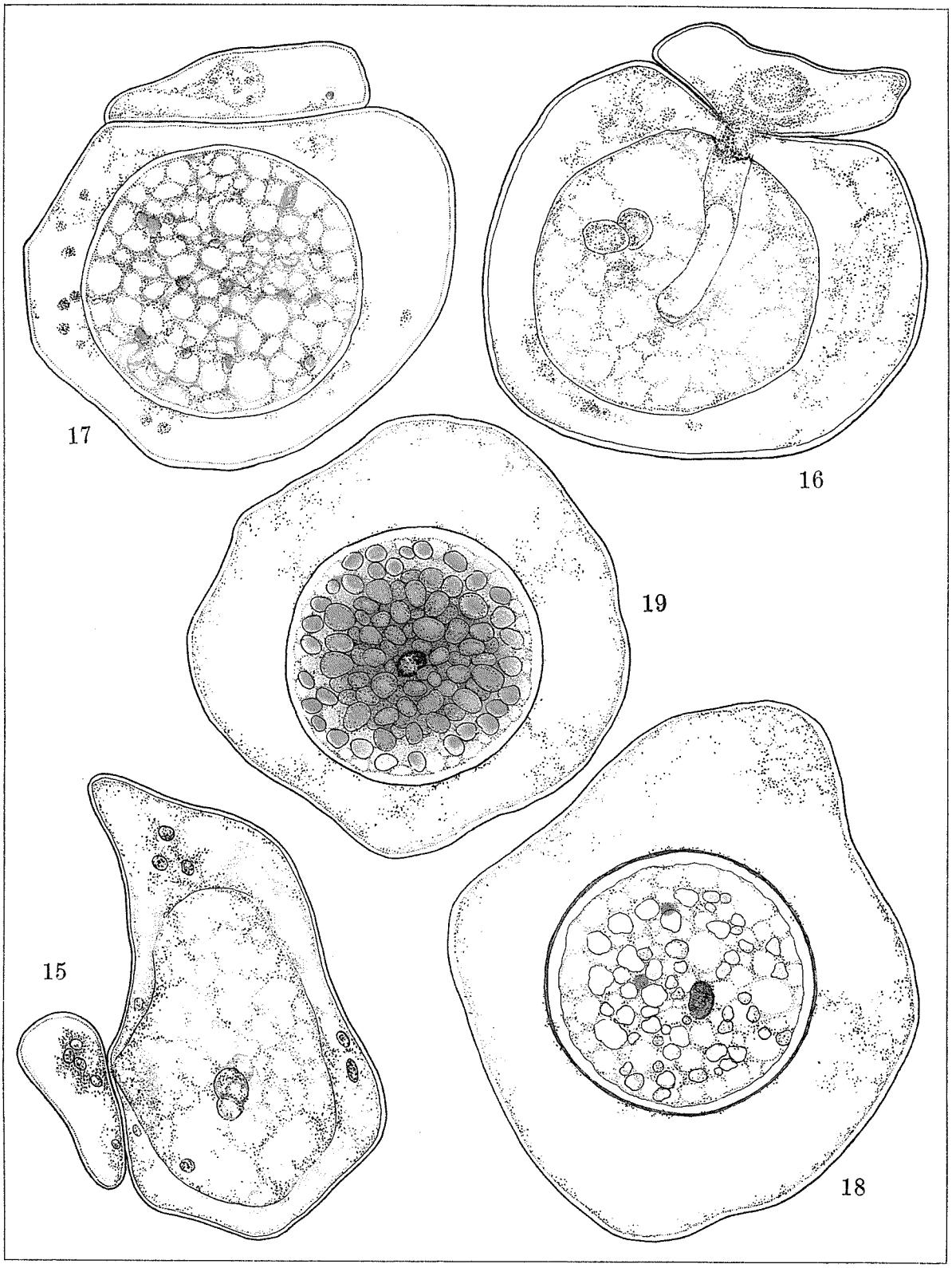

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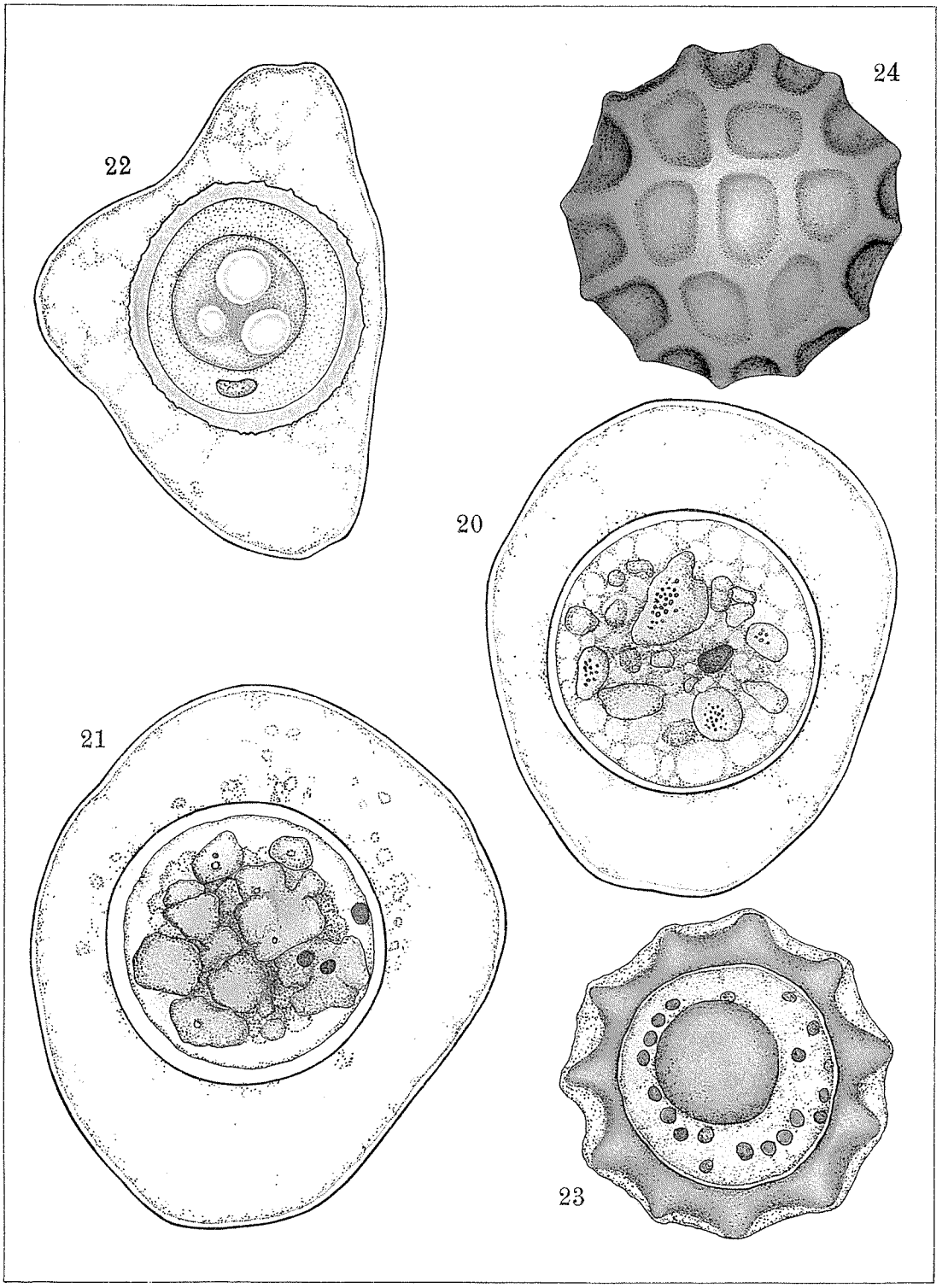

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