On the Structure and Reproduction of *Cystopus Candidus*, Lév.

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With Plates **XV** and **XVI**.

IN the year 1887, while working in Dr. Scott's laboratory at South Kensington, I undertook at his suggestion an investigation into the structure and mode of fertilization of *Cystopus candidus* (Lév.). A large number of observations and sketches were made, and a number of interesting facts determined, which tended to disprove the observations made by Fisch in 1885 as to the nature of the cytological structure and fertilization; but, owing to lack of material, I was at that time unable to complete the investigation. Since then, however, I have collected considerable quantities of fresh material in various parts of the country, and am now able to give a fuller account of the karyology and fertilization of this plant.

By the application of the new methods of study initiated by the introduction of the paraffin-method to botanical technique, by the improved methods of staining, and the use of the beautiful apochromatic lenses of Zeiss, a great

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impetus has been given to the study of the cytology and minute structure of the lower forms of plants, especially of the Fungi. Extremely interesting results have already been obtained, but the difficulty of prosecuting researches into the minute structure of parasitic Fungi is very great, mainly owing to the small size of these plants and their component parts. The work of staining sections is very difficult and demands much patience, owing to the great care which has to be taken in the application of the reagents and stains necessary to bring out the various details of structure, and still greater difficulties arise in the interpretation of the structures observed. To this must be due the conflicting statements made by various observers concerning the structure and development of the sexual reproductive organs in *Cystopus* and allied plants.

I am very pleased, therefore, to be able to record here that Mr. Trow has independently observed many of the facts mentioned in this paper, and in a letter to me states that 'the results so far obtained by me are, so far as I can judge from your descriptions, concordant with your own. In particular, I have no doubt as to the structure of the nucleus (excepting possibly the presence of a nucleolus which I have not observed), the number of nuclei in the oogonia, the number found by me averaged ninety-one, and the uninucleate character of the oosphere.'

Cystopus candidus is found in large quantities on various Cruciferous plants, especially on the Shepherd's Purse, *Capsella Bursa-pastoris*. It is difficult to find a patch of this common plant which does not show some of its members covered with the white spots and swellings so characteristic of the appearance of the Fungus, although it may easily be mistaken for *Peronospora parasitica*. It occurs on many other plants of the same order however, and Mr. Massee has been good enough to give me a list of the thirty-four species on which it has been found up to the present time.

It has a very wide geographical distribution, being found, as Mr. Massee informs me, in Europe, Asia, South Africa,

Ceylon, Victoria, New Zealand, Brazil, Cuba, the United States, Canada, the Falkland Islands, Surinam, and British Guiana.

The number of British species in addition to *C. candidus* is not numerous. In Masee's 'Phycomycetes and Ustilagineae' the following are given:—

C. Tragopogonis, Schroet.

C. Tragopogonis, var. *spinulosus* (*C. spinulosus* of De Bary).

C. Lepigoni, De Bary.

De Bary was the first to give us an account of the life-history of the plant, although, in the light of our recent knowledge, it is wanting in completeness. As his description is exceedingly clear and interesting however, I propose to give here a short account of the observations which he made in following out the life-history on fresh specimens.

The Fungus is commonly known as White Rust, and appears as spots or pustules on all parts except the roots and seeds. It attacks especially the stems and seed-capsules. It causes generally a large amount of hypertrophy of the organ attacked, which is sometimes visible even before the white patches are seen¹. The filaments of the mycelium penetrate the tissues of the plant in the intercellular spaces. The walls are composed of cellulose, and are often thick and gelatinous. Small globular haustoria are produced frequently and penetrate the walls of the parenchymatous cells of the host, and come to lie just inside the cell-wall. They are spherical bodies about equal in diameter to the filaments of the mycelium or not so large. They are at first full of protoplasm, but later they contain only a watery fluid, and their membrane becomes thick and highly refractive, which makes them appear on a cursory examination like grains of starch. These organs are never wanting, and are so characteristic that one can often determine the presence of the mycelium by means of them.

The oogonia are formed by terminal or intercalary swellings

¹ I have often found such swellings to contain numerous sexual organs. H. W.

on the mycelium. Branches of the mycelium which do not carry oogonia apply their obtuse extremities against the oogonium, swell up and are cut off to form the antheridia. When the oosphere is formed, the antheridium puts out a narrow and erect tube from the middle of its face which is pressed against the oogonium, which perforates the wall of the oogonium, and traverses the peripheral protoplasm towards the oosphere. As soon as it touches the oosphere it ceases to elongate; the oosphere becomes surrounded by a cellulose membrane and takes a regular spheroidal form. The tube put out by the antheridium must be regarded as a fertilizing tube, but it is remarkable that it never opens, effecting fertilization by contact only; until the maturation of the oospore the antheridium retains the aspect which it presented at the moment of fertilization.

The membrane of the oospore becomes thickened: the epispore is derived from the peripheral protoplasm, which gradually disappears, and finally there remains only a quantity of granules suspended in a watery transparent liquid. At the period of maturity the epispore is a thickened membrane, very resistant, coloured yellowish brown and finely punctate. The surface of it is nearly always furnished with brownish warts, large and obtuse, sometimes isolated, sometimes confluent, forming irregular crests. The warts are composed of cellulose and are coloured deep blue by the known reagents, while the membrane which carries them retains its primitive colour. One of the warts larger than the others forms a sheath around the fertilizing tube. The endospore is a thick membrane, smooth and uncoloured, composed of cellulose; it encloses a layer of protoplasm finely granular, which surrounds a large central vacuole.

The conidial sporangia form zoospores. When sown in a drop of water so that they become soaked, they absorb water rapidly and swell; an obtuse papilla is developed on one side, and vacuoles are formed in the finely granular protoplasm, which disappear later: fine lines of demarcation at the same time divide all the protoplasm into five to eight polyhedral

portions, each of which shows in the centre a small feebly coloured vacuole.

Some minutes after the division, the papilla swells, opens, and the zoospores are pushed to the outside through the opening one by one, without giving the least sign of movement; they take a lenticular form, and group themselves before the opening of the sporange in a globular mass. They soon commence to move, vibratile cilia show themselves and the whole mass is set oscillating, the zoospores ultimately become free and move away through the liquid. The zoospores are of the form of a plano-convex or slightly concavo-convex lens. Under the plane or concave surface is a disc-like vacuole, and the cilia are attached on the edge of the vacuole, a short one in front, a long one behind, both on the same side. The generation of zoospores in the sporangia commences in $1\frac{1}{2}$ to 3 hours after being placed in water. They appeared always in sporangia recently formed, or which had been kept a month and a half. But sporangia which were kept longer than that would not produce zoospores, and did not develop in any other way.

The oospores only change after a repose of several months. The oospore becomes a large sporange: placed in water the epispore bursts and the endospore protrudes. It contains a mass of protoplasm in which are to be seen several large vacuoles, which are constantly changing in form and volume. Soon this fluctuation is stopped, and in an instant the whole protoplasm is divided into polyhedral portions similar to the zoospores produced in the conidia. The endospore swells up to a much larger size and ultimately the zoospores are grouped in this vesicle in the form of a globular mass, which offers nearly the same phenomenon as in the case of the ordinary zoospores. Finally the spores separate from one another; for some minutes they swarm in the vesicle, then this bursts and disappears and the zoospores become free in the surrounding water.

The number of zoospores thus produced is very considerable; it is almost impossible to count them, but one may fairly

estimate them at about one hundred. They are similar to the ordinary zoospores. Their movement in the water goes on for two to three hours. Then they slow down, the cilia disappear, the spore becomes immovable, takes a globular form and is surrounded by a cellulose membrane. Then a thin tube is put out, curved or straight, which attains a length of two to six times the diameter of the spore on the object-glass. The apex of this tube is swollen, and all the protoplasm of the spore gradually passes into it. There is never any further development of the tube on the object-glass. The tubes never penetrate roots. They enter only through the stomata on leaf or stem. When a drop of water containing zoospores is placed on the surface of the leaf of a cruciferous plant, the spores will be found after the lapse of some hours fixed on the stomata, their movement having ceased. A germ-tube is produced on the side of the spore nearest the pore, which it enters immediately, and plunges the swollen extremity into the air-cavity of the stoma. The spore never passes through the stoma.

No further development of the tube takes place, however, and no mycelium is formed. It is only in the case of germ-tubes which enter the cotyledons that a mycelium is produced which spreads as the plant grows, and ultimately produces the characteristic white spots and pustules.

LITERATURE.

The phenomenon of the formation of zoospores was described by Prévost as early as 1807. He describes them in a species found on the cabbage, which is evidently *C. candidus*. His description is given very clearly and with much detail, and there is no doubt that he had under observation the formation of zoospores in this plant.

L. R. and Ch. Tulasne in their memoir on the Ustilagineae and Uredineae ('47) refer to *C. candidus* (*Uredo candida*), and point out that the epidermis underneath which the

spores are immediately placed is often only slightly raised and irregularly torn by their development, and its projecting torn fringe surrounds the group of spores as a sort of indusium.

Léveillé ('47) founded the genus *Cystopus* with the following characters:—Receptacle composed of very small irregular cells, forming a sort of disc covered with cylindrical vesicles, terminated by several spores arranged in a chain. The spores are spherical or cubical. The following species were placed in the genus:—*Uredo candida* (Pers.), *U. cubica* (Mart.), *U. Portulacae* (D. C.), *U. Bliti* (Bivon.), *U. floriformis* (Mérat), &c.

Berkeley ('48) gave a short account of the structure of the mycelium and the sporangia of *Cystopus candidus*, illustrated by four figures, in which the moniliform character of the arrangement of the spores is described. This account was anticipated and confirmed by Léveillé.

L. R. Tulasne ('54) gave a description with figures of *C. Portulacae*, Lév. (*Uredo Portulacae*, D. C.). The mycelium is shortly described and its mode of ramification. The method of formation of the sporangia in *C. Portulacae* and *C. candidus* is described and good figures are given in Pl. VII, Figs. 1–5 of the appearance of the mycelium and spores of *C. Portulacae*. He states, however, that there are two kinds of spore developed in *C. Portulacae*—(1) shortly cylindrical spores, united into long chains, form the larger number; they have a smooth cell-wall and are sometimes nearly colourless, sometimes tinged with brown, and (2) spores with a spherical and trigonous form, larger, and tinged a yellowish brown which is always more or less deep. They are developed at the apex of the chain and are the first spores formed therefore. In *C. candidus* the spores are nearly always similar to one another, but sometimes one distinguishes larger ones with a manifestly three-sided form. The dimorphism which is very evident in *C. Portulacae*, is also present to a less degree in *C. candidus*. He was not able to confirm Prévost's observations as to the zoospore formation, but states that

when sown in water the large spherical spores of *C. Portulacae* germinate in six to ten hours, but he had never seen the other spores germinate, and once only had he observed a spore of *C. candidus* to germinate. In germination a filament of uniform diameter is developed.

Hoffman ('59) supported Tulasne's observations on the development of the sporangia, and stated that he believed he had seen the direct germination of spores of *Cystopus*, and was unable to accept the account given by Prévost.

De Bary ('60) confirmed Prévost's observations by observations on *C. candidus* and *C. cubicus*, and pointed out that the spores are really sporangia and produce in *C. candidus* from five to eight spores, and in *C. cubicus* from eight to twelve. He was not able to confirm Tulasne's observations as to the direct germination of the spores.

De Bary's larger memoir, which has already been quoted, appeared in 1863, in which the sexual organs were for the first time described.

Cornu ('72) in his Monograph on the Saprolegnieae referred to the growth of the cell-wall in *Cystopus*, and pointed out that at first the membrane of the oospore is thin and the contour perfectly regular. Later it is composed of a thin exospore and a thick endospore, and by examination of oospores of different ages it is seen that the external diameter increases in size while the internal one diminishes. There is, therefore, growth in two directions, centrifugal and centripetal. He objects to De Bary's observation that the episporium is formed from the periplasm, and points out that he has omitted to note the decrease in the internal diameter of the spore, which is even more easily seen than the increase in the external diameter. He regards it as more logical and more conformable with what is known as to the growth of cell-wall in other cases, to admit that the oospore formed at the expense of a part of the cellular protoplasm should be nourished afterwards by the remainder which it first of all absorbs. He very pertinently asks why, if the periplasm has the power of secreting a wall without being elaborated

previously, it does not deposit a layer also on the wall of the oogonium. If there is a necessity for elaboration, it would be more in accordance with what ordinarily occurs that it should be previously absorbed by the spore and afterwards be used up to form the external layers as well as the internal, and he therefore suggests as more probable that the episporium is formed by growth from the internal protoplasm of the spore.

In the oospores of *C. Bliti* the endospore is very thick, and is formed of concentric layers, which are coloured intensely by chlor-zinc iodide, while the intermediate spaces remain uncoloured. These layers are two or three in number. The upper one is moulded exactly in the crests of the episporium, and is coloured strongly blue-violet. The episporium is not coloured and is a brown encrusted layer.

In 1879 Schmitz gave an impetus to the study of the cytology of the Fungi by his memoir on the nuclei of the lower plants. Although he does not actually deal with *Cystopus*, he describes the presence of nuclei in *Peronospora calotheca*, and states that he has been able to observe them in many other members of the Peronosporaceae.

Büsgen ('82) gave an interesting account of the formation of the zoospores in the conidia. He pointed out that the protoplasm of the conidia of *C. candidus* contains but few granules as compared with *Phytophthora*, in which the granules are more numerous. He evidently refers here to the nuclei which, as is now known, are much more numerous in the conidia of *Peronospora* than in *Cystopus*. His description of the formation of the zoospores is very carefully done, and I give here a short account of his observations. He points out that from one to one and a half hours after being placed in water, the sporangium becomes flask-shaped. Vacuoles of various sizes appear, and between them dark granules which become arranged in more or less regular lines. The protoplasm tends to contract away from the neck of the sporangium and from other parts of the wall, leaving clear spaces filled with a strongly refractive substance. The vacuoles unite together

and finally disappear. The protoplasm swells up again and becomes divided into spores by lines of granules which soon become converted into continuous cell-walls. Each of the spores thus produced contains a clear spot as already described by De Bary. The spores then escape from the sporangium, remain in connexion with one another for a short time, and then swim away.

Zalewski ('83) described the formation of the sporangia. The rounded summit of the basidium becomes superficially constricted and swells up until it attains the size of the basidium. At the level of the constriction a thin layer or border of cellulose appears, which grows gradually towards the centre and forms a transverse partition. When this division wall has attained its definite thickness, it divides into three layers, one of which belongs to the basidium, and another to the sporangium. These are separated by a third, which becomes gelatinous. This jelly-like membrane colours rose-yellow with iodine and sulphuric acid; the membranes of the sporangia and basidia are coloured blue. This jelly increases in volume and acquires its largest development when the sporangium is individualized. It becomes gradually resorbed as the sporangia develop, until only a very small portion is left between them in joining them together. On the addition of a small quantity of water the jelly is entirely dissolved and the sporangia become free. The jelly is also capable of absorbing moisture from the air, and is thus able to swell up and set the sporangia free. The chain of sporangia is now surrounded by a thin membrane which grows in and gradually separates them by forming the gelatinous substance which connects them together as De Bary at first thought.

In the same year ('83) Zalewski, in another memoir, gives an account of his observations on the structure and life history of *Cystopus*, in which he describes the formation of the conidia (sporangia) again, and the structure and development of the oospore as observed on living specimens. In connexion with the formation of zoospores he makes the interesting

observation that they appear in summer in from two to three hours, but in autumn not until one to two or three days after the sporangia have been placed in water.

His observations on the oospores were made on a *Cystopus* found on the undersides of the leaves of *Cirsium arvense*. He kept sections of the leaf with oospores in a damp chamber, but found that the culture could not go on longer than three to four days, as after a longer time the oogonia became degenerated. In addition therefore to observations made in this way, living oogonia in various stages of development were observed and drawn at the same time, and the author concludes that the exospore is undoubtedly formed from the periplasm. As he could only keep them three days, he was obliged to begin each third day with different stages, so that, as he says, his observations are probably only approximately correct. Once only was he able to observe a spore for five days.

In the oogonium, soon after it has become filled with protoplasm and cut off from the mycelium, the oospore is differentiated and is surrounded by a thin cellulose membrane. In three days more the wall has trebled or quadrupled itself in thickness by a deposition of the periplasm. The periplasm, shortly after the formation of the membrane around the oospore, has a dense, frothy, reticulate appearance. The vacuoles surrounded by this reticulate protoplasm are somewhat angular. They grow considerably while the periplasm slowly streams into the meshes towards the wall of the oospore in order to form the exospore. The meshes break down here and there; the vacuoles unite with one another, and at last the oospore remains suspended to the wall of the oogonium by means of a few protoplasmic strands. These break through also, and the whole periplasm, or a great part of it, falls down upon the wall of the oospore, whilst the remainder clings to the wall of the oogonium or floats about in the sap and slowly becomes brown and dies. At first the wall of the oospore is formed uniformly throughout, but when the streaming of the protoplasm becomes more rapid so

that it spreads unequally, then the wall of the oospore becomes unequally thickened, and the ridges may become so pronounced as to look like warts. The fertilizing tube of the antheridium also becomes surrounded by a thick exospore.

The exospore begins to take a brown colouration when the projections are yet small; it is already quite dark brown when these are uncoloured, the latter requiring a longer time to attain to the brown colouration.

So far as the author's observations go it takes probably from eight to ten days from fertilization to the ripening of the oospore.

He does not accept Cornu's observations as to the structure of the wall of the oospore. The endospore consists of pure cellulose. The exospore is cuticularized. In the majority of cases, as in *C. candidus*, the exospore consists of four layers.

The thickness of the separate layers differs in the different species. The inner layer is thin homogeneous and cuticularized—yellow or brown with iodine and sulphuric acid. The next layer consists of cork, but is seldom homogeneous (as in *C. Convolvulacearum*) and usually finely granular; it is formed of thin, closely packed, round or angular rods, and in *C. candidus* is the thickest layer. In all other species except *C. sibiricus* it is very thin, and is completely wanting in *C. Portulacae*, *C. Amarantacearum* and *C. Bliti*. The third layer is the cellulose-like layer. It is most strongly developed in *C. sibiricus* and *C. Convolvulacearum*. In *C. candidus* it forms its thick upper ridges without spreading out into an unbroken layer, and in *C. cubicus*, *C. Lepigoni* and *C. Bliti* it only forms the inner part of the projecting ridges and warts.

Outside this cellulose layer is a fourth (or third) thin, commonly dark brown cuticularized layer. There is no distinct line of demarcation between this and the other cellulose layer however, and it appears to be merely the cuticularized outer portion of the latter.

The cellulose layer is dissolved by long treatment with sulphuric acid. This is best seen in *C. candidus*, where the projections of the surface then appear quite hollow.

The appearance of the contents of a ripe oospore of *C. candidus* is as follows:—The large central space contains a globule of oily matter about half to two-thirds the diameter of the lumen. It is surrounded by a coarsely granular protoplasm, the outer layer of which contains clear, round spots in variable number, three to four or more.

In 1885 Fisch gave an account of the structure and fertilization in species of *Pythium*, and stated that, according to his incomplete observations, *Cystopus* appears to behave in the same manner. In the young oogonia of *Pythium* there are usually from ten to twenty nuclei. In the formation of the oosphere these fuse together to form a single large nucleus. In the antheridium he always found a single nucleus, but thinks that this may be due to the fusion of several. The nucleus of the antheridium passes into the oosphere with the gonoplasm, and disappears there with the egg-nucleus.

In 1888 Chmielewsky gave a description of the process of fertilization in *C. candidus* which is very different from that given by Fisch. The protoplasm of the young oogonium is net-like, and the knots of the net, which consist of granular collections of plasma, stain strongly. Fisch must (says the author) have mistaken these knots for nuclei. In reality the young oogonium contains only one nucleus, and that is parietal. It is usually large, and has the form of an ellipse, in one focus of which the very small nucleolus is generally placed. The nucleus is very poor in chromatin, and stains feebly. The nucleus passes later into the centre of the oogonium, and the distinction between epiplasm and gonoplasm becomes visible. Shortly before fertilization the nucleus contracts a little and becomes strongly stainable (but even now is larger than the nuclei of the vegetative hyphae). The antheridium contains one nucleus only, which is similar in size and staining properties to the nucleus of the oosphere, a peculiarity which is to be noted. The passage of the male

nucleus and gonoplasm takes place through a fertilizing tube, which often penetrates deeply into the oosphere. The author observed the two nuclei in the oosphere, and found that they finally become fused. The ripe oospore contains only one nucleus.

Dangeard ('90) gives an account of the histology of *C. candidus* Pers., and *C. cubicus* Strauss.

In *C. candidus* the haustoria are very small, and contain only one nucleus or none at all. The nuclei in the mycelium are fairly well spaced. The conidia (sporangia) contain a hyaline protoplasm, and a number of nuclei, five to seven, as many as the number of zoospores which the conidia form. It appears that the nuclei all come from the mycelium and there is no division in the basidium. After the formation of a number of conidia, the membrane of the basidium becomes thick, and only two to four nuclei are left in it.

The oogonia are generally produced at the ends of the branches, but may also be intercalary. They contain numerous nuclei which appear to possess nucleoli. The structure described by Chmielewsky as a single large nucleus is considered by Dangeard to be an oil-globule. After the differentiation of the protoplasm of the oogonium into oosphere and periplasm, a corpuscle is found in the centre of the oosphere of a variable form and more or less stained. In the periplasm several small nuclei are to be observed. Later the ripe oospore contains a large corpuscle in the centre, which stains strongly. It is nothing else than an oil-globule which is found also in other oospores, particularly in the Saprolegnieae and Peronosporae. Sometimes it is a sphere, sometimes a ring, sometimes an arc of a circle. If chloroform is allowed to act long enough it disappears, and there is no longer any colouration. Sometimes when the chloroform has not acted for a sufficiently long time it takes the form of a sponge.

The nuclei according to Dangeard are masked during the fertilization, and reappear again distinctly in the old oospore, in which from eight to ten nuclei can be observed placed in the layer of protoplasm between the globule of oil and the

endospore ; they resemble by their size and other properties those which have been seen in the vegetative organs.

He arrives at the conclusion that of the nuclei of the oogonium some remain in the oosphere, some in the periplasm ; to the nucleus in the oosphere are probably added those of the antheridium, although he found it impossible to observe their passage. In any case the ripe oospore contains about twenty nuclei. The oil globule grows little by little until it forms the large central globule of the ripe oospore.

He was not able to observe any fertilization, and did not observe the division of the nucleus in the oogonium nor in the oospore. He accepts Zalewski's observations on the structure of the wall of the ripe oospore.

Mangin ('91) describes the disarticulation of the sporangia in *C. candidus*. When a sporangium is about to form, a thin ring of callus appears near the apex of the basidium. This gradually grows inwards until a transverse partition has been completely formed, of a convex or conical shape, the concavity being towards the sporangium. The cellulose wall in contact with this transverse partition at the edge becomes resorbed, and a constriction is produced by an apparent contraction and thickening of the callus partition. This proceeds rapidly, and ultimately the callus forms a little cup-like formation at the base of the sporangium, by which the latter is attached to the basidium. The author was not able to observe the division of this layer into three as observed by De Bary and Zalewski. Ultimately the callus-like substance becomes reduced to a short cylindrical mass connecting the sporangium to the basidium, and the cellulose wall both of the basidium and the sporangium ultimately extends all round on both sides of the callus. The portion of callus left between them slowly acquires the property of dissolving in water by which the sporangia are set free.

When the basidia have formed a number of sporangia their activity ceases, and as the hyphae are interrupted here and there by masses of callus which stop communication with the

other parts of the mycelium the formation of sporangia ceases.

In 1892 I gave a description of the structure of the nuclei of *C. candidus*, and pointed out the multinucleate nature of the oogonium and antheridium. Like Dangeard I was, however, unable to observe fertilization or the fate of the nuclei in the oosphere, and agreed with him that Fisch had mistaken the central oil-globule for a nucleus. Since then I have been able to disprove this, as will be seen later.

Peglion ('93) in a paper on the hypertrophy of the tissues produced by *C. candidus* on *Raphanus Raphanistrum*, points out that large accumulations of starch are produced where the conidia pustules are formed. The epidermal cells and the cortical parenchyma are increased in size, the former especially tangentially, and the vascular bundles are modified.

Macallum ('95), in his paper on the distribution of assimilated iron in animal and vegetable cells, shows that a substance in which iron is firmly held appears to be a constant constituent of the nucleus and of the cytoplasm of non-nucleated cells and those possessed of apparently rudimentary nuclei. He says that in *Cystopus candidus* the whole of the protoplasm in the mycelium and gonidia, except the mature gonidia, is chromophilous, that is, contains chromatin. The nuclei in the mature gonidia are of the more regular form, but in the mycelium are chiefly, if not wholly, small masses of chromatin substance, like those forming the 'nucleoli' in the abjointing gonidia. Mitotic phases were not seen.

The disposition of the assimilated iron corresponds closely with the distribution of the chromophilous substance. The small masses of chromatin and the nucleoli gave abundant evidence of its presence, the remaining portion of the nucleus in the case of the latter containing very little, relatively less than the cytoplasm.

In the subsequent development of the abjointed gonidia, the nuclei appear to take up from the protoplasm all, or nearly all, the substance containing iron, and with this the character of the nuclei seems to change. The nucleoli first of all are

converted into fine granules distributed through the nuclear cavity, and finally in the mature gonidia the nuclei appear to be simply more or less homogeneous masses of iron-holding substance, while the cytoplasm does not contain a trace of the metal.

In 1895 Istvánffi observed the multinucleate character of the vegetative hyphae in *Cystopus Portulacae*, *Peronospora Ficariae*, *P. Chlorae*, *Phytophthora infestans*, &c., and also pointed out that the conidia contain several nuclei.

He investigated also the nuclei of the sexual organs in *Cystopus Portulacae*. The material was hardened in alcohol, by which the fatty matters were dissolved out.

The protoplasm, as it passes into the swollen end of a thread to form the oogonium, possesses a peculiar structure. It streams into the oogonium from the hyphae, and exhibits at the base of the oogonium a radiating structure which gradually passes over into a network. In the meshes of the network the nuclei are found; only occasionally are nuclei to be found in the streaming portion. The oospore is formed out of the network of protoplasm, and appears as a large spherical body inside the oogonium; its contents are formed of a quite dense protoplasm, and the network structure is no longer visible. The space between the oosphere and the wall of the oogonium contains epiplasm, which forms the brown, net-like thickening of the oosphere. The formation of the oosphere seems to take place only after fertilization, for the antheridium is already attached to the oogonium in the early stage, when its protoplasm still shows the reticulate structure. It contains many nuclei, and empties itself before the oosphere is rendered visible. It is probable that fertilization takes place by the fusion of the male and female nuclei.

No reference is made to the separation of the nuclei in the formation of the oosphere, as to whether one or several nuclei remain in the oosphere, but in his explanation of Fig. 25—an oogonium with oosphere in a young stage—he states that ‘a part of the nuclei remain in the epiplasm,’ and figures also a large number of nuclei in the oosphere.

METHODS.

I have always found it necessary to make a preliminary investigation of the piece of stem infested by the Fungus in order to determine the presence of the sexual organs. Sections were taken here and there and examined, and, when a suitable region of the stem had been discovered, it was cut up into quite small pieces not more than about $\frac{1}{16}$ to $\frac{1}{8}$ inch long. Each piece was halved longitudinally, which allows the satisfactory penetration of the re-agent, and was then placed in the fixing fluid. As a fixing and hardening re-agent, I have found corrosive sublimate (HgCl_2) the most useful and successful. This is used in saturated solution, and the pieces of stem are placed in it for from one or two hours to a whole day. I have also used absolute alcohol and chrom.-osmium acetic acid, but corrosive sublimate is the most satisfactory in every way.

The pieces of tissue are then well washed in water, transferred to 30 % spirit, 50 % spirit, and 70 % spirit, about two or three hours in each, and finally transferred to 90 % spirit.

The tissues may now be stained *en bloc* and embedded in paraffin; or they may be first embedded in paraffin and the sections stained on the slide. I have tried both methods, and found the latter the more successful, as it allows the greatest amount of latitude in determining the extent to which it is advisable to allow the stain to act, and the whole operation of staining and clearing can be controlled under the microscope.

For embedding previously to staining, the tissues are transferred to absolute alcohol for half an hour or so, then to a mixture of alcohol and xylol, and are finally placed in melted paraffin, where they remain for an hour or so.

The sections were then cut in the ordinary way by a Cambridge rocking microtome. The ribbon was cut up into suitable lengths, which were mounted on the slide in the following way. The pieces of ribbon were first floated on the surface of warm water, which causes them to spread out perfectly flat. A piece was then floated on to a slide, the

superfluous moisture sucked up with blotting paper and the slide allowed to dry at the ordinary temperature of the room or in a gentle heat. There is no need to use any kind of cement if one is very careful to allow the drying to be complete. The paraffin is then melted and dissolved in xylol. From xylol the slide is transferred to absolute alcohol and then to 90 % spirit, where it may remain until a convenient time for staining.

The methods of staining adopted have been various, but the one which gives most beautiful and satisfactory results is a slight modification of one kindly communicated to me by Prof. Hartog and devised by him. The solutions used are those recommended by Hartog, somewhat modified for my purpose, viz.

1. 50 % spirit, 4 vols. ; glacial ac. ac. 1 vol.
2. To solution 1 enough nigrosin is added to make it opaque in the bottle and transparent in a half-inch layer.
3. To solution 1 enough nigrosin is added to make it blue, but transparent in a layer two inches thick.
4. 50 % spirit, to which a small quantity of 2 is added to make it quite light blue in colour.
5. Mayer's alcoholic solution of carmine.

The sections are placed for about five to ten minutes in the mordanting solution 4.

They are then placed in Mayer's carmine for a few minutes until they become stained distinctly red, then washed in 30 % spirit and placed in solution 3. If a deep stain is required solution 2 must be used.

The sections remain in solution 3, called by Prof. Hartog a toning solution, until the required effect has been produced. The exact stage at which the operation should be stopped can be easily determined by observing the sections under the microscope. In order to produce a very clear and well-differentiated stain, I next transfer the sections to solution 1, in which a good part of the stain becomes washed out, leaving the nuclei beautifully differentiated and every part clearly shown up. It requires considerable practice to enable one

to judge of the length of time which these stains should be allowed to act, but I have found that the best results are obtained by a very short stay in carmine. I have occasionally found it useful to differentiate in Hydrochloric acid alcohol.

The sections are then transferred to 70 % spirit, methylated spirit and absolute alcohol. They are next transferred to xylol or carbolized xylol and mounted in xylol balsam.

I have used in the investigations the apochromatic lenses of Zeiss; 4.0 mm., apert. 0.95; 2.0 mm., apert. 1.40, and oculars 8, 12, and 18. In order to obtain the best illumination possible, I used a high-angled oil-immersion condenser, and recently the Welsbach Incandescent light, which surpasses anything I have previously worked with.

STRUCTURE OF MYCELIUM AND HAUSTORIA.

The mycelium is not so easily seen, and its structure is more difficult to observe than is the case in *Peronospora parasitica*, which commonly occurs with *Cystopus* on the same host-plant, and it may be easily overlooked unless it is present in abundance. The hyphae are irregular in size and outline, according to the shape of the intercellular spaces through which they pass, and branch frequently. The branching is very irregular, depending upon the presence of the intercellular spaces, and it occasionally happens that when a branch reaches a larger intercellular space than usual it expands considerably and produces branches radiating from it in all directions. The protoplasm contained in the hyphae is granular, and loosely arranged in a network with numerous meshes. It is sometimes very scanty in amount, and forms just a thin layer on the wall of the hypha with a few strands stretching across here and there. It is on the other hand occasionally very dense indeed, completely filling the lumen of the hypha. This occurs where probably active growth is taking place, and is often found in those portions of the mycelium on which the basidia are borne. In the normal condition the protoplasm stains reddish blue in the carmine-

nigrosin stain; where it is very dense, however, the red stain predominates. The haustoria are small, spherical projections produced here and there on the hyphae, occasionally in considerable numbers. They often indicate the presence of the mycelium before this has been observed, and it is often very difficult to see their connexion with the hyphae upon which they are borne owing to the small size of the connecting tube. The protoplasm in the haustoria takes always a distinctly blue colouration in the carmine-nigrosin stain, with no tinge of red. In some cases it is very dense, stains deeply, and nearly completely fills the cavity; in others it forms a peripheral layer of deeply stained granules around a central vacuole. I have never been able to observe nuclei in the haustoria, although Dangeard ('90) states that they sometimes contain one. This is perhaps interesting from the point of view of the function of the nucleus, as showing that the presence of a nucleus in the haustoria is not absolutely necessary in order to enable them to properly perform their function of absorption of food-material. It must be pointed out, however, that as these haustoria always remain small, and that the whole of their protoplasm therefore is not far removed from the influence of the nuclei of the hyphae upon which they are formed, there may be no necessity for the actual presence of a nucleus in them. In *P. parasitica*, where the haustoria are very large and sometimes nearly completely fill the cells of the host-plant, they always contain numerous nuclei. The protoplasm of the haustoria in these stained sections is generally shown slightly contracted away from the wall equally all round, which gives them a very characteristic appearance, as if they possessed a very thick cell-wall.

The nuclei of the mycelium are generally very loosely arranged in the protoplasm, but where the protoplasm is abundant and where probably active growth is taking place, the nuclei are more numerous. In the former case the structure of the nuclei can be easily observed, but not in the latter. In the resting condition each nucleus consists of a nuclear membrane, network, and nucleolus. The network

stains light blue, the nucleolus reddish blue. The network of the nucleus appears to be distinctly granular.

I have never been able to clearly observe nuclear division in the mycelium, although I have constantly seen appearances which gave me the impression that they were due to nuclei in a state of division. But the nuclei are so small and the amount of chromatin so insignificant that, judging by the difficulty I experienced in making out nuclear division where the nuclei are much larger, as in the oogonia, it will, I think, be extremely difficult to satisfactorily observe it here.

FORMATION OF GONIDANGIA.

The gonidangia are produced on basidia, which are formed in large numbers just underneath the epidermis of the stem, leaves, and fruits. Protoplasm and nuclei pass into the basidia from the mycelium. The mycelium is very much branched and abundant in the region where the basidia are given off, and the cell-walls, especially in the later stages, are very much thickened and stain easily in haematoxylin. No fusion of nuclei has been observed in the basidia, such as has been described in the Basidiomycetes by Rosen, Dangeard and myself, and in the Asci of Ascomycetes by Dangeard and Harper. This is interesting, and tends to emphasize the different nature of the basidia in *Cystopus* as compared with those in the Basidiomycetes and Ascomycetes, which are to be regarded as exhibiting a kind of sexual fusion of nuclei (as has been shown by Dangeard, '95; Harper, '95; and myself, '93). In *Cystopus*, the formation of the conidia is preceded neither by fusion nor by division. Some five to eight nuclei, together with a quantity of protoplasm, accumulate at the apex of the basidium, which is then cut off by a transverse wall to form the gonidangium, as shown by Zalewski ('83) and Mangin ('91). The four to eight nuclei remain undivided, and each one becomes the nucleus of a zoospore. Each nucleus is surrounded from the beginning by a quantity of dense granular protoplasm, which tends to render it invisible,

as the protoplasmic granules stain deeply. The nuclei possess a nuclear membrane, nucleolus and nuclear network, and are perhaps a trifle larger and more distinct than those in the mycelium. The protoplasm, both in the basidia and in the sporangia, stains deeply with all the ordinary nuclear stains. The formation of the zoogonidia has been described by Prévost ('07), De Bary ('63), and Büsgen ('82).

Macallum's observations ('95), already quoted, appear to accord very well with my own as to the distribution of stainable (iron-containing) substance. But I have been able to observe that the small chromatin-masses in the hyphae are the nucleoli of the nuclei. No figures are given by Macallum of the mycelia and haustoria, and it would be interesting to know whether the author had been observing the mycelia of *P. parasitica*, in which the nuclei are very rich in chromatin-substance and the haustoria very large, or the mycelia of *C. candidus*, in which the nuclei do not contain so much chromatin and in which the haustoria are very small, as both fungi commonly occur on the same host-plant and the mycelium of one might be mistaken for that of the other.

FORMATION OF THE SEXUAL ORGANS.

The sexual organs, described by De Bary in 1863, are found in considerable abundance in favourable specimens. It is impossible to say what induces their formation, or at what stage in the life-history of the fungus they appear, or under exactly what conditions. So far as I have been able to observe, a large supply of nutrient matter, such as occurs in the more succulent parts like the stem, is a general concomitant of their appearance. It is not safe to trust too much to the abundance of the conidia on the surface of the stem, or to the decay of the stem, leaves, or fruits, as an indication of their occurrence. I have often found stems in an advanced state of decay, completely covered with the old basidia, to contain no sexual organs. On the other hand,

I have often found portions of the stem, very much hypertrophied, but with few or no basidia and gonidangia, to contain them in abundance. One may look for sexual organs in vain in some localities and find them plentifully in others, and one may find them in stems the surface of which is almost completely covered with the asexual reproductive organs of *Peronospora parasitica*. They may be accompanied by much hypertrophy or by very little, with many asexual organs or with very few. I have generally found it a pretty certain indication of the presence of sexual organs if the portion of the stem on which they are suspected to occur is brittle, and easily broken when slightly bent. Where they do not occur the stem may be bent double before it breaks. In cutting sections by hand, one is also able to judge fairly easily whether sexual organs are present or not. When present, the sections come off beautifully smoothly, and without the least suspicion of tearing or toughness; but if not present, there is a larger resistance to be overcome, and unless the razor be in good condition a certain amount of tearing is the result. Generally speaking the sexual organs are to be found more easily towards the end of the season than at the beginning.

In many cases, and perhaps in the most favourable condition for observation as a rule, that part of the stem in which they are to be found in the early stages of their development is coloured a dark reddish purple and is somewhat swollen. When the oospores are mature, the stem becomes brown and rotten, and the tissues almost completely broken up. In the earlier stages of their formation, although they may be found in great abundance in some parts of the stem, there is no apparent degeneration visible in the cells of the host-plant such as appears later. The cells contain protoplasm, nuclei, and chlorophyll grains, and present a normal appearance, except for the presence of haustoria which are to be found lining the cell-wall in some cells in considerable numbers. Peglion ('93) has given a very careful account of the action of the fungus upon the tissues of the host-plant.

In the formation of oogonia, protoplasm and nuclei pass from the mycelium into an expansion, generally produced at the end of a filament, in large quantities. The protoplasm appears to pass in very rapidly, and there is always a dense mass of it at the opening leading into the young oogonium, and radiating from this region into the oogonium are striae indicating the flow or streaming of the protoplasm (Fig. 4), a phenomenon which Istvánffi ('95) also describes and figures. The oogonium at this stage often presents a contracted or somewhat crumpled appearance, and the protoplasm is arranged in an irregular network in which the foam-structure visible at a later stage cannot be seen. As the nuclei pass into the oogonium they also present a crumpled, irregular appearance, looking very much like knots in the protoplasm (Fig. 4), and it would be very easy to mistake them for such, if it were not that in very carefully prepared sections the nuclear structure can in some cases be seen. When a sufficient quantity of protoplasm and nuclei have passed into the oogonium, a transverse wall is formed separating it from the hypha upon which it is formed: the oogonium immediately expands, as if it suddenly became turgid, loses its irregular crumpled appearance and becomes smooth, and, so far as the surrounding tissues will allow, spherical in outline (Fig. 5). The nuclei also regain their shape and are then seen to be spherical in outline and similar to those in the mycelium, or perhaps a little larger. Even now, however, in badly prepared sections, it would be perfectly easy to mistake them for knots in the protoplasm as Chmielewsky ('88) did. The protoplasm at this stage has a distinct foam-structure which is very clearly shown in well-stained preparations, and the nuclei occur more or less regularly spaced in the protoplasmic reticulum (Fig. 6). The nuclei possess a nuclear membrane, a small nucleolus, and a network which is, if anything, slightly more distinct than in the nuclei of the mycelium. This is probably due to changes which appear to be set up as regards the supply of nutriment as soon as the oogonium becomes delimited from the mycelium. The number of nuclei present

at this stage varies according to the size of the oogonium. I have counted, in different oogonia by an examination of serial sections, and by making allowances for nuclei cut in two, and so observed twice,—though the section is rarely of such equality as to deceive one in this way,—64, 75, 88, 97, and 115 nuclei, and numbers varying from 70 to 110 are common in all stages up to division¹. The protoplasm and nuclei at this stage stain with a decidedly reddish tinge, similar, though not quite so red perhaps, to what is observed as they pass into the oogonium. Before very long, however, owing probably to changes in the supply of nutriment, both nuclei and protoplasm become more blue, the nucleolus only retaining its decidedly reddish tinge.

At about this time the antheridia attach themselves closely to the oogonia. They possess a number of nuclei varying from six to twelve or perhaps more. Both nuclei and protoplasm have the same structure and appearance as in the oogonia, and the changes in the appearance of the nuclei in an oogonium are accompanied by similar changes in the antheridium. The protoplasm is, however, denser than in the oogonium and renders observation of the nuclei correspondingly more difficult. Soon after the antheridium comes into contact with the oogonium a change takes place leading to the appearance of a granular, more or less homogeneous, mass of protoplasm just beneath the wall of the oogonium on the side nearest the antheridium (Fig. 5). This, as will be shown later, is the place where the receptive spot appears and is the first indication of it.

From this stage onwards considerable changes take place in the oogonium. The nuclei increase in size, the network becomes more distinct, and the protoplasm becomes more vacuolate and exhibits more clearly the foam-structure already mentioned (Fig. 6). These changes lead up to the division of the nucleus and the formation of the oosphere.

¹ Mr. Trow informs me in a letter that the average number found by him was ninety-one.

FORMATION OF THE OOSPHERE AND FERTILIZING TUBE.

In the denser and more granular protoplasm on the side of the oogonium nearest to the antheridium, already noticed, a hyaline space appears, from which a papilla with a deeply stained apical spot projects towards the antheridium, tending to bore its way through the wall of the oogonium and causing the wall to become somewhat thinner at this place. The protoplasmic contents of the antheridium appear to be contracted at first in front of this projection and a slight depression is produced (Fig. 6); but soon a dense granular mass appears in the antheridium, on the side nearest this projection, and a fertilizing tube is put out which penetrates the oogonium at this spot (Fig. 10). This however does not take place until the formation of the oosphere has commenced. The receptive papilla, as we may call it, is very clearly located in the oogonium by the contraction of the protoplasm from all parts of the wall except at this point, where it appears to be securely attached, and remains so during the whole of the time required for the development of the fertilizing tube. While these changes are taking place the nuclei, both of the oogonium and antheridium, increase considerably in size. The network becomes more distinct and stains more deeply, so that its granular nature can be easily made out.

The differentiation of the oosphere now begins. The protoplasm contracts towards the centre into a roundish mass, connected to the wall of the oogonium by thick protoplasmic strands (Fig. 8). This central mass contains all the nuclei; none are to be found in the thick peripheral strands of protoplasm. It gradually becomes further differentiated into a central vacuolate and reticulate mass which stains with a reddish tinge, and an exterior peripheral layer of very dense non-vacuolate protoplasm, the periplasm, which stains with a bluish-red tinge. The whole of the protoplasmic contents of the oogonium outside the oosphere become finally condensed into this periplasm, with the exception of a few

strands which still connect it to the wall of the oogonium (Fig. 9). The nuclei at the same time undergo changes leading up to division. The nucleoli disappear and the network appears to divide up into chromosomes and contracts towards the middle of the nucleus in a very characteristic manner (Figs. 8 and 9). The network of chromosomes takes on a reddish tinge instead of a blue one and the nuclear membrane is very distinct. There can be no doubt, as will be seen later, that this contraction of the nuclear network, which gives the nuclei a very characteristic appearance at this stage, is preliminary to the division of the nuclei which takes place shortly afterwards. The majority of the nuclei are now to be found in the periplasm, and they gradually become more and more restricted to this layer. Meanwhile, however, nuclear division actually begins. Each nucleus divides into two, karyokinetically, and the appearance presented by an oogonium at this stage is shown in Fig. 9, in which a number of nuclei are to be observed in the periplasm which have undergone division, as well as nuclei in the centre still undergoing division. The various stages of division, so far as I have been able to observe them, are given in Fig. 28 (1 to 5), and are typically karyokinetic.

While the division of the nuclei is taking place, a dense, very deeply stained blue, mass of protoplasm appears in the centre of the not yet completely differentiated oosphere. This appears at first sight to be completely homogeneous, but on closer examination is found to be made of a dense mass of granules closely packed together and more or less sharply marked off from the rest of the protoplasm. This is probably what was described by Dangeard ('90) as an oil-globule, and was mistaken by Chmielewsky ('89) for a nucleus. Dangeard's observation that this substance disappears on soaking the tissue for a long time in chloroform, was probably due to the fact that it is not so apparent in some oogonia as in others, although it is generally present, even when casual observation of a thick section might lead one to the conclusion that it was absent. That it is not oil is proved by its structure and by the fact that oil is not present except

in minute drops at this stage, and that it will not dissolve even after a long soaking in alcohol, ether, and chloroform. It is of the same nature as the dense protoplasmic mass which appears in the fertilizing tube at the moment when it begins to grow, and is produced probably by an accumulation of stainable granules from the protoplasm. This dense mass of protoplasm can be observed in oogonia of all stages, such as are figured in Figs. 8 to 22. Shortly after its appearance, one of the nuclei produced by the division in the oogonium comes into close contact with it and gradually becomes more or less completely embedded in it. All the other nuclei pass to the periplasm, leaving this single nucleus in the centre as the nucleus of the ovum (Figs. 13 and 14). We may consider that at this stage the oosphere has become differentiated, although its cell-wall has not yet appeared. It is interesting to note that the wall of the oosphere does not form until just at the time when fertilization takes place, or shortly afterwards.

THE DIVISION OF THE NUCLEUS.

As already pointed out, the division of the nucleus in the oogonium is karyokinetic and is very similar to the normal process as described in the higher plants. The more the structure of the nucleus is investigated in the lower plants the more clearly does the fact become apparent that, instead of a much simpler and more rudimentary type of nucleus than that so well known in the higher plants, it appears to be almost exactly similar, the former differing from the latter in no essential respect, except perhaps as regards the presence of centrospheres. Indeed it appears to me that in some cases, certain details of structure come out more clearly in these lower plants than in the higher, provided a sufficiently good magnifying power be used. In any case I think we may fairly arrive at the generalization that simplicity of vegetative structure does not necessarily mean simplicity of cytological structure, and that it is probable that further

investigations will tend to bring the structure of the nuclei of the lower plants still more into accordance with those in the higher plants and animals. All the evidence we have of the details of nuclear division in the fungi strongly support this conclusion, and it may be useful here to give some short account of the observations which have so far been made bearing directly on this question.

Sadebeck ('83) describes nuclear division in the Asci of *Exoascus* as distinctly karyokinetic, and gives figures of nuclei in a late stage of division in which the spindle-formation is seen.

Strasburger ('84), in an account of the formation of the sporangia in *Trichia fallax*, describes a nuclear division which follows the ordinary karyokinetic method, and in a more recent paper ('94) he states that these nuclei each contain twelve chromosomes.

Fisch ('85), in his paper on the development of *Ascomyces*, gives a description of nuclear division in which he points out that at the commencement of the division granules appear in the nucleus, that this is immediately followed by the spindle-stage, and that in many respects the division resembles that which takes place in the higher plants.

Eidam ('87) describes the division of the nucleus in *Basidiobolus Ranarum* as indirect, and states that the details of the division can be brought into accord with those known in the higher plants.

Hartog ('89 and '95) regards the division of the nucleus in *Saprolegnia* as essentially a special case of karyokinesis, intermediate between direct division and the commoner process. According to him the nuclein-mass of the nucleus separates into four rods which form the nuclear plate. They undergo longitudinal division, so that the plate consists of eight rods, and finally these separate into two crescentic groups which become the daughter-nuclei.

In 1889 I was able to show that the division of the nucleus in the oogonium of *Peronospora* showed some details of karyokinesis, including the probable appearance of a spindle.

Rosen ('92) describes the process of nuclear division in the Fungi as not altogether indirect, and simpler than in the higher plants. He was not able to observe any of the details of spindle-formation, &c., and states that the smaller the nuclei the simpler their division. I have already pointed out ('93) that his observations on nuclear division in the Basidiomycetes are incomplete.

Gjurasin ('93) shows that, in the division of the nucleus in the Asci of *Peziza vesiculosa*, a spindle-figure appears with protoplasmic radiations at the poles, that the granules in the nuclear network pass to the equator of the nucleus and divide into two halves, which move very rapidly to the poles of the spindle to form the two daughter-nuclei.

Lister ('93), in his paper on the division of nuclei in the Mycetozoa, describes the division in the formation of the swarm-cells, and gives beautiful figures illustrating the various stages, which conform in general to what is known in the higher plants.

In 1893-4 I showed that in the Hymenomycetes we have a very clear case of karyokinesis in the basidia; spindle-figure, equatorial plate, protoplasmic radiations at the poles of the spindle, and probably centrosomes, being produced.

Harper ('95) describes and figures beautifully the nuclear division in *Peziza* and *Ascobolus*, which is seen to follow almost exactly the normal course.

Trow's description of nuclear division in *Saprolegnia* ('95) is obviously incomplete. He states that the nuclei divide directly in the germinating zoospores, but that in the oogonia the division is indirect. The nucleus contains one chromosome of irregular shape, which divides into two, producing two 'half-chromosomes.' He regards the division therefore as a reducing one. In a recent letter to me, however, he states that 'a long study of the work which has been done on the behaviour of the nucleus in gameto-genesis has led me to the conclusion that the network I have figured is a linin-network impregnated with chromatin, and the division figured

simply a very special case of ordinary karyokinetic division brought about possibly by the small size of the nucleus.'

Finally, Isatschenko ('95) describes a process of indirect nuclear division in the cells of the pileus and stipe of *Pholiota aurea*, Fr., in which the chromatin substance collects at the equator of the nucleus and divides into two portions which gradually approach the poles of the nucleus, where small round bodies (centrosomes?) can be seen. The nucleus becomes constricted in the middle and two daughter-nuclei are formed.

In the process of nuclear division, as shown in the oogonium of *Cystopus* (Fig. 28, 1 to 5), the nucleus first of all loses its nucleolus (1) and the network becomes at the same time clearly visible and is seen to be granular. Its network then appears to be transformed into a number of granular chromosomes (2), which stain reddish, and among which a faintly stained blue network is to be seen. The appearance of the nucleus at this stage gives one the impression that the chromosomes are very numerous, but on counting them, as well as one is able, there appears to be about 12 or 16. It is very difficult to be sure of this however. I have counted them in the stages 2, 4, 5 (Fig. 28) and the impression always given is that 12 to 16 is the number.

In 3 (Fig. 28) the nuclear membrane is seen to be slightly irregular; the chromosomes have contracted towards the middle, and a blue network is seen surrounding them irregularly and is also seen between them. Then the nucleus begins to elongate in one direction and becomes oval-shaped. The chromosomes begin to take up a transverse position to form the equatorial plate, and at the same time the nuclear spindle appears, and the fact is strongly impressed upon one that it is formed out of the faint blue-stained network of the nucleus. This is supported by the fact that the nuclear membrane remains intact. In 5 (Fig. 28) the equatorial plate is seen and a well-formed spindle. The nuclear membrane becomes, at this stage, more indistinct and presents a granular appearance, which is probably the first indication of its

breaking down ; but later than this I have not been able to follow the division. The number of nuclei contained in the oogonium after division is doubled, 143 and 218 having been counted.

It will be seen that although the general type of nuclear division conforms, to a greater or less extent, with that observed in the higher plants, there is one body concerned about which we are not quite so clear. The centrosphere which appears to be generally present both in animal and vegetable cells, has not been observed in the Fungi as satisfactorily as one would like. Observations have been made, however, which lead one to suspect that this is only due to the fact that our present methods of investigation are not sufficiently perfect to enable us to cope with the difficulties of observing structures, which even now cannot be observed without much careful manipulation in connexion with the very large nuclei to be found in the higher plants. It is probable that further investigation with improved methods of staining will confirm what has been already observed in connexion with the nuclei of the Hymenomycetes and Ascomycetes, and will demonstrate centrosomes in connexion with other nuclei also. With this exception I think we can safely say that there now remain only the Bacteria and Cyanophyceae in which a normal process of karyokinetic division of the nucleus has not been indicated.

The division which occurs in the oogonia and antheridia of *Cystopus* and *Peronospora* previously to fertilization is very interesting, and does not, so far as I am able to judge at present, admit of an explanation. The same phenomenon has been described by Eidam ('87) and Chmielewsky ('89) in *Basidiobolus*, one of the Entomophthoraceae, but not so far as I am aware in any other Fungus, except *Saprolegnia*, recently described by Trow ('95). In *Basidiobolus Ranarum* the vegetative cells contain only a single nucleus. In order to produce a zygospore, two cells lying together in the same filament put out a projection on each side of the common partition ; the nucleus of each cell passes into the projection

thus formed, and each nucleus divides karyokinetically into two. The apical portions of these projections, each with a single nucleus, are then cut off by a partition-wall, and these two nuclei then degenerate and disappear. The partition-wall between the two contiguous cells is then resorbed, and the two masses of protoplasm, each with its nucleus, fuse together to form the zygospor. Chmielewsky ('89) was able to observe the ultimate fusion of the nuclei into one egg-nucleus. The fusion of the nuclei takes place very slowly, but in zygo-spores four weeks old they are always found fused together. The author makes the extremely interesting observation that quite ripe zygo-spores with fused nuclei would not germinate, but that unripe zygo-spores with the nuclei as yet not fused would germinate very easily when placed under suitable conditions. He explains the fact that the ripe uninucleate zygo-spores would not germinate because, obviously, they require a longer resting period.

This division of the sexual nuclei previously to fusion is exactly similar to what occurs in *Peronospora* and *Cystopus*, and according to Trow in *Saprolegnia* also. It would be interesting to know if it takes place in *Pythium*. The readiest explanation of the phenomenon would of course be that it is a reducing division, but this does not appear probable in the light of recent investigations on the reduction of the chromosomes in plants and animals, and also from the fact that, so far as I was able to observe in *Cystopus*, the number of chromosomes in the dividing nuclei in the oogonium is less than the number in the dividing nuclei of the fully formed oospore, as will be seen later. Moreover the explanation of this phenomenon, whatever it may be, is probably bound up with the explanation of the post-sexual process of reduction described by Klebahn ('88) in *Cosmarium* and *Closterium*, and by Chmielewsky in *Spirogyra*.

That 'we can only regard the nuclear divisions in oogonium and antheridium as phylogenetic reminiscences of the formation of gametes by cell division,' as suggested by Hartog ('91), is possible, but it seems to me that more facts are

required before a perfectly satisfactory explanation can be obtained.

FORMATION OF OIL-DROPS.

Dangeard's observations ('90) are interesting and his figures of the appearances presented are correct as far as they go, but his interpretation is wrong. The central mass in his figures 6, 7, 8 is not an oil-globule, but the central, deeply stained, very dense mass of protoplasm already mentioned, containing a nucleus. The globules of oil at these stages are very numerous. In his figures 9, 10, 11, the central mass is undoubtedly oil. It is a pity that Dangeard took oospores at such very different stages to illustrate his general observations on the formation of oil. If he had carefully followed the gradual development of oil-drops, he would not probably have made the mistake of regarding the large central body found in the early stages of the development of the oospore as an oil-globule.

In sections cut from fresh specimens and from specimens soaked in corrosive sublimate and preserved in methylated spirit, small oil-drops in considerable numbers are to be seen soon after the separation of the oosphere from the periplasm. When the oosphere-wall has been distinctly formed, the oil-drops are very distinct.

These oil-drops gradually fuse together, and at certain stages one large oil-drop with a number of small ones is to be seen. In others, there are two large oil-drops and many small ones. The oil-drops are in cavities or vacuoles in the protoplasm and are much smaller than the vacuoles when they are of considerable size. This is due probably either to the contraction of the oil-drops or to the loss of a certain quantity of oil through soaking in spirit. The drops are coloured yellowish or blackish-brown in osmic acid solution.

All the oil-drops gradually fuse together into a somewhat irregular lumpy mass, and finally into the large central oil-sphere of the ripe oospore. At this stage, the large drop appears dense black after staining in osmic acid for some time.

The sections observed and described above were stained in osmic acid for twenty-four hours and mounted in dilute glycerine.

In the earlier stages, before the formation of the oosphere and just after its delimitation, a number of small, bright, refracting spheres were observed. These stained light yellow in osmic acid, and were probably small oil-globules in process of formation.

The antheridium is sometimes observed cut transversely in an oosphere. The dense protoplasm contained in it then nearly always presents the appearance of a crescent, owing to the protoplasm being more on one side of the tube than the other, and this is what Dangeard has probably mistaken for oil-crescents, and which I was inclined at first also to regard as due to the presence of oil.

FERTILIZATION.

While the changes already described have been taking place in the oogonium, the nuclei of the antheridium have been undergoing division, and the number of nuclei at this stage is also now, so far as can be observed, about double the number at the beginning. The fertilizing tube has grown, and has at this stage pushed its way through the periplasm into the gonoplasm. A single nucleus and a small quantity of densely stained protoplasm passes from the antheridium into it down the side of the fertilizing tube (Fig. 11) to the apex (Figs. 12 and 13). The remainder of the tube is occupied by a thin lining layer of protoplasm and a large vacuole (Fig. 13).

The fertilizing tube now grows towards the centre of the young oosphere, around which a limiting membrane has not yet been formed. As it grows, the dense mass of protoplasm becomes reduced in amount, being used up probably to form the new growing wall. The apex of the fertilizing tube expands considerably, and it looks just as if this took place

in order to cover as much space as possible so as not to miss coming in contact with the nucleus of the oosphere (Fig. 14). In my preparations, the fertilizing tube was nearly always seen slightly contracted away from the protoplasmic layer immediately outside and in front of it, leaving a space. This was probably due to the contraction consequent on the addition of re-agents (Figs. 11, 13, 15 and 16). Occasionally, however, this contraction had not taken place (Figs. 12 and 14).

The fertilizing tube grows until it comes into contact with the central mass of dense protoplasm. This is shown in Figs. 15 and 16, two successive sections from the same oogonium; one showing the nucleus of the oosphere, the other the male nucleus in the fertilizing tube. The fertilizing tube has contracted slightly away from the protoplasm in its immediate neighbourhood, but its original position with respect to the nucleus of the oosphere can be distinctly seen. Unfortunately this oogonium was cut very obliquely, so that the antheridium itself could not be seen; nevertheless from examination of numerous sections at about this stage it is evident that the antheridium contains numerous nuclei. The apical part of the fertilizing tube is very thin and probably soft, and as soon as it has come into contact with the ovum-nucleus, the male nucleus is expelled and the tube immediately contracts, or rather collapses, and is withdrawn from the oosphere, leaving a large vacuole (α , Fig. 17) to mark its position. The two nuclei are left in close contact with one another (Fig. 17). The male nucleus is nearly always slightly smaller than the female. A delicate membrane, already indicated in Figs. 15 and 16, becomes now visible around the oosphere, separating it from the dense protoplasm. The two nuclei remain in contact for a short time and then fuse together (Fig. 20) to form the nucleus of the oospore.

It may be well to refer here to previous observations on the fertilization of the Peronosporae. De Bary points out ('84) that in *Pythium*, nearly the whole of the contents of the

antheridium pass over into the oosphere; and if there is more than one antheridium, they all usually, but not always, empty their gonoplasm one after another into the oosphere.

Marshall Ward also ('83), in a very careful description of the process of fertilization in various species of *Pythium*, points out that 'the contents of the oogonium contract away from the wall towards the centre, strings and bands of protoplasm being left attached to the inner wall.' At a later stage, the central mass becomes rounded off, leaving a small quantity of protoplasm between it and the wall of the oogonium, the periplasm. This appears to be much smaller in amount than that found in *Peronospora* or *Cystopus*. The protoplasm of the antheridium, which contains a number of brightly refractive granules, sends out a fertilizing tube, which comes in contact with the oosphere or egg-cell. The contents of the antheridium then pass over almost entirely into the oosphere, a very small quantity only being left in the antheridium. A thin skin appears round the oospore and ultimately a thick envelope which is derived from the periplasm.

In *Phytophthora* again De Bary ('84) points out that the process of oospore formation is much the same as in *Pythium*, except that only a very small quantity of protoplasm passes over into the oosphere through the fertilization tube, and this portion is not distinctly separated beforehand. In *Peronospora* and *Cystopus* the passage of protoplasm could not be seen ('63-'84).

Neither Fisch ('85) nor Chmielewsky ('89) appear to have clearly observed the process of fertilization in *Cystopus*, and Fisch is not quite clear as to fertilization in *Pythium*.

It would of course be useless to offer at the present time any extended generalization from a comparison of the mode of fertilization of *Cystopus* as described in this paper with those methods described by earlier observers on other members of the Peronosporae, as the latter are obviously incomplete; but it may be useful to point out that my observations on *Cystopus* are so far in general agreement with the observations on *Phytophthora* described by De Bary,

that the amount of protoplasm which passes over is very small; but do not agree with the observations made on *Pythium*, that nearly the whole of the protoplasm passes over. If it be found that the antheridium of *Pythium* contains numerous nuclei, and that these all pass over into the oosphere, as would appear to be clearly indicated by Marshall Ward and De Bary, and perhaps Fisch, it will afford an interesting comparison with *Cystopus*, and will probably lead to interesting results.

That fertilization takes place in the Saprolegniae as described by Trow, or as indicated by me for *Peronospora*, without the penetration of a fertilizing tube into the oosphere, would appear to be extremely doubtful in the light of these observations on *Cystopus*. If fertilization does take place in the manner described, then we have simply the passage of a male nucleus from the fertilizing tube through the protoplasm of the oosphere to the female nucleus without the intervention of a fertilizing tube, a state of affairs which is now known to take place, with the exception of a few little-investigated cases, only when antherozoids are formed. In all other cases, where the conditions under which the plant is grown do not allow the formation of antherozoids or motile spermatia, the male nucleus is carried right into the oosphere by a fertilizing tube: whilst in the archegoniate plants and in *Vaucheria*, a substance is excreted or formed which conducts the antherozoid down to the oosphere. Such evidence as this tends to show therefore that fertilization as is described in *Saprolegnia* by mere contact of a fertilizing tube with the oosphere, and subsequent passage of the male nucleus through it into the oosphere, does not take place as a rule.

Another interesting point to which it may be well to call attention is that the process of fertilization as here described does not differ in any essential particular from the process as it takes place in the Angiosperms, if we except the part played by the centrospheres, which I was not able to observe in *Cystopus*. In the Angiosperms, for example, according to

Guignard ('91) the extremity of the pollen-tube swells up on coming into contact with the embryo-sac, and pushes its way towards the nucleus of the oosphere. As soon as it comes near the latter the male nucleus passes rapidly through the membrane and comes into contact with the nucleus of the oosphere so quickly that it is rare to find it at any distance from the latter; a state of affairs which is almost exactly paralleled by what takes place in *Cystopus*.

In the Angiosperms the male nucleus, as it passes through the extremity of the swollen pollen-tube, is accompanied by a thin layer of protoplasm probably belonging to the generative cell. In *Cystopus* also the nucleus is accompanied by a small quantity of protoplasm at the time of fertilization, but whether or not any part of this passes with it into the oosphere could not be observed. In Angiosperms the male nucleus does not show any differentiated structure until it comes into contact with the female nucleus, when it swells up and assumes the normal structure of a resting nucleus. The two nuclei can be distinguished as separate structures up to the time of the formation of the chromatic segments. It is somewhat different in *Cystopus*; the male nucleus as it passes into the oosphere is certainly smaller than the female nucleus, but it appears to show much the same structure. It increases in size, and then fuses with the female nucleus; so far as I was able to observe the fusion is complete, and one nuclear membrane only can be seen at a stage much earlier than the appearance of the chromatic segments. These are however mere differences of detail, the important fact remains that the processes described are essentially similar in their chief features.

MATURATION OF THE OOSPORE.

The fertilizing tube on its withdrawal from the oosphere is very much crumpled, as if by a sudden loss of turgidity due to the expulsion of its contents; but it soon regains to some

extent its original shape, although its apex appears in some sections to be disorganized (Fig. 18). In most cases a portion at least of the dense protoplasm which accompanied the male nucleus in its passage down the tube can be observed after the withdrawal of the latter.

The fusion-nucleus in the oospore now begins to undergo changes. It increases in size; the network becomes more visible and stains more deeply, and the dense mass of protoplasm in its immediate neighbourhood begins to disappear, probably being used up to nourish the growing nucleus. The nucleolus then disappears, and the nuclear network divides up into chromosomes, which contract into an irregular spherical mass at the centre of the nucleus (Figs. 21, 22, 23), and presents the same appearance as occurs in the nuclei of the oogonium just previously to their division. The nucleus then divides, and although I have not been able to observe the details satisfactorily, it appears to follow the normal course of karyokinesis. The number of chromosomes present in the equatorial plate before division appears to be considerably in excess of the number observed in the nuclei of the oogonium, but it is very difficult to make sure. By counting as carefully as possible twenty to twenty-four or even more appear to be present, and the impression is produced that the number is certainly much larger than that observed in the oogonium.

Meanwhile the membrane around the oospore—the exospore—becomes distinctly visible as a moderately thick cell-wall. The columnar wall has appeared as minute projections on the inner layer (Figs. 21, 22), and further development of the wall accompanies the changes going on in the contents of the oospore, so that it is perfectly easy to follow the changes which take place in its further development in consecutive order, by using the formation of the wall as an index.

The two nuclei of the oospore (Figs. 24, 25) now undergo changes similar to those described for the primary nucleus, and finally each one divides into two, so that we have now

four nuclei in the oospore (Fig. 26). These are distributed irregularly in the protoplasm, which is very irregularly vacuolate, showing a large number of oil-vacuoles also. The four nuclei then divide again into eight, and successive divisions take place until thirty-two nuclei have been produced, at which stage the division stops. By the time this stage is reached, the oil-globules have united together into the single large central mass of oil, the protoplasm forming a layer around it in which the thirty-two nuclei are arranged in a single layer (Fig. 27). The oospore now appears to be mature, and the formation of the exospore and endospore to be complete. In the former three distinct layers can be seen; an inner comparatively thin homogeneous layer, a middle thick columnar layer, and an outer irregular warty layer. The endospore consists only of one very thick layer (Fig. 27). The oospore now enters upon its resting period, and it is interesting to note that it has already germinated to the extent of producing what we may regard as a multinucleate cell or sporophyte with thirty-two nuclei. I have never been able to observe a larger number of nuclei than this even in quite old oospores taken from very rotten material, so that it is probable that I am quite right in stating that this is its normal condition in the resting stage. Unfortunately I have never been able to observe the germination of the oospore, so that I cannot say at what period or under what conditions it begins to germinate; but De Bary ('63) has shown that in germination the whole of the cell becomes a zoosporangium; and, if his observation is correct, as is probably the case, that 100 or more zoospores are produced; and if we assume that each zoospore possesses one nucleus, as is also probable, then each of the thirty-two nuclei by dividing into four would give us 128 zoospores, a number sufficiently near De Bary's estimate to render it probably correct; we may therefore regard each of the thirty-two nuclei together with the protoplasm in connexion with it as the mother-cell of four zoospores, and the ripe oospore with thirty-two nuclei as the sporophyte. The life-

history of *Cystopus candidus* might then be represented by the following diagram.

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EXPLANATION OF FIGURES IN PLATES
XV AND XVI.

Illustrating Mr. H. Wager's paper on *Cystopus candidus*.

All the figures have been drawn with the aid of the camera lucida, and the apochromatic 2.0 mm. apert. 1.40 of Zeiss with ocular 8 ($\times 1000$), except Fig. 28 which was drawn with ocular 18 ($\times 2250$), and Fig. 3 drawn with ocular 12 ($\times 1500$).

Fig. 1. Small piece of mycelium showing nuclei and haustoria.

Fig. 2. Mycelium with sporangiophores containing nuclei, previous to the formation of zoosporangia. Fig. 2 a is a sporangium.

Fig. 3. Sporangiophore with seven nuclei and three more passing in from the hypha. Oc. 12.

Fig. 4. Young oogonium just forming; the irregular shape of the nuclei clearly seen and the lines produced by the streaming in of the protoplasm at the base.

Fig. 5. Young oogonium soon after its delimitation from the mycelium. An antheridium is seen attached to one side and the accumulation of a denser protoplasm is to be seen commencing in the oogonium on the side near the antheridium. In this oogonium there were 115 nuclei altogether, and about six in the antheridium.

Fig. 6. A later stage than Fig. 5. The nuclear network is distinctly visible, and the germinal papilla projecting from the oogonium towards the antheridium.

Fig. 7. Portion of section of an oogonium at a later stage than Fig. 6. The nuclei have much increased in size and the nuclear network is very clear.

Fig. 8. Section of an oogonium in which the protoplasm has begun to contract towards the centre. The nuclei show their chromosomes contracted in the centre joint preliminary to division. Thick strands of protoplasm are seen connecting the central mass of protoplasm to the wall of the oogonium.

Fig. 9. Later stage than Fig. 8. Nearly all the nuclei have divided, and are collecting at the periphery of the central mass. A deeply stained dense mass of protoplasm is seen in the centre, in contact with which is the ovum nucleus, produced by the division of one of the original nuclei of the oogonium.

Fig. 10. An antheridium attached to the wall of an oogonium, just beginning to push out its fertilizing tube. A dense mass of protoplasm and several nuclei are seen in and near the projection. This dense mass of protoplasm stains deeply in a similar manner to that in the centre of the oogonium or young oosphere.

Fig. 11. Further stage of development of an antheridial fertilizing tube. The antheridium now contains a large number of nuclei produced by the division of those originally present, but the fertilizing tube contains only one nucleus, together with a mass of deeply stained protoplasm; these are passing down the sides of the tube towards the apex.

Fig. 12. A fertilizing tube at a slightly later stage than preceding, showing the male nucleus with protoplasm at the apex.

Fig. 13. Oogonium showing ovum-nucleus with its deeply stained mass of protoplasm, and a fertilizing tube with nucleus at the apex. Owing to the action of re-agents the fertilizing tube is shown slightly contracted away from the protoplasm into which it is pressing. The central mass of protoplasm which will become the oosphere shows, as well as in previous sections, a very characteristic foam-structure.

Fig. 14. Later stage than Fig. 13. The apical wall of the fertilizing tube is now becoming very thin, and the tube itself is expanding at the apex into a bladder-like structure. The wall of oosphere has not yet begun to form.

Figs. 15 and 16. Two successive sections of the same oogonium, showing the fertilizing tube in contact with the central or ovum nucleus.

Fig. 15. Ovum nucleus shown, and fertilizing tube which has contracted away from it, probably owing to the action of re-agents.

Fig. 16. Shows the apex of the fertilizing tube with nucleus, the deeply stained mass of protoplasm in contact with the ovum nucleus is shown on the wall of the space produced by the contraction of the fertilizing tube. These two sections taken together show that the male nucleus is brought quite into contact with the ovum nucleus by means of the fertilizing tube.

Fig. 17. Section showing the male and female nuclei in close contact with one another. The male nucleus is expelled from the fertilizing tube in some way, probably by a gradual thinning and ultimate rupturing of its apex, the fertilizing tube being immediately withdrawn, a large vacuole (*a*) being left to indicate the place where it was. The two nuclei in the egg are still surrounded by a dense mass of protoplasm. The wall of the oosphere can now be seen.

Fig. 18. Portion of a section of an oogonium immediately after fertilization, showing the fertilizing tube withdrawn to the outside of the young oosphere, and presenting an appearance as if ruptured or disorganized at the apex.

Fig. 19. An older oogonium with young oosphere showing the empty fertilizing tube on the outside of the latter. The antheridium contains still a small quantity of protoplasm, and a number of small degenerating nuclei.

Fig. 20. Section of an oogonium at a later stage than Fig. 17, showing the nucleus of the oospore shortly after the fusion of the male and female nuclei. Two nucleoli can be seen, and the nucleus is of an oval shape and still surrounded by the dense mass of protoplasm. The wall of the oosphere, the exospore, is now distinctly visible.

Fig. 21. Oospore nucleus at a later stage than in Fig. 20. It contains a considerable number of granules, probably chromosomes, and a very distinct nuclear membrane. It is surrounded by a small quantity of the dense deeply stained protoplasm only. As the latter disappears the nucleus takes a more intense stain and increases in size. The wall of the oospore just begins to show the appearance of the columnar layer of the exospore.

Fig. 22. Oogonium with oospore, slightly later stage. The columnar layer of the exospore is now visible as a number of minute projections on the inner wall. The chromosomes of the nucleus are contracting towards the centre, probably preparatory to division.

Fig. 23. A still later stage of oospore formation. The columnar layer is more strongly developed. The endospore has not yet begun to develop. The chromosomes are still more contracted towards the centre. An antheridium attached to the wall of the oogonium shows a number of small degenerating nuclei. The periplasm

is still more or less granular, but the nuclei are not so clearly visible in it; they are probably in process of degeneration.

Fig. 24. Portion of section of an oogonium showing the oospore with two nuclei. Each nucleus possesses a distinct network and a small nucleolus. The protoplasm is losing its characteristic foam-structure and is becoming more irregularly vacuolate. Some of the vacuoles indicate oil-spaces.

Fig. 25. Portion of an oogonium with contained oospore, showing two nuclei just after division. This oospore contained four nuclei, produced by the division of two nuclei similar to those in Fig. 24. The columnar layer of the exospore is more highly developed. Spaces in which oil was contained are shown.

Fig. 26. Oospore with four nuclei. The endospore is just beginning to form, as a thin layer just inside the innermost layer of the exospore. The columnar layer has apparently been completed and the third is now being formed. The whole or nearly the whole of the periplasm has been thrown down upon the wall of the spore in the form of a dense homogeneous irregular layer, only here and there is to be seen any connexion with the wall of the oogonium.

Fig. 27. Ripe oospore in section, showing seven nuclei in the parietal layer of protoplasm. The section is taken from an oospore containing thirty-two nuclei. Each nucleus with its protoplasm may be regarded as a mother cell of four zoospores. The central space shown is filled, in the fresh oospore, with oil. The endospore is very thick. The fertilizing tube of the antheridium is also shown surrounded by an exospore.

Fig. 28. Five stages in the division of the nuclei of the oogonium. Four and five show the spindle-figure. It will be seen that the nuclear membrane persists at least until the formation of the equatorial plate and probably later.

Fig. 1.

Fig. 2.

Fig. 2 a.

Fig. 3.

Fig. 6.

Fig. 8.

Fig. 7.

Fig. 9.

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Fig. 5.

Fig. 4.

Fig. 10.

Fig. 13.

Fig. 11.

Fig. 12.

Fig. 14.

Fig. 1.

Fig. 2.

Fig. 2 a.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 8.

Fig. 10.

Fig. 13.

Fig. 11.

Fig. 14.

Fig. 12.

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Fig. 16.

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Fig. 19.

Fig. 25.

Fig. 26.

Fig. 27.

Fig. 28.

Fig. 15.

Fig. 16.

Fig. 17.

Fig. 18.

Fig. 19.

Fig. 20.

Fig. 22.

Fig. 25.

Fig. 26.

Fig. 24.

Fig. 23.

Fig. 21.

Fig. 28.

Fig. 27.

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