Cell-Division in Sporangia and Asci.

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With Plates XXIV-XXVI.

THE most thoroughly studied case of spore-formation in sporangia is that of *Saprolegnia* and *Achlya*. Here the slenderness and transparency of the objects made accurate observation of the processes taking place in the dividing protoplasm possible, without the employment of any very refined technique. The behaviour of the nuclei has not been sufficiently investigated in this case, but the observations of Humphrey¹, Trow², and others leave little doubt as to the main facts. The sporangium, when cut off from the parent hypha, is multinucleate, and the ultimately formed zoospores are probably uninucleate. In the light of Belajeff's³ observations on the formation of the cilia in antherozoids, it would be extremely interesting to determine

¹ Saprolegniaceae of the U.S., Trans. Am. Phil. Soc. Vol. xvii, 1892-3.

² The Karyology of *Saprolegnia*, Ann. of Bot. ix, 1895, p. 609.

³ Über die Cilienbildner in den spermatogenen Zellen, Ber. d. D. Bot. Gesellschaft. Bd. xvi, p. 140, 1898.

the relations of the nuclei to the formation of the cilia. As to the actual method of cell-division whereby the protoplasm of the sporangium is cut up into the swarm-spores, the earlier observers seem to have been too much influenced by theories of cell-formation in general. A brief summary of the views of Nägeli, Unger, Schleiden, Meyen and others, is given by Pringsheim¹ in the introduction to his account of the development of *Achlya prolifera*. Büsgen² also reviews briefly the views of the older authors as to the nature of the spore-formation and its bearing on cell theories.

Unger held that the sporangium was formed simply by the building of the basal septum, and was an example of simple meristematic cell-formation.

Nägeli interpreted the same phenomena of sporangium-formation as indicating the building of a complete new cell in the end of the hypha with a new wall just inside the sporangium wall. It was for him a case of 'wandständige Zellbildung,' and was used to still further support the doctrine that all new cells arise in old ones by free cell-formation. Nägeli further believed, in agreement with the views of Schleiden, that the individualizing of the spores was due to preceding formation of cytoblasts (nuclei), centres around which the cell is differentiated. According to Pringsheim's own account of the process in *Achlya prolifera* (*Saprolegnia ferax*, Ktz.) the septum which cuts off the sporangium is a double membrane running up on the sporangium wall and down on the wall of the hypha for a short distance, thus forming in optical section three-cornered intercellular spaces. The septum becomes convex downward. A lumen now appears in the sporangium due to influx of water (areola of Unger).

The spores appear first as prominences pushing into the sporangium-lumen. No boundary line about them is visible.

¹ Die Entwicklungsgeschichte der *Achlya prolifera*, Nov. Act. Acad. Caes. Leop. Carol. Nat. Cur., vol. xxiii, p. 1, 1850.

² Die Entwicklung der Phycomycetensporangien. Jahrbücher für Wiss. Bot., Bd. xiii, 1882.

Their surface consists of granules just like those in the mass of the protoplasm. A boundary line appears when they separate from each other and from the sporangial wall. They are thus completely bounded off from each other before a cellulose cell-wall appears, though this is formed shortly after their separation.

The protoplasm cleaves simultaneously, and not by successive bipartitions breaking into larger and then smaller portions. There is no deposition of slime between the plasma masses during the process of division. The septum now becomes convex upwards, due to the pressure of the protoplasm below.

No cytoblast (nucleus) is present in the spores. They are bounded by a cellulose wall, and beneath this the protoplasm is covered by a primordial utricle. The oospores are formed in the same fashion as the swarmers. The primordial utricle of the sporangium and oogonium is used up in spore-formation, but cannot be considered as dividing to form the entire new primordial utricle for each spore. Pringsheim recognizes that Nägeli's distinction of 'wandständige Zellbildung' and free cell-formation is dependent entirely on the degree to which the sporangium is filled with protoplasm.

Büsgen¹ investigated the sporangia of the Phycomycetes after Strasburger's description of the cell-plate in the Phanerogams, and is evidently much influenced in his interpretation by Strasburger's work. He describes the cleavage as occurring simultaneously by means of cell-plates. These cell-plates which are formed at first break down, and are later reconstituted for the definitive separation of the spores.

This period of disappearance of the cell-plate, as we shall see later, doubtless corresponds to the period of swelling when the spores are so closely pressed together that their boundaries become indistinct. The whole process is supposed to be analogous to full cell-formation, as described by Strasburger for *Eranthis hiemalis*.

¹ l. c.

Berthold¹ describes the cleavage essentially as follows, basing his description chiefly on a study of the oogonia, and assuming that the processes in oogonium and sporangium are identical. The first step consists in differentiating a dense granular wall-layer around a central vacuole out of the, at first, more homogeneous protoplasmic contents of the oogonium. This layer of protoplasm then, at a certain definite number of centres, begins to form rounded protrusions into the central vacuole, the protoplasmic layer between these eminences becoming as a consequence thinner. These centres of aggregation become constantly larger, pushing out more and more into the central vacuole. The whole protoplasmic mass also contracts so as to be set free from the oogonial wall. Finally the different masses become entirely separated and round themselves up, though threads of protoplasm may connect them for a time after they have become well individualized.

These balls of protoplasm now swell so as to become pressed together and flattened against each other, but ultimately round themselves up again and ripen into the mature oospores.

From another point of view, the cleavage, according to Berthold, might be interpreted as proceeding from the central vacuole outward by means of very broad furrows which finally cut from the vacuole to and through the plasma membrane. No furrows from the surface inward are found. Berthold sums up the process for sporangium and oogonium under three heads: first, the separation of the contents of the sporangium into a wall-layer and a central vacuole; second, the loosing of this wall-layer from the cell-wall, and its division into a number of distinct heaps which finally separate entirely; third, the forming of these masses into swarm-spores or eggs.

Berthold's object throughout is the explanation of the mechanics of the process. He regards the position of the spores as determined by a number of new centres of attraction

¹ Studien über Protoplasma-Mechanik, p. 308: Leipzig, 1886.

toward which the protoplasm of the wall-layer is drawn together.

He notes very clearly a preliminary contraction followed by swelling, and a second final contraction of the spore masses.

Berthold¹ regards the process of cleavage in *Saprolegnia* as derived from free cell-formation and as representing a higher stage of development than the latter, since, in this case, the spores are able to separate out of the parent plasm and maintain their independence without the sacrifice of materials involved in the formation of periplasm. This view involves of course a reversal of the process as conceived by Brefeld, who holds that the ascus has been derived from the sporangium. The sporangium, according to Berthold's view, has been developed from types which form periplasm rather than the reverse. The ascus being in the latter group represents the more primitive type. It is to be noted that, while insisting definitely on the derivation of the sporangium as seen in *Saprolegnia* from some periplasm-producing type, Berthold does not go into the question of establishing relationships between the groups of fungi on this basis, his interest in the matter being wholly from the mechanical physiological standpoint. Berthold's doctrine of the stratification of the cell-body, with polarity determined by the position of the nucleus, influences throughout his interpretation of the spore-formation in the sporangium. Also the position of the developing oosphere, he thinks, is determined by the appearance of certain new and characteristic relations in the symmetry of the wall-layer of the oogonium.

The differentiation of the sporangium-contents into a central vacuole and a peripheral protoplasmic layer is perhaps an example of stratification in a cell. The position of the forming spores is ultimately determined, as we shall see, by the position of the nuclei. Whether the distribution of the nuclei is influenced by diffusion streams, I shall discuss more fully later on.

¹ l. c. pp. 313-4.

Rothert¹ gives a very full account of the formation of the basal septum of the sporangium. He finds that the dense protoplasm at the apex of the sporangiophore excretes a hyaline plasma in the region where the septum is to be formed. This hyaline mass is at first in the form of a ring which gradually widens and cuts off the sporangium. It is sharply bounded below and passes gradually into the dense plasma above. It may be thicker than the breadth of the sporangium at that point. A few seconds suffices for the process of building this zone. The lower part of the hyaline zone condenses next to a cross-wall without sharp contours at first. Cellulin granules are abundant in this region, and are dissolved in the hyaloplasm and used to form the wall.

Pringsheim² had already pointed out a similar participation of cellulin bodies in the formation of cell-walls.

In sporangia which are full of protoplasm, Rothert finds that the hyaline disk may be built simultaneously across the entire diameter of the hypha. Cleavage occurs in sporangia with a central vacuole by clefts which extend from the vacuole outward. They are not formed by a gradual progressive splitting, but simultaneously in their entire depth. These clefts become connected to form a continuous net. They contain cell-sap and not slime, and divide the protoplasm into polygonal areas of equal size. The net appears simultaneously throughout the sporangium. The plasma is not first divided into larger and then progressively smaller portions. At first numerous protoplasmic connexions are present between the spores, but these soon disappear for the most part.

In full sporangia the process is the same, except that clefts appear in the midst of the protoplasm and are enclosed by it on all sides. In sporangia with little protoplasm aggregation occurs at certain points as in the case of the oogonia. The polygonal masses which are to form the spores now begin to contract and round up, but are still connected next the

¹ Die Entwicklung der Sporangien bei den Saprolegnieen. Beitr. zur Biologie der Pflanzen, Bd. v, p. 291, 1892.

² Ber. d. D. Bot. Gesellsch., Bd. i, p. 303, 1883.

sporangium-wall, as can be shown by plasmolyzing the whole contents of the sporangium. After this stage of greatest contraction, twenty-five minutes after the full differentiation of the spore rudiments, swelling begins. The central 'lumen' disappears, vacuoles appear in the spore masses, and they become polygonal by mutual pressure but do not fuse. It was this swollen condition which led Büsgen to suppose that the spores, after separating, fused once more into a homogeneous mass. Cleavage is now completed and the whole sporangium shrinks, due to the escape of cell-sap. The stages of contraction and rounding up of the spores and subsequent swelling followed by another contraction in ripening as described by Rothert seem to be of general occurrence, as will be seen from my own observations described later.

Humphrey, in the paper already referred to, confirms most of Rothert's observations as to cleavage in the sporangium. He also describes the cutting off of the sporangium by a basal transverse wall. The process of wall-formation is preceded by the formation of a disk of hyaloplasm which arises by the withdrawal of the microsomes from the originally granular protoplasm of the region just below the forming sporangium. A comparison of his description with that of Oltmanns, relating to the cutting off of the oogonium of *Vaucheria*, leads to the surmise that Humphrey may have mistaken a flattened lens-shaped vacuole for a disk of hyaloplasm.

Pringsheim¹ first observed and figured the process of progressive cleavage from the surface inward in the sporangia of *Pythium entophytum*. In this plant, according to the author, the protoplasm escapes from the sporangium before the spores are formed, but remains attached to the end of the sporangium as a spherical mass enclosed in a very delicate membrane. Division begins by furrows on the surface of this mass, which advance towards its centre and finally separate it into a mass of swarm-spores in a fashion very much like that described below for *Synchytrium*. As already noted, however, he takes

¹ Beitr. zur Morph. u. Phys. der Algen, ii. Die Saprolegnieen. Ges. Abhandl., Bd. ii, p. 63.

quite a different view of the process as seen in the sporangia of *Achlya* and *Saprolegnia*.

The sporangia of the Mucorineae have been much less studied than those of the Saprolegniaceae on account of their extreme density and opaqueness. Only very fragmentary and unsatisfactory references to the cleavage are to be found in the older literature. All of these older observations were made on the living material, in which the differences of refractive index of the various parts of the protoplasm are so slight as to preclude the possibility of recognizing with certainty the parts played by vacuoles, nuclei, plasma-membrane, &c. The accounts of spore-formation in these sporangia given in the standard text-books¹ are extremely inadequate and most of the details given are inaccurate, as will be seen later. The relatively large and opaque sporangia of *Mucor*, *Pilobolus*, *Sporodinia* and *Synchytrium* can only be satisfactorily studied by means of microtome sections, and this explains the worthlessness of the earlier observations.

Corda² describes and figures the sporangial development in *Ascophora Mucedo*³. Prior to spore-formation, the plasma-mass is filled with transparent vesicles. In the lower part of the sporangium the columella is differentiated out, and at the same time in the upper part ill-defined and isolated cells begin to appear. These cells multiply and finally fill the entire sporangium. These cells are the spores. They are arranged in radial rows and probably connected in chains. Büsgen⁴ gives a very brief account of the cleavage in *Mucor*, based on a study of small living sporangia. He considers that the cleavage is accomplished here as in *Saprolegnia* by the formation of granular cell-plates, and notes that the surface of the sporangium is first marked off by the cleavage lines into larger areas, which are progressively cut up until the size of the spores is reached. This progressive division has been regarded as including the idea of successive bipartitions of

¹ De Bary's *Morph., Physiol. u. Biol. d. Pilze u. s. w.*, pp. 74-5.

² *Icones Fungorum*, Bd. ii, p. 19, 1838.

³ *A. Mucedo*, same as *Rhizopus nigricans*, Ehrbg.

⁴ *l. c.* pp. 278-9.

the spore-plasm, though this is by no means involved in the description given by Büsgen.

Recently Maurice Léger¹ has undertaken a very extended investigation of the cytology of a large series of Mucorineae. Léger points out the difficulties in technique which beset the investigation of the Mucorineae, and a careful reading of his paper is sufficient to convince one that the technical methods which he has employed are inadequate for overcoming these difficulties. A real insight into the cleavage phenomena was impossible from the preparations he was able to obtain. Léger himself is aware (p. 3-4) that there are very considerable gaps in his account.

Léger used alcohol for fixing, and cut sections in celloidin. In some cases he relied on results obtained from preparations of crushed sporangia. In my experience alcohol is a very unsatisfactory fixing agent for these materials, and not to be compared with those of Flemming, Merkel and Wilson.

To his reliance on alcohol for fixing is probably due Léger's inability to distinguish nucleolus and chromatin. He describes the nucleus as spherical, provided with a central nucleolus, which stains strongly, and a membrane; between the nucleolus and the nuclear membrane is a colourless zone which takes no stain. It is probable, judging from its relative size in his figures, that Léger's nucleolus is a fused mass made up of nucleolus and chromatin together. Léger is convinced that the nuclei regularly divide by direct division, and that karyokinesis is only met with in the germinating spore. He does not tell us just how thick his sections cut in celloidin were. The difficulty of obtaining very thin sections in this medium may well have been responsible in part for his poor nuclear figures.

Léger studied spore-formation in a very considerable series of forms from the genera *Sporodinia*, *Mucor*, *Rhizopus*, *Chaetocladium*, *Thamnidium*, *Pilobolus*, *Pilaira*, *Mortierella*, *Syncephalis*, and *Piptocephalis*.

In *Sporodinia* the young sporangium is filled with homo-

¹ Léger, Rech. histol. sur les Mucorinées, 1897.

geneous protoplasm in which the nuclei multiply rapidly by direct division. He notes nothing in the cutting off of the columella, except that the plasma is divided into an outer denser sporogenous part, and an inner less dense part with fewer nuclei about which the columella-wall is laid down. For the formation of the spores Léger quotes Van Tieghem's account, with the correction that the nuclei are continuously present during the process. He accepts the doctrine that the cleavage of the spore-plasm is due to a differentiation into a granular portion, which condenses itself into polyhedric masses which gradually round themselves up, and a hyaline intersporal plasma which fills the spaces between the polyhedric masses and forms a continuous layer beneath the sporangium-wall. The spores are formed by a process of free formation, and enclose themselves in a cellulose-wall by secretion from their surfaces. The masses are separated simultaneously, and Léger figures the separating zones of intersporal substance as being plates of equal thickness throughout. It is hard to see how he could have failed to observe the furrows which cut into the protoplasm gradually from the surface, and which can be observed readily on living material with an ordinary lens.

Léger studied the sporangia of *Mucor Mucedo*, and the remaining forms with large sporanges, at the stage of spore-formation, chiefly by means of crushed specimens and optical sections, and concludes that the process is the same as in *Sporodinia*, a differentiation of the plasma into polyhedric spore-bodies and intersporal substance which separates them.

In *Rhizopus nigricans* he notes a stage where the polyhedric masses are formed before the intersporal substance appears, and says he has not observed this elsewhere. In *Mucor racemosus* the plasma cleaves into sporogenous polyhedric bodies containing three to seven nuclei. In small sporangia with few spores, each contains a larger number of nuclei than do the more numerous spores in larger sporangia. The remaining forms investigated show nothing especially characteristic in their method of spore-formation.

In the case of *Mortierella candelabrum* Van Tieghem's and Monnier's views as to the appearance and disappearance of nuclei are opposed by Léger. Three to eight nuclei are present in the spores, and the conditions in this respect are the same as in other genera.

For *Syncephalis* the author accepts Van Tieghem's and Monnier's view that the spores are formed inside sporangia whose walls are absorbed after spore-formation. The nuclei divide directly at the base of the sporangium. Each spore contains from four to five nuclei or rarely three to six. The spore rows are held together after solution of the sporangial-wall by intersporal protoplasm.

In *Piptocephalis* the sporangium-rods divide into two spores by differentiation of intersporal plasm. The sporangial walls disappear as in *Syncephalis*. Léger distinguishes three types of spores in the Mucorineae: sporangiospores, chlamydospores and zygosporos. In germination stages neither nucleolus nor nuclear membrane is present; the nuclei are granular chromatin-like bodies. They divide once while still in the spore by indirect division. Rarely this division takes place in the germinating tube, but more than one indirect division is never observed. With the beginning of the independent nutrition of the mycelium the nuclei begin to divide directly.

In all these cases Léger considers cleavage as the direct formation of the polyhedric sporogenous bodies which have the same number of nuclei as the ripe spores.

Strasburger¹ gives a brief account of the spore-formation in *Mucor*. He considers that the cleavage occurs here as in *Saprolegnia* by means of cell-plates. The entire mass of protoplasm is used for forming spores, and there is no epiplasm. He considers that the 'Zwischensubstanz' of Brefeld and others is derived from the cell-plates.

Dangeard² has studied the sporangium-formation in *Synchitrium Taraxaci*. He finds that the single primitive nucleus of the fungus-body divides by direct division to form the

¹ Zellbildung und Zelltheilung, 3rd Ed.

² Dangeard, Rech. hist. sur les Champignons, Le Botaniste, ii. sér. pp. 77-86.

numerous nuclei which appear prior to sporangium-formation. In assuming the existence of direct division here he confirms the observations of Rosen¹, who finds that the nuclei of *S. Taraxaci* regularly divide directly, and not by karyokinesis. Dangeard, to be sure, thinks karyokinesis occurs in some cases, but his figures give little evidence that he had really seen mitotic figures. Dangeard describes the division of the primitive cell to form sporangia as a process of simultaneous fragmentation, whereby the multinucleated mass is at once cut into a larger or smaller number of polyhedric multinucleated portions. These are the sporangia. They become enclosed in a membrane, round themselves up, and later the zoospores are formed equalling in number the nuclei in the sporangium.

Dangeard notes merely this simultaneous fragmentation of the protoplasmic mass. The portions are separated from each other by a layer of colourless protoplasmic liquid.

Very recently C. M. L. Popta² has studied spore-formation in several of the so-called group of the Hemiasci, with special reference to the comparison of the phenomena in these forms with those in the ascus and in the sporangia of the lower Fungi. According to Popta, the process of delimiting the spores in *Ascoidea rubescens* is initiated by the appearance of abundant vacuoles in the protoplasm. These vacuoles are separated from each other by numerous protoplasmic plates and threads. This reminds us of the condition in *Pilobolus* as I have described it further on, but according to the author these angular vacuoles, instead of cutting through the plasma-plates and fusing edge to edge to divide the plasma into spore-masses, gradually become rounded up and disappear. The protoplasm now becomes charged with oil drops and granules. Later, hyaline non-granular protoplasm begins to gather round the nuclei, and these non-granular plasma-masses become delimited by membranes and, ultimately, uninucleated spores. Almost at once the single spore-nucleus

¹ Cohn's Beitr. zur Biol. d. Pfl., Bd. vi.

² Beitrag zur Kenntniss der Hemiasci, Flora, Bd. lxxxvi, pp. 1-46, 1899.

divides into at least four daughter nuclei. Gradually the granules between the spores are absorbed, and the latter are separated only by an oil-like *Zwischensubstanz*. The spores are then pressed out by the growth upward of a new sporangium from below. These processes were studied by the author on living material and on sporangia stained and studied *in toto*, and I can but believe that the process would show more resemblance to that in *Pilobolus* if studied on abundance of material in all stages and prepared in serial sections. The appearance of the angular vacuoles may well be a stage in the cleavage process, and their disappearance later may be due to the swelling of the already separated spores, whereby they are so closely pressed together that their boundary lines are invisible except in thin and differentially stained sections. It is to be remembered that Büsgen similarly was convinced by a study of the living material that the spores of the Saprolegnieae are formed completely only to immediately fuse once more into a homogeneous mass which is later cut up a second time into the definitive spore-bodies. It is doubtless the case in *Saprolegnia* that this apparent fusion after the spores have been once cut out is merely due to the swelling, whereby they become so closely pressed together that the plasma-membranes bounding each are indistinguishable without special preparation. If it could be assumed that the formation of angular vacuoles brings about the separation of the protoplasm into spores in *Ascoidea* the process would agree with what I have described below for a number of sporangia. If, however, the definitive cleavage is brought about by aggregation of hyaline plasma about the nuclei as centres, these hyaline masses becoming in some unknown fashion surrounded by a wall, the process is so far unique, and its homologies must be sought in some forms other than the asci or sporangia which have yet been studied.

In *Protomyces Bellidis*, according to Popta, the contents of the sporangium become differentiated into a wall-layer and a central vacuole. The wall-layer is divided then simul-

taneously into a single palisade layer of rod-like spores. This spore layer is bounded inside and out by a plasma-membrane (Hautschicht). Later the spores migrate from their first position and collect in a ball at the apex of the sporangium. No intersporal protoplasm is to be observed, but an outer and inner boundary layer of protoplasm about the spore-mass is present. In *P. macrosporus*, instead of a single layer three layers of spores are formed at once from the wall-layer of protoplasm in the sporangium. The spores here also migrate to form a ball at the apex of the sporangium. This spherical mass of spores is surrounded by a space containing cell-sap and numerous radially arranged protoplasmic threads and plates. These spores contain from four to seven nuclei. The protoplasm outside the spore-mass is also multinucleated. In this last point can perhaps be noted something similar to the multinucleated periplasm in the oogonia of *Cystopus*. Popta describes the cleavage in these cases as simultaneous rather than progressive, which would suggest division by cell-plates rather than by superficial furrows.

My own observations concern the phenomena of cleavage and spore-formation in certain sporangia of the lower Fungi, which perhaps may be taken as typical at least of the groups to which they belong, and further an account for purposes of comparison of the process of free cell-formation in the asci of *Lachnea scutellata*.

I shall describe the process in species from three genera of the lower Fungi, *Synchitrium*, *Pilobolus*, and *Sporodinia*. The sporangium of *Synchitrium* may represent a type of the cyst-like reproductive bodies of the one-celled fungal organisms, while *Pilobolus* and *Sporodinia* are types of the vegetative reproductive bodies of the Phycomycetes. Whether these sporangia are homologous with the zoosporangia of the Algae is perhaps still an open question, as we shall see later. In all three cases spore-formation consists in the cleavage of a multinucleated mass of protoplasm and the formation of numerous smaller reproductive bodies.

SYNCHITRIUM.

In *Synchitrium decipiens*, parasitic on the hog peanut, the swarm-spore, after penetrating an epidermal cell, develops the orange-coloured sorus in the hypertrophied host-cell. The so-called initial cell contains a single nucleus until it has reached practically its full development. It is then a mass of protoplasm visible to the naked eye (Fig. 1), and its nucleus has a diameter several times as great as that of the nuclei of the adjacent cells of the host-plant. This nucleus now divides with great rapidity till some hundreds of much smaller daughter nuclei have been produced, which are still, however, much larger than the nuclei of most Fungi. These daughter nuclei lie irregularly distributed in the initial cell which has thus passed from the single nucleated to the multinucleated condition. It is a question whether this process is to be regarded as a part of reproduction rather than the formation of a multinucleated vegetative thallus.

Since nuclear division does not begin until the initial cell has reached nearly its full size, I am inclined to regard the division as the beginning of spore-formation and the multinucleated cell as a sporangium, the uninucleated cell being the typical vegetative body of the *Synchitrium* plant.

Cleavage of the protoplasm of the sporangium now begins. This does not take place by repeated bipartitions nor by simultaneous precipitation of cell-walls about each nucleus. The process is rather analogous to that in the dividing protoplasm of the germinal disk of the chick, or still more nearly like that in those insect eggs where a series of nuclear divisions precedes protoplasmic segmentation¹. The cleavage is progressive from the surface of the initial cell inwards, and divides the mass into successively smaller portions (Fig. 2). The actual division of the protoplasm is accomplished by furrows formed on the surface, and growing deeper and deeper in a more or less exactly radial direction. These grooves are in reality so narrow as to appear as plates, which grow wider

¹ Hertwig, Die Zelle und die Gewebe, p. 187.

by additions along their inner margins till they intersect, and thus divide the protoplasm into irregular blocks or sometimes pyramids with their bases in the surface of the initial cell. (See Figs. 1, 2, 3, which are median sections through an initial cell in which cleavage has just begun.) Only at the very periphery the separation of the cut surfaces of the protoplasm to form a shallow notch, as it appears in section, reveals the true nature of the process as a pushing in of the free surface to form a deep though extremely narrow constriction.

In many cases at first there is no separation of the newly-formed surfaces; they remain closely appressed, up to the periphery of the cell. The groove appears in section, merely as a single line which the Zeiss apochromatic lens 140 ap. fails to resolve into two closely appressed surfaces (Fig. 3). The position of the line is further emphasized by the arrangement of the vacuoles, which are pushed aside and form in section two more or less regular rows in the plane of the newly-formed surfaces on each side of the furrow (Fig. 3). Such a line might be taken for a cell-plate which subsequently splits to form the boundaries of the protoplasmic segments or which is metamorphosed into the cellulose walls of the spores. That this line, however, in reality represents from the start two closely appressed surfaces is abundantly shown in many cases. Shrinkage in volume of the protoplasm goes on during the early stages of cleavage. This shrinkage is probably associated with the throwing off of water which has been seen to accompany sporangium-formation in many Zygomycetes. As a result of this shrinkage we frequently find that the cleavage of the protoplasmic mass is followed at once by the separation of the newly-formed surfaces, so that open furrows appear. This is regularly the case in *Pilobolus*, as we shall see later. There can be no doubt that the processes in forming the broad open furrows and the narrow cleavage lines are identical. In one case the surfaces have remained so closely appressed as to be indistinguishable, while in the other they have been pulled apart at once. The two conditions can hardly be regarded as distinct stages

in cleavage, since in many cases the grooves open as fast as they are formed, while in others they remain closed till a much later period (Fig. 4). The separation of the surfaces thus formed need not proceed from the periphery inward. Frequently furrows are found closed at the periphery and widely open deeper in the mass of the cell. The formation of the cleavage furrows is the essential process; whether the surfaces thus formed separate earlier or later is a matter of secondary significance. That these cleavage lines are not long meridional furrows dividing the initial cell symmetrically, as is the case in the sea-urchin's egg for example, is clearly seen from surface sections of cells in the first stages of segmentation, as shown in Fig. 5. We see here that the cleavage surfaces intersect each other at very varying angles, so as to mark off the surface of the cell by an irregular network of grooves in which the meshes are of very irregular shape and of unequal dimensions. The grooves seem to appear first, at least in some cases, on one hemisphere of the cell, and to spread gradually outward over the other. In other cases cleavage seems to begin almost simultaneously over the whole surface of the cell. In the first set of cases I endeavoured to determine whether cleavage began on the superficial or deeper surface of the cell, as it lies in the epidermis of the host, but I could discover no regularity in this respect.

The cleavage is progressive from the surface inward, the furrows deepening in general in a radial direction. Still they may be curved, and are inclined to each other at very varying angles and frequently form intersections at points near the surface of the cell, thus cutting off superficial blocks of protoplasm of varying shapes and sizes. Sometimes this tendency to cut off a superficial layer of segments is very marked (Fig. 2), so that we have a central solid mass or cell of protoplasm surrounded by a layer of superficial cells; in other cases the furrows grow radially inward without intersecting till near the centre, thus forming narrow cones and pyramids with their bases outward. As the process of cleavage goes

on the contraction of the protoplasm becomes much more marked, the furrows open widely and the masses of protoplasm tend to round themselves up.

The cleavage-segments produced in this way are seen to be unequal and as a rule multinucleate. The cleavage-furrows have so far shown no tendency to orient themselves with reference to the nuclei. But as the process advances, and the protoplasm is cut into smaller and smaller masses, we find the nuclei more evenly distributed, and it is seen that none of these portions are left without nuclei. In the end the result is always the separation of the protoplasm into uninucleate masses (Fig. 6). The process which at first seemed to be independent of the nuclei is seen finally to be directed solely from the standpoint of their distribution. The cleavage, as has been already noted, is attended by a very pronounced shrinkage of the protoplasm. This began while the segments were still multinucleate, and by the time cleavage is complete the segments are so reduced as probably not to occupy more than one-third of the volume of the primordial cell. An exact estimate of this reduction is very difficult. Rothert¹ estimates the shrinkage of a *Saprolegnia* sporangium at a corresponding stage at thirteen per cent. It is to be noted that the cell does not entirely fill the membrane at these stages, sections always showing a considerable open space on one side or the other (Figs. 4, 5). This indicates that loss of water and volume begins prior to cleavage. To be sure, this reduction prior to cleavage may be artificial and due to fixation, though the structure and appearance of the protoplasm give no evidence of this. The cavity around the protoplasmic mass prior to cleavage must be filled with water or at least by solutions which are not precipitated in fixation nor stained by ordinary reagents, but at the end of cleavage the uninucleated segments are floating or, better, imbedded in a liquid which contains an abundance of dense oily droplets, which blacken with osmic acid and take up stains (especially orange G.) with considerable avidity. There is generally a layer of

¹ l. c., p. 315.

segments around the inside surface of the sporangium-wall. The remaining segments are not evenly distributed, but are gathered in clumps which stick together and press upon each other even to the extent of giving the normally spherical segments a polygonal outline. Frequently there is one large central clump of segments, and between this and the surface layer appears the emulsion-like oily liquid mentioned. I have spoken of this liquid as thrown off by the protoplasm by a process of drying, connected with ripening. Why it should contain oily matter is not apparent. It perhaps should be mentioned that it is not at all protoplasmic in its apparent nature, and cannot in any sense be compared with the epiplasm of the ascus or the periplasm of the oogonium of the Peronosporaeae. It is apparent already that the method of spore-formation by constriction from the surface, as we find it in *Synchytrium*, precludes the possibility of a true periplasm in these forms. With the stage shown in Fig. 6 the process of cleavage is completed, and the uninucleated plasma masses so produced may be taken as typical vegetative spores homologous with those of *Saprolegnia*, and the process as representing typical asexual reproduction. Still their formation marks the completion of only the first stage of development of the reproductive bodies of *Synchytrium decipiens*. This first stage has been a process of cleavage, by which the multinucleated secondarily developed vegetative body of the fungus has passed back for purposes of reproduction to the primitive uninucleated condition. The bodies so formed may be called protospores. We now find a second series of changes initiated in the sporangium, sharply distinguished from the cleavage processes, and which I think we shall see are to be considered as initiatory stages in the germination of the bodies formed by cleavage. The first step in this second stage of intrasporangial development consists in a growth or swelling of the protospores. This proceeds until the primordial cell-wall is completely filled once more with its protoplasmic contents, as it was in earlier stages prior to cleavage. The segments as a result of this growth become pressed together, and take on

as a consequence a sharply angled polygonal outline in cross sections. The appearance of the sections at this stage is characteristically different from that in the last. The segments seem to be delimited and separated from each other by rather broad granular plates, and if one had not observed the preceding stages it would be easy to believe that these figures indicated a stage in cleavage where the protoplasm was being cut up simultaneously by granular plates into uninucleated spores. The granular plates are very conspicuous in stained sections (Fig. 7), and careful observation shows that, in the case of every cell, cleavage is complete rather than in progress at this stage, the granular plates being really double, and composed of the closely appressed membranes of the swelling protospores. Still I am inclined to believe that the appearance of this stage in unsectioned and unstained material of *Saprolegnia* such as Büsgen studied was what led him to advance the doctrine of a fusion of the once separated spores, followed later by a second more definitive cleavage by granular cell-plates. As a result of this growth in volume the liquid in which the primary segments floated disappears. Whether it is taken up again as food by the spores from which it has been extruded or simply is forced out of the sporangium, or whether both of these processes take place, is not easy to determine. The spore-mass at this stage fills the sporangium. The protoplasm of the protospores is evenly vacuolar and not apparently more or less dense than in the cleavage stages.

The next further step in development consists in a rapid multiplication of the nuclei by division. The divisions do not proceed as simultaneously as in the case of the earlier nuclear multiplication prior to cleavage, and we find spores containing 1, 2 or 4 nuclei in the same section (Fig. 7). The apparent irregularity is due in part to the fact that the spore appears in several sections. The process of division continues however till each segment contains from eight to twelve or possibly more nuclei. There is no evidence to be gained that the different spores finally contain exactly the same number of nuclei nor that each nucleus is removed by the same number

of generations from the original single nucleus of the protospore. It seems rather that the number of divisions in each spore depends on individual conditions of nutrition, &c., the same which determine the slight variability in size of the ripe spores. This is to be noted especially as showing that we have in the divisions of this spore-nucleus nothing directly comparable to the determinate number of divisions passed through by the ascus-nucleus to form the eight ascospore-nuclei.

This period of nuclear multiplication is followed by the third stage, in which the spores ripen preparatory to being set free from the sporangium for distribution. In this stage they become for the first time enclosed in a true cell-wall. Through the preceding stages they have been mere naked bits of protoplasm bounded only by their plasma-membranes. Fig. 8 shows the spores when the wall first appears as a very delicate membrane which stains strongly with orange G. At first it is only to be detected in cases where slight plasmolysis has occurred in fixation, so that plasma-membrane and spore-wall are somewhat separated. Later it becomes much thicker and shows a double contour. With the formation of the spore-wall the protoplasm of its body undergoes contraction once more, and the spores which were closely pressed together and polygonal in outline during the period of nuclear division draw apart from each other and become more rounded in outline (Fig. 8). With these changes the spores are finally ripened and ready for dissemination. The spore germinates as a sporange. The stages of germination consist in the breaking up of the multinucleated spore into a number of uninucleate swarm-spores. This may occur after a resting period in the case of *S. decipiens*. In *S. Taraxaci*, as is well known, the spores germinate to form swarm-spores at once. This formation of swarm-spores must be considered as a continuation of the stage of nuclear division in the spores which was interrupted by the ripening period. The whole may be roughly characterized as an embryonic stage in the life of the fungus.

The changes which take place in the process of cleavage of the protoplasm into the swarm-spores I have not as yet been able to study. The functional analogy between the nuclear divisions and growth of the protospore which takes place in the mother sporangium and the development of the egg in the tissues of the parent in the higher plants is sufficient to justify the distinction of an embryonic stage in the life-history of the fungus.

In *Synchytrium Taraxaci* this whole process of cleavage is abbreviated by the omission of the protospore stage. The multinucleated segments formed by the first cleavage-furrows round up, their nuclei become still more numerous by division, and they are then set free at once and germinate as sporangia. The life-history of the fungus is thus much shorter, and it is able to spread with great rapidity when conditions are favourable. As is known, provision is made in this species for its existence during unfavourable periods by the formation of the so-called resting spores. The relation between the cleavage processes in *S. decipiens* and *S. Taraxaci* is the same as that between the segmentation in *Pilobolus* and in *Sporodinia*, as we shall see later.

The question arises whether the protospore or the swarm-spore is the homologue of the gamete in the higher plants, but the resemblances are too remote, and the probability of any genetic relationship between these particular lower forms and the higher plants is too slight, to justify any speculation on this point. If we now summarize the life-history of the fungus we can roughly, and for purposes of comparison with other forms, distinguish five stages.

1. The multiplication of the nuclei by which the uninucleated vegetative body becomes multinucleated as a preparation for the formation of a corresponding number of reproductive bodies.

2. The progressive cleavage from the surface inward of the multinucleated mass into uninucleated masses or protospores.

These two processes constitute the original and fundamental elements in spore formation.

3. Embryonic development, in which the spores increase in size and become multinucleated.

4. Ripening, in which a protective wall is formed about the spore and the protoplasm passes into a denser resting condition with abundant inclusions of reserve food materials.

5. Germination, which is a mere continuation of embryonic development by which a sporangium produces a number of embryos (swarm-spores).

A mechanical explanation of the cleavage I have just described is by no means easy. The formation of superficial constriction-furrows cutting into the protoplasm is analogous to what is seen in the division of the animal cell especially, as noted above, to the phenomena of cleavage of certain insect eggs. Heidenhain¹ and later Kostanecki² have attempted to refer the constriction which divides the animal cell to the contraction of fibres arranged about a centrosome. Such sets of fibres are certainly not concerned in the division of the *Synchytrium* sporangium. The gradual cleavage, first into large multinucleated segments and only later into uninucleated spores, which are formed in *S. Taraxaci*, could not well be referred to any such simple systems of contractile fibres as the above-named authors discuss.

The decrease in volume of the protoplasm during the process might suggest that the cleavage is connected with the loss of water, and the formation of superficial furrows might be compared to the cracking of the surface of a drying mass of a colloidal substance. Such an explanation however in itself alone is entirely inadequate to explain the definiteness and certainty with which the final result of the cleavage is attained. The nuclei are seen to be the centres of the ultimately formed segments, which would by no means necessarily be the case as a result of simple cleavage by drying. We might indeed assume that the loss of water was least in the neighbourhood of the nuclei, which would meet the demands of the case without however simplifying the

¹ Archiv für Mikr. Anat. 1894, p. 423.

² Ibid. 1897, p. 651.

matter. The nuclei are certainly the centres about which the cleavage planes ultimately orient themselves, and it seems necessary to assume that some influence has passed outward from them and directed the whole process of the cleavage.

Strasburger¹, in connexion with Noll's² doctrine that the protoplasmic membrane is the direct controlling organ in the growth of the coenocytic Algae, has pointed out that the nuclei may be the real controlling centres working through the protoplasmic membrane, even in resting cells, by means of impulses transmitted through the kinoplasm. My own observations on spore-formation in the ascus point to the assumption that the plasma-membrane is itself kinoplasmic in its origin.

If in *Synchitrium* the nuclei are centres for the formation of kinoplasm, and it proceeds outward from them by diffusion in all directions till it reaches the plasma-membrane, this will be correspondingly increased in thickness, and if the mass be decreasing in volume by loss of water and tending to split up like a mass of drying starch, the membrane might perhaps press into the furrows thus formed, so as to become the surface layer of the forming segments. The first furrows will show no specific orientation with reference to the nuclei, but as the process continues the nuclei forming the centres for building up and distributing this membrane-material will by reason of this very fact tend to occupy the centres of cleavage-segments, until ultimately each nucleus will have furnished sufficient material for enclosing itself in an independent membrane. The evidence in favour of the explanation of cleavage just suggested is by no means adequate; still it will perhaps serve to bring out more sharply the elements of the problem to be solved.

PILOBOLUS.

The method of spore-formation in the Zygomycetes is of especial interest, since we have here one of the most

¹ Über die Wirkungssphäre der Kerne und die Zellgrösse. Hist. Beitr. v, p. 981, 1893.

² Die Wirkungsweise von Schwerkraft u. Licht auf die Gestaltung der Pflanze. Nat. Rundschau, Bd. iii, 1888.

widely distributed, commonest, and in some cases such as *Pilobolus* one of the most highly perfected methods of asexual reproduction and spore distribution known among the Fungi.

Brefeld¹ has given very full accounts of the external anatomy of *Pilobolus* and has also described the behaviour of very numerous forms under cultivation with varying conditions of temperature, &c. He has not especially investigated the phenomena of spore-formation. He attempts however to maintain and strengthen the doctrine that the mucilaginous jelly in which the spores of many Mucorineae are imbedded is identical with the epiplasm of the ascus.

The study of these forms of the Zygomycetes, apart from their interest as illustrating the mechanics of protoplasmic division in coenocytes, has gained a certain further importance since, as is known, the doctrine has been advanced by Brefeld that they illustrate in their vegetative sporangia the ancestral types in the development of the ascus which is, according to this view, only a sporangium which has become definite in its size, form, and in the number of spores which it produces. Evidence for or against this doctrine can perhaps be obtained by an exact and complete study of the processes in the two structures, and I hope to contribute something to this end in the following account.

I have investigated especially the phenomena in the sporangium of *Pilobolus*, and have used the common species *P. crystallinus*, from which all the figures illustrating spore-formation are made. I have, however, confirmed the principal points in the process by studies on *P. oedipus* and *P. microsporus*. *Pilobolus* is very readily cultivated in the manner described by Brefeld. The fungus always appears on horse-dung left for a few days under a bell jar, but to get an abundance of material at the proper stage of development it is better to resort to the simple culture methods described by Brefeld. These consist simply in infecting a mass of dung,

¹ Botanische Unters. üb. Schimmelpilze, I. Heft, 1872.

partially sterilized by repeated exposure to the temperature of boiling water, with spores from a culture which has appeared spontaneously. The sporangia can be caught on a clean slide as they are thrown off, and the spores are sufficiently free from bacteria when simply rubbed in a drop of water to use in infecting the partially sterilized dung. The culture can conveniently be made in a crystallizing dish of any size. In seven or eight days the sporangiophores begin to appear. Successive crops of sporangia are matured for several days, and thus abundant opportunity is given to secure any desired amount of material at any stage in development. The sporangiophores stand in dense turfs, completely covering the surface of the substratum. Not the least favourable feature of this fungus for cytological study is the exact regularity with which the different stages succeed each other through the day and night. The well-known yellow bulb-like swellings of the mycelium from which the sporangiophores arise appear in abundance on the dung in the early afternoon. This is a favourable period for studying nuclear division in this fungus. The vegetative nuclei in these bulbs divide rapidly to form the numerous nuclei which are afterwards cut off in the sporangium to form the spores. A little later the sporangiophores bud out from the bulblets and grow toward the light. Late in the afternoon the ends of the sporangiophores begin to swell to form the sporanges. The columella wall is formed about midnight. Segmentation occurs between one and four o'clock a.m. Embryonic development of the spores goes on from four to seven, and the ripe sporanges will be thrown off from nine till twelve a.m. The whole process is repeated at approximately the same daily periods for three or four days, when the culture is exhausted. The above periods of course vary somewhat with differences in temperature, moisture, &c., and the development of different sporangia is also not exactly parallel. Still, material fixed at the periods mentioned has always shown in my experience the majority of the sporangia in the stages noted. The general facts as to the development of *Pilobolus* have been very well worked out

by Cohn¹, and the whole process, as noted above, has been described and figured by Brefeld, so I need only mention the more especial points bearing on spore-formation. The mycelium of *Pilobolus* is one-celled and multinucleate, and when the sporangiophores are to be formed, the protoplasm collects at certain points in the mycelium, producing barrel-shaped swellings. These swollen portions are cut off by both peripheral and proximal walls. The division of the protoplasm is accomplished by simple constriction-furrows, and in the bulb so formed, as noted above, a rapid multiplication of nuclei takes place². This dividing period probably corresponds to the dividing period in *Synchitrium*, whereby the one nucleated cell becomes multinucleated. The protoplasm of *Pilobolus* is already multinucleate, but preparatory to reproduction these vegetative nuclei multiply rapidly, apparently simply as a preparation, for the production of a sufficient number of spores. The protoplasm in the bulbs becomes much more densely packed with nuclei than that in the adjacent vegetative portions of the mycelium. This period of division is completed before the budding out of the sporangiophores. I have never observed nuclear division going on in the growing tip of the sporangiophore nor in the young sporangium before the cutting off of the columella. The growth of the sporangiophore is apparently largely accomplished by the flowing out of the material stored up in the bulb. As the sporangium becomes larger the bulb is more and more emptied of its protoplasmic contents, though a layer sufficient for maintaining the turgor of the central vacuole is always maintained, and in this layer also numerous nuclei are present. The sporangiophore grows in length, and is bright yellow throughout its upper portion, due to the presence of yellowish, perhaps fatty, granules in the protoplasm. At first it is slightly narrowed at the tip, but after

¹ "Die Entwicklungsgeschichte des *Pilobolus crystallinus*." Nova Acta Acad. Caes. Leop. Carol. Nat. Cur., vol. xviii, pt. 1.

² A detailed description of these and some other nuclear divisions in the fungi I expect to give in the near future in another connexion.

reaching a height of one to several millimetres the tip becomes rounded and swells rapidly to form the young sporangium (Fig. 9). No especial aggregation of the nuclei at the apex is to be noted at any time during this growth. They are rather evenly distributed throughout the sporangiophore-tube, and are present also in the hyaline plasma at the very tip. The growth is largely apical, rather than by stretching and deposition of new layers of cellulose on the inner surface of the stalk already formed. Well-fixed specimens show the paths of the streaming protoplasm in the sporangiophore very distinctly; each streaming thread is seen to have marked a path for itself through the protoplasmic structure. It is bounded by continuous delicate films, quite distinct from the spongy structure of the adjacent plasma. Whether these streams flow upward only cannot be determined from fixed material. That these delicate threads are really the paths of streaming plasma-currents is evident from the further fact that after the sporangium is full grown and the columella has been formed, all trace of the threads disappears from the protoplasm of the sporangiophore. The paths of the protoplasmic streams in the young sporangium are very well shown in sections such as are given in Figs. 10, 11.

As the currents passing up in the stalk reach the enlargement of the sporangium the streams seem to widen and thicken, indicating a checked velocity in the suddenly enlarged channel. This gives us the thickened ridge about the upper edge of the stalk shown in Fig. 2. From the upper edge of this ridge the currents spread into what in section is seen to be a fan-shaped series, distributing themselves to the surface of the sporangium from its apex through about 90° , thus building up the thickened layer which is to constitute the spore-plasma. In the lower half of the sporangium there is indicated a slight backward flow toward the mouth of the sporangiophore, thus filling the space gradually around the ridge which is built up around the mouth of the sporangiophore. The whole process of the growth of the sporangiophore and the inflow of protoplasm into the young sporangium

is seen to be analogous to the process in the growth of a pseudopod on an *Amoeba*, and the same distinction of central outflowing currents and slight peripheral backward currents can be observed. Noll has already pointed out that the growth of a coenocytic alga like *Caulerpa* is to be compared to the creeping of an *Amoeba*, and the comparison is equally striking here.

The central portion of the young sporangium is largely filled with cell-sap, only a few strands of protoplasm cutting through it. Outside this central area the protoplasm is spongy and thready, becoming denser toward the periphery. The outer layer is dense and quite homogeneous from the start, and is slightly thinner at the apex of the sporangium. The appearances in these young sporangia are suggestive with reference to the question as to the gross structure of protoplasm. In Figure 11 for example the plasma is not vacuolated in the ordinary sense, though an immense amount of cell-sap is present. This sap is not contained in more or less rounded cavities like the vacuoles as commonly described. The protoplasm forms rather a spongy framework in whose meshes is the cell-sap. The whole mass of sap occupies the interstices of the plasma-net, and is not divided off in isolated vacuoles. The sap is a *continuum*, in which the plasma-strands float as a net or framework. Later, after the upward flow is complete, the plasma passes into a truly vacuolated condition, when the cell-sap is contained in rounded cavities whose outlines are determined by surface tension. A similar transition from a foamy vacuolated structure to a spongy structure will be described later in the account of the germination of the spores. The whole sporangium continues to grow in size for a considerable period (5 p.m. until about midnight), and its wall in the apical region becomes thickened and dark coloured. The layer of spore-plasma becomes thicker by the increase in density of the spongy plasma on its inner surface. The circular ridge becomes higher, and to the outside, between it and the spore plasma, an open spongy mass is maintained which already indicates the outline of the

columella wall. The radiating strands from the upper edge of the ridge are shorter as the distance from the edge of the ridge to the dense spore-plasma is less. By midnight the layer of spore-plasma reaches its definitive thickness. The dome-shaped columella cavity is still a large cell-sap cavity, and the inner surface of the dense spore-plasma passes gradually into this by means of a spongy layer which is rapidly becoming typically vacuolar as the streaming ceases. In the outer portion we can now roughly distinguish three parts in the protoplasm of the sporangium; the outer dense spore-plasma, passing into a spongy layer on its inner surface, and the central cavity of the columella. In the outer part of the spongy layer the split between the spore-plasma and the columella-plasma is to be formed. The first indication of this cleavage is seen in the gradual appearance of a layer of vacuoles larger than the rest, and lying in the curved surface which marks the outline of the columella. The vacuoles become flattened in their radial axes parallel to the surface of the sporangium, and form thus disk-like openings which tend to fuse at their edges. At the same time a circular cleft is seen to start from the edge of the sporangiophore opening just outside the protoplasmic ridge above noted, and to develop upward, cutting into the vacuoles so that they become connected into a continuous furrow (Fig. 12). Whether this furrow is continued upward to enclose the whole dome-shaped columella, or whether the vacuoles in the upper portion fuse edge to edge before the cleft reaches them, is difficult to determine. The process is a progressive one, the cleavage being complete in certain portions sooner than in others, and at a very late period strands of protoplasm are seen connecting the spore-plasma with that in the columella. It is not impossible that many of the apparently disk-shaped vacuoles are sections of curved openings which burrow through the plasma from below upwards. Frequently vacuoles which are distinct in one plane are seen, by focusing up or down, to be connected. There can be little doubt however that a considerable part of the cleavage of the columella is accomplished

by flattening and lateral fusion of originally ellipsoidal or spherical vacuoles, that is, the cleavage is not entirely by a furrow from the plasma-membrane at the mouth of the sporangiophore, but is at least in part a process of separation by excretion of liquid into vacuoles and their fusion side by side *in situ*. These vacuoles are not situated on the extreme boundary of the protoplasm adjacent to the large central vacuole, but are placed where the dense spore-plasma first becomes characteristically spongy. At the base of the sporangium indeed they cut through plasma as dense as the densest spore-plasma of the sporangium. Why the cell-wall of the columella could not be deposited on the surface of the central vacuole as well as on the surface of the small vacuoles, and thus enclose all the protoplasm in the sporangium, is an interesting question. The necessity is evident that the cleavage should proceed through a tolerably dense plasma, and this is perhaps due to the need of two protoplasmic surfaces in contact, in order to form a cell-wall. It would seem otherwise quite natural that the spore-plasma should simply bound itself off by a wall against the central vacuole of the columella, in which case only the plasma at the mouth of the sporangiophore would need to be cut through, and the cleavage process would be apparently simplified.

The cleft separating the spore-plasma from that of the columella is quite wide and irregular at first. Later, the turgor of the two separated plasmic masses causes them to be firmly pressed together. This whole process of the delimitation of the spore-plasma is accomplished without the formation of a cell-wall, and consists simply in the establishment of new protoplasmic membranes. The cell-wall is deposited later, in the cleft between the two membranes.

With the cutting off of the columella, the so-called collar begins to form on the basal wall of the sporangium, just outside the mouth of the sporangiophore. It appears first as a thin layer of homogeneous material which, with Flemming's triple stain, becomes faintly blue in colour (Figs. 14 and 15). After the columella-wall is laid down it increases rapidly in

thickness and finally extends up the base of the columella. It is thickest at the angle which the columella makes with the sporangial wall and thins out toward the edges in both directions. The substance of which it is composed is the same in appearance and staining reactions with the intersporal substance (Fig. 24) which appears later, and which Brefeld has attempted to homologize with the epiplasm of an ascus. It is plainly to be seen here that this collar-substance is not protoplasmic, and is an excretion deposited outside the plasma-membrane bounding the spore-plasma. It is an excreted material, and designed for a special function in distributing the sporangia as Brefeld has pointed out; but its perfectly homogeneous structure and its position outside the plasma-membrane of the spore-plasma prior to spore-formation show that it is in no way comparable to the protoplasm left behind in the ascus in the process of free cell-formation as it takes place there.

It is to be noted that the columella-wall has the same dome-shaped outline when it is first formed as it has later, when the spores are ripe. It is not at first a flat transverse septum which is later pushed up into a dome as the spores ripen. Cleavage of the spore-plasma begins very shortly after the columella is complete. The protoplasm becomes somewhat vacuolar, and the nuclei are rather evenly distributed through its mass. Cleavage-furrows then appear around the base of the sporangium, cutting the surface of the protoplasm into irregular polygonal areas (Fig. 14). At the same time in the interior of the protoplasm, where hitherto only rounded or spherical vacuoles have been found, angular vacuoles, frequently three cornered in optical section, appear, and their edges cut outward through the protoplasm to meet similar cleavage-furrows from adjacent vacuoles (Fig. 14). The surface-furrows have also been growing deeper, and meet and become continuous with the edges of the vacuoles. The surfaces of the vacuoles from being convex outwardly have become concave from the pressure of the adjacent plasma-masses, and the latter appear as intercellular spaces between the proto-

plasmic cleavage-segments. The spore-plasma is thus roughly marked out into irregular blocks apparently without reference to their size or the number of nuclei they contain (Figs. 14-15). Further progress of this furrowing cuts the first-formed blocks into oblong rounded sausage-shaped masses generally containing two to four nuclei in a row (Fig. 16). These oblong bodies are now divided transversely to form rounded or spherical masses each with one or few nuclei (Fig. 17). With this the primary cleavage phenomena are complete. The multinucleated spore-plasma has been cut up into units with one or few nuclei, corresponding to the protospores produced by the cleavage in the sporangium of *Synchitrium*.

But these are by no means the definitive spores of *Pilobolus*. A further period of growth and cleavage is to be passed through before the spores with protective walls are formed ready for dissemination. These uninucleated masses are perhaps morphologically the equivalents of the *Saprolegnia* swarm-spores, but they have no cell-walls, and are but a transitory developmental stage in the *Pilobolus* sporangium. The succeeding periods are, however, at once distinguished from the cleavage stages so far described. The protospores enter on a period of growth and division, and since this growth occurs in connexion with the parent tissue and nourished by it, it may be fairly regarded as an embryonic stage. The first step in the further development of the protospores is a period of nuclear division, wherein the nuclei divide rapidly, so that the masses become once more multinucleated (Fig. 18-19). After the nuclei have thus divided the cell divides by constriction, the nuclei being separated into two groups in the halves so formed. The nuclei may then divide still further, their divisions being followed by further cell-divisions, but finally nuclear division ceases. Cell-division, however, continues until the masses are cut up into regularly oblong binucleate cells. A series of these final cell-divisions, whereby an octinucleate mass is cut up into binucleate spores, is shown in figures 20 to 23. After each cell-division the nuclei are separated into two equal groups in opposite halves of the

cell (Figs. 20-21). The equatorial plane between the groups becomes more hyaline, and constriction of the plasma-membrane gradually cuts the cell in two (Fig. 22). The primary cleavage is complete about four o'clock, and the period of embryonic growth and division lasts until about seven a.m. The binucleated spores are at first naked masses of protoplasm, but soon are covered by a wall. Their protoplasm passes into a resting vacuolar condition, and reserve food products appear as drops of oil. Frequently a large oil drop occupies the centre of the cell, and the nuclei lie at its ends. The spores in this ripened condition are difficult to stain, their walls being extremely resistant to fixing and staining reagents. The best figures were obtained by killing in Flemming's solution, and staining with methylene blue. By this method very sharply defined nuclear figures are brought out in different shades of blue. The nuclear sap is colourless, the nucleolus deep blue, and the chromatin lighter blue. The spores are killed, fixed to the slide with albumen-fixative, and exposed about five minutes to one-tenth of one per cent. methylene blue solution. Spores that have been imbedded and sectioned on the microtome are not as favourable for the above staining as those freshly killed and fixed without dehydration and imbedding in paraffine. After staining, the preparations are dehydrated, cleared in clove-oil, and enclosed in balsam. The ripe spores lie imbedded in a shining mass of intersporal substance which can be stained readily with gentian violet. It is similar in all respects to the material in the so-called collar, and is presumably an excretion of the protoplasm during the ripening of the spores. As was noted above, the method of spore-formation as it occurs here, by cleavage of the entire protoplasmic mass from the surface inward, precludes the possibility of any comparison between this intersporal substance and the epiplasm of the ascus.

Before passing to the germination of the spores we may note the second enlargement of the sporangiophore, just below the sporangium, which is to become the explosive vesicle for firing off the ripened sporangium. It appears first

late in the afternoon, and continues gradually to enlarge during the night (Fig. 10). It is lined by a protoplasmic layer no thicker nor more numerously supplied with nuclei than the remainder of the sporangium. The cell-wall of the narrow neck between this vesicle and the sporangium is very thin, indicating the line of fracture when the vesicle finally bursts. The central vacuole of the vesicle contains only cell-sap, the protoplasmic primordial utricle forming only a lining layer just inside the wall. The throwing off of the entire sporangia during the morning hours by the swelling of the collar and bursting of the vesicle beneath, and the wonderful heliotropic reactions they show in the direction of the discharge, have been fully described by Brefeld. During ripening the sporangial wall has become very much thickened and brittle over the top of the sporangium. It thins out rapidly, however, and is light-coloured beneath the collar, and offers less resistance to the swelling of the latter at the time of the explosion.

The germination of the spores in a decoction of horse-dung can be readily studied in all its phases by fixing in Flemming's solution, fastening them to the slide with albumen-fixative, and staining with Flemming's triple stain or Mayer's 'Carmalaun.' The spores swell tremendously before pushing out a germ-tube, and the two nuclei divide to form eight or more. 'Carmalaun' gives the best results for staining the swelling stages, but Flemming's stain serves better after the germ-tube is formed. Figs. 25, 26, and 27 show these germinating stages. The nuclei may be readily demonstrated at these stages, but the plasma is too dense and the wall too impenetrable to allow a full study of their division. The development of the living spores can be well watched in their earlier stages under an immersion lens, and Figs. 28 to 29 show the appearance of spores of an undetermined species of *Pilobolus* during germination in water. As soon as the spores swell the nuclei can be made out without staining. They undergo considerable amoeboid changes of form, and seem even to change position in the spore by this means. In this case

nuclear division does not precede the pushing out of the germ tube as it does in *P. crystallinus*. The gross structure of the plasma is at first typically vacuolar. The vacuoles are spherical or flattened against each other, and are entirely distinct, each maintaining its outline apparently by surface tension of the contained liquid. So soon, however, as the germ-tube is pushed out from one side the whole structure is transformed, the vacuoles become elongated and fuse, the protoplasm is gathered into strands, forming a loose spongy net in which streaming is begun at once, and continues as long as the cells can be kept under observation without suffering from lack of oxygen. This change of the structure of the protoplasm can be followed with the greatest ease and accuracy, and is entirely similar but in reverse sequence to what takes place in the sporangium just before the columella is formed. It is apparently a necessary change if streaming is to take place in the germinating spore, and is doubtless a result of the same influences that lead to those phenomena.

Figs. 30-32 show the appearance of living spores under the 2 mm. apochromatic lens when germinating in a decoction of horse-dung. The change from vacuolar to thready structure is shown just beginning in Fig. 32. The thicker germ-tubes produced when the spores are germinated in a nutrient solution instead of water have already been noted by Brefeld.

The processes of cutting off the sporangium and dividing the multinucleated spore-plasma into spores may be summarized as follows:—

1. Arrangement of a series of vacuoles in a dome-shaped, curved stratum on the inner boundary of the dense spore-plasma.
2. Flattening of these vacuoles and the formation of a cleft through them, beginning at the edge of the opening into the sporangiophore.
3. Smoothing of the new plasma-membranes thus formed, by turgor of spore-plasma and columella-plasma.
4. Formation of a columella-wall in the cleft between the new plasma-membranes and vacuolization of the spore-plasma.

5. Formation of surface-furrows which deepen and meet the vacuoles, and thus cut the plasma into irregular and finally oblong sausage-shaped multinucleated bodies, which are further divided to form one or few nucleated energides, the protospores.

6. Nuclear division followed by cell-division, constituting embryonic development of protospores into mature binucleated spores.

SPORODINIA.

Sporodinia is a further type of the Zygomycetes, in which the asexual reproductive apparatus is considerably modified. The fungus occurs in nature as a parasite on various Hymenomycetes, especially *Boleti*, and, as described by Brefeld, is very easily grown on bread cultures. It is a very favourable object for the study of spore-formation in the sporangia. The whole development and ripening of the spores is here passed through in a very brief space of time, one hour from the first swelling of the end of the sporangiophore sufficing for the development of the ripened spores. The spores are thin-walled, variable in size, short-lived, and by no means so resistant to unfavourable environmental conditions as those of *Mucor* and *Pilobolus*. They retain their capacity for germination only a week or two. Zygosporangia are produced much more abundantly by this fungus than by other members of the group, and serve the purpose of maintaining the existence of the fungus through a period of unfavourable conditions.

The sporangiophores are here dichotomously branched, and with the ripening of the spores become regularly septate.

The ends of the sporangiophore-branches swell to the form of flattened spheroids, whose transverse diameter is only about twice that of the extremely thick sporangiophores. The spore-plasma becomes aggregated in the form of a cap-shaped mass, thinning at its edges and lining the upper two-thirds of the spheroid. In sections the spore-plasma shows a regularly crescentic outline. The central and lower parts of the

sporangium are filled with a characteristically foamy protoplasm. Between this foamy plasma and the spore-plasma there is at first a very gradual transition (Fig. 33). Later, the difference in density is more sharply defined, the transition being sudden, on the lower surface of the dense spore-plasma, to a very open foamy structure. Just along this line of changed structure a series of larger vacuoles become arranged which gradually flatten in a curve, outlining the surface of the columella (Figs. 34, 35). It is perfectly evident that these are ordinary vacuoles which have grown larger and begun to flatten. At first it is not easy, in many cases, to say whether a particular vacuole is to take part in the formation of the series or not. As they grow larger they mark very sharply the boundary of the dense plasma. One wall of any one of these vacuoles will be dense plasma, while the opposite wall abuts on a number of smaller vacuoles. The nuclei¹ are somewhat more numerous in the spore-plasma, but not strikingly so. The mechanism of the flattening of these vacuoles is not easy to understand, except in so far as the denser resistant spore-plasma might be supposed to resist the action of surface-tension of the cell-sap on the side adjacent to it.

These vacuoles become more and more flattened and tend to fuse side by side (Fig. 35). Especially large vacuoles are to be seen at the edges of the crescent-shaped section of the spore-plasma. These come to lie closer and closer to the cell-wall at that point, and finally break through the plasma-membrane, thus bringing the cell-sap in direct contact with the sporangial wall through which it doubtless filters out and is evaporated. I have detected no cleavage-furrows cutting in to meet these vacuoles at the boundary of the sporangium, as was the case in *Pilobolus*. The remaining vacuoles in the series now fuse, thus separating spore-plasma and columella-plasma by a wide irregular cleft filled with cell-sap (Fig. 36). The turgor of

¹ In the figures given of *Sporodinia* the magnification is not sufficient to show the structure of the very minute nuclei clearly, and hence I have represented them merely as dots.

the two protoplasmic masses very soon brings the two membranes together and the contents of the sporangium are thus cut off from the sporangiophore by the new plasma-membranes. Between these a cell-wall is soon laid down. Cleavage of the spore-plasma begins at once by surface-furrows and clefts, which are rather wide and without the aid of vacuoles cut from below upward (Fig. 36) and, later, from both the upper and lower surface of the sporangium, thus dividing its contents into irregular polygonal blocks. There are two or three layers of these blocks in the apical thicker portion of a well-developed sporangium. At the edges of the spore-mass it is cut into only a single row of blocks (Fig. 37). These masses are very variable in size, and each contains a large but variable number of nuclei. They correspond morphologically to the blocks into which the spore-plasma of *Pilobolus* is first divided by the surface-furrows and vacuoles. They generally, however, contain many more nuclei than do the latter. With this the process of cleavage is complete in *Sporodinia*. A thin wall is built about each of the cleavage-masses which becomes at once a spore. The sporangial-wall is never thickened as in *Mucor* and *Pilobolus*, and bursts immediately for the escape of the spores. I have not detected by stains any intersporal substance, though the spores tend to adhere slightly when the sporangium is broken on a microscope slide. In nutrient liquids the spores round themselves up and put out a germ-tube very soon without any noticeable swelling. They contain from ten to fifty or so nuclei.

Sporodinia shows thus a very interesting abbreviation of the process of spore-formation as compared with *Pilobolus* or *Synchytrium decipiens*. It is seen, however, that the process in *Sporodinia* is related to that in *P. crystallinus* very much as the cleavage in *Synchytrium decipiens* is related to that in *S. Taraxaci*, except that in the latter the spore germinates as a sporangium. In *Sporodinia* and in *Synchytrium Taraxaci* the process stops with what is the initial stage of the process in the other two forms, and

it is plain that the ripe spores in the two types are not exactly homologous structures. Such variations in closely related forms are rather surprising, but in each case the abbreviated cleavage process is evidently an adaptation to meet the demand for a more rapid method of multiplication in the species in which it appears. Investigation will doubtless lead to the discovery of still further modifications of the process. It may be noted that the absence of winter spores in *Synchytrium decipiens*, as compared with *S. Taraxaci* where they are present, may be correlated with the longer process of cleavage and embryonic development in *S. decipiens*. It is hardly worth while to propose changes in nomenclature to distinguish the different forms of spores already described until a larger number of forms have been thoroughly studied. It is plain, however, that the term sporangiospore, as used by Léger, includes several structures quite distinct as to the method of their formation. The use of the term protospore for the ultimate product of cleavage in such forms as *Synchytrium decipiens*, *Pilobolus* and probably *Saprolegnia*, is certainly conducive to clearness. The ripened product of the sporangium, whether the cleavage process is more or less complete, and whether embryonic development is present or absent, may still be loosely designated as a spore or sporangiospore.

Cell-division also occurs in *Sporodinia* in the cutting off of the gamete-cells from the suspensors in sexual reproduction. This is an interesting case, since the mass of protoplasm to be cut in two is nearly as great in diameter as in the sporangium when the columella-wall is formed. There, as we have seen, the cleavage is accomplished by a row of vacuoles that flatten and fuse edge to edge. Here in the gamete-formation, however, the cleavage is accomplished entirely by a circular furrow proceeding from the surface to the centre, as in the cutting off of the bulb in *Pilobolus*. The nuclei are extremely numerous and are distributed in the protoplasm without any apparent relation to the plane of cleavage. The ingrowth is simply a deep narrow furrow and not the growth inward of a ring of fungus-cellulose. The cellulose-wall is formed

later. The process is exactly like that described by myself for the cutting off of the conidia in *Erysiphe*.

It is interesting that two such modifications in the method of cell-division should occur in the same plant. It is possibly evidence against the homology of the sporangium and gamete that the two are cut off so differently. It may be that the two methods are adapted to the difference in the form of the cell-wall to be developed in the two cases. Division by vacuoles may be better adapted to the formation of a dome-shaped cross-wall, while constriction suffices to form a plane cross-wall.

LACHNEA SCUTELLATA.

I have already described the process of spore-formation in the ascus as represented in *Erysiphe communis*, and have given some figures indicating that the process in *Ascobolus* and *Peziza* is essentially the same as in *Erysiphe*¹. The process is so unique in the method of cutting out the spore from the protoplasm of the ascus that I have continued the investigation of it in a number of other forms. *Lachnea scutellata* has proved especially favourable, from its abundance and the relatively large size of its nuclei, for investigating the phenomena in the ascus. And since, as I have already noted, the doctrine is widely accepted that the ascus is a more highly developed and specialized member of the same group of endogenous spore-producing cells as those I have been describing, it will be worth while to describe the process here once more as a basis for comparison, as well as to note minor differences from what has been already described for the asci of the above-mentioned forms.

L. scutellata is a very common cup-fungus growing on decaying wood in moist, shady places. The whole plant is bright red and the disk is bordered with long, stiff septate and pointed bristles. The material fixed in Flemming's solution is clear and transparent, neither so dense nor so

¹ Jahrbücher für wiss. Bot., Bd. xxx, p. 249.

much blackened as is the case with many Discomycetes. I will first describe some of the stages in the nuclear divisions in the ascus which I had failed to find or had been less successful in fixing and staining in the forms of cup-fungi already studied. In my earlier paper, for the sake of showing details on a larger scale, only the nuclei and the protoplasm immediately adjacent to them were figured. In the present case I have figured in each case the entire upper part of the ascus (from one-half to one-third) so as to show the appearance of the entire spore-bearing portion as a whole.

In the almost fully developed ascus of *Lachnea* there is a differentiation of the protoplasmic contents such as was described in part by De Bary and Strasburger. The lower two-thirds is filled by a very foamy vacuolated protoplasm which is convexly rounded off at its upper end against a denser granular plasma which fills most of the remainder of the ascus. The very apex of the ascus is filled by a cap of fine granular protoplasm, between which and the dense granular spore-plasma there is an oval foamy mass like that found in the lower portion of the ascus. Through the centre of this upper foamy area a vertical dense strand of protoplasm extends, at certain stages of development, from the under surface of the granular cap tapering downward to the upper surface of the spore-plasma. It is regularly present in the uninucleate stage of the fully developed ascus (Fig. 38), and again after the spores are fully delimited, and suggests the possibility of a fountain-like streaming of the protoplasm in the upper end of the ascus analogous to that described by Klebs¹ for the cells of some higher plants.

In my figures of the late diaster stages of the first division in *Peziza Stevensoniana* I was unable to make out a system of polar rays. In *Lachnea scutellata*, however, the asters attain a very noticeable development in this stage (Fig. 38). The rays from the upper nucleus extend almost to the apex of the ascus, and those from the lower one stretch far down into

¹ Form und Wesen der pflanzlichen Protoplasmabewegung, Biol. Centralblatt, Bd. i.

the spongy plasma, beyond the dense special spore-plasma. They are straight, unbranched and fine through their whole length. Some are much longer than others, and these longer rays, as they pass between the vacuoles of the spongy plasma, modify the shape of the latter noticeably. The vacuoles are pinched into a slightly ovoidal shape with flattened sides, the narrowed end being directed toward the centre of the aster, indicating that the system of rays is of some rigidity as compared with the other structures in the protoplasm. This deformation of the vacuoles is especially apparent where the rays pass from the denser spore-plasma into the foamy protoplasm below it. The appearance is given here as if the dense plasma was carried out around the rays as a rapidly narrowing sheath for each, suggesting that the rays have pushed forward from the dense plasma into the foamy plasma as the daughter nuclei separated, and that the dense protoplasm adhered to them and was carried along a certain distance. The connecting fibres, the old nucleoli, and the dense chromatin-masses of the daughter nuclei appear here essentially as in *Peziza Stevensoniana*. Abundant fine red-staining granules are scattered throughout the protoplasm, analogous to the so-called extra-nuclear nucleoli of cells in the higher plants.

In the earlier stages of division of the ascus-nucleus of *Lachnea* I have found nothing to add to my previous accounts. Also the reconstruction of the daughter nuclei proceeds in *Lachnea* essentially as in *Peziza Stevensoniana*, except that the polar rays continue for some time after the diaster stage, and there is no appearance of a disk-like central body on the equatorial side of the daughter nucleus. Figures 37, 38, 39 show stages in the nuclear divisions that follow and give the appearance and distribution of the protoplasm in the upper third of the ascus during these stages.

Figure 39 shows the equatorial plate stage of the division of the first two daughter nuclei. It is seen here, as is frequently the case in *Lachnea*, that the long axis of the spindle does not lie in the long axis of the ascus, though it quite regularly takes that position in *Peziza Stevensoniana*. Fig. 40 shows a diaster

stage of the same division. Here also the old connecting fibres do not lie in the long axis of the spindle. The diasters here duplicate in all particulars the diaster of the first division, but on a smaller scale. In this and the following figures the tip of the ascus is bent in such fashion that the section is not median, and the apical cap of dense protoplasm shown in Fig. 38 does not appear. Fig. 41 shows three of the spindles of the third division lying transversely to the axis of the ascus and the fourth at some distance off and parallel to the ascus-wall. This displacement of the fourth nucleus at one end or the other of the series was observed several times, and indicates the independence of the individual nuclei of each other and of the general proportions of symmetry in the ascus. The ends of all these spindles in the equatorial plate stage are decidedly broad and blunt, and the central body in which they end is flat and disk-shaped, as in *Peziza Stevensoniana* and *Ascobolus*. It stains more densely than the rays or spindle fibres, but there is no indication that it is more than a denser mass of kink-plasm formed by the meeting of the spindle and ray fibres. Figure 42 shows five daughter nuclei of the octinucleate stage of development in the ascus. They are in a late (anaphase) stage. The chromatin is still aggregated near the central body. The nucleolus has not yet appeared. The vesicular nuclear membrane encloses a clear space into which at a later stage the chromatin will be distributed. The remaining three nuclei appear in the next section of the ascus, which is here split lengthwise. It is seen that the systems of rays which formed the polar asters in the last divisions are still present. No indication of a beak-like elongation is to be noted. The central body lies close on the membrane of the spherical nucleus. The nuclei in almost every case lie quite near the wall of the ascus, though this is not shown in the figure, since the figures have been somewhat displaced vertically in drawing in order to bring them all into one plane. The aster in general lies toward the plasma-membrane, the rays here quite generally ending in the latter. The whole effect is somewhat as if the nuclei were hung up on the plasma-membrane by the

aster-rays. I am inclined to think this may have significance as indicating a resemblance in constitution between rays and membrane. It might be taken to indicate a connexion of the rays with the cell-surface such as is assumed, by Heidenhain and others, to exist between the astral rays and cell-boundary in the animal cell. It might be further assumed then that the rays give an anchorage for the spindle poles as claimed by Van Beneden and others, and that the daughter nuclei are drawn up to the walls in the position indicated above by the contraction of these rays during the early anaphases. However, such a connexion of the rays to the membrane is plainly lacking in the first and second divisions and in the divisions in the ascus of *Erysiphe*, as I have pointed out in my earlier paper. The spore-plasma at this stage again shows rather sharp boundaries against the foamy plasma above and below it, a condition which is obscured during the second and third divisions by the appearance of a considerable number of vacuoles in it. These vacuoles perhaps represent the nuclear sap set free by the disappearance of the nuclear membranes during division. Ultimately, however, this sap is either absorbed or extruded into the foamy epiplasm, and at the period just before the beginning of the delimitation of the spores, the spore-plasma is quite dense and homogeneous. There is no indication at this stage of a tendency to aggregate in denser layers about the nucleus. Such aggregation as was described in my former paper¹ represents in reality a later stage after the plasma-membrane is established, although I was not able to demonstrate the membrane at this stage from the preparations I then had. In *Lachnea* it is perfectly clear that the spore-boundaries are in no way outlined at the stage shown in Fig. 43. There is nothing to indicate that the nuclei are centres of any particular limited areas of cytoplasm about them. The further reconstruction of the daughter nuclei proceeds in the fashion described for *Erysiphe*. In Fig. 43 we have a stage in which the chromatin has been distributed through the nucleus and a nucleolus has appeared. The

¹ Ber. d. D. Bot. Gesellsch., Bd. xiii, p. 67, 1895.

chromatin lies for the most part on the nuclear membrane and is still in lumps and masses rather than threads or granules. A beak-like prolongation of the nuclear membrane beneath the central body and aster has appeared, and the nucleus has come to lie a little further from the plasma-membrane of the ascus and may be inclined at almost any angle to it. It will be readily seen that the nuclei being distributed on the inner walls of the cylindrical ascus, with the axis of the nuclear figure placed in general radially to the plasma-membrane, the beaks and asters will appear in polar view, in the case of such nuclei as have the beak-like prolongation extending vertically upward or downward from the plane of the section. Of the six nuclei appearing in this figure this was the case with the second and third nuclei from below, which extended almost vertically downward from the upper surface of the ascus, and their centres and asters do not appear in the figure. In this section some irregular openings appear in the spore-plasma and frequently red-staining granules are associated with them, lying on their borders but never free in their interior. In such cases the appearance suggests that the openings are due to breakage of the protoplasmic framework, whose elements have then shrunk together in the form of dense granules on the margins of the rents. This suggests that these red-staining extranuclear nucleoli may be formed artificially by partial breaking down of the protoplasmic structure in fixation. Whether this is a general explanation of this very common appearance in the cells of higher plants, I have not been able to satisfy myself. The process of metamorphosis of the aster in cutting out the spore is essentially the same as in *Erysiphe*. The rays revolve on the central body, and the cone-shaped opening, triangular in optical section, which indicates the first motions of the aster-rays is well shown in the case of the lower nucleus in Fig. 43, and in the upper nucleus in Fig. 44. The asters being in contact with the plasma-membrane, the latter seems to be pulled in as the fibres fold over, and thus a depression is produced in the surface of the protoplasm of the ascus. At first the central

body is very close to the plasma-membrane, but as the process of folding over of the rays continues, the whole figure migrates somewhat toward the centre of the ascus, as is seen by comparing the upper two nuclei in Fig. 44. An examination of Figs. 44 and 45 shows that the lower nuclei of the series tend to be a little in advance of the upper nuclei in their development, though this is by no means a general rule. Fig. 44 shows stages in the cutting out of the spores from the early stages in the displacement of the aster-rays in (*a*) to the completion of the plasma-membrane (*f*). In *d* and *e* the enclosure of the spore is not quite complete, and it is seen that the last part to be enclosed need not necessarily be antipodal to the central body. The shape of the mass first enclosed is also seen to be quite variable. In Fig. 44 (*c*) the nuclear figure is seen to be at the side rather than at the end of an ellipsoidal spore-mass. Of the two spores (*e* and *f*) the latter lies somewhat higher in the section and overlaps *e*, though they are drawn as if lying in the same plane. Irregularities of shape and in the position of the nucleus in the forming spore are much more numerous in *Lachnea* than in *Erysiphe*, and are due no doubt to greater crowding in the formation of the eight spores before they become evenly distributed in the elongated, relatively narrow ascus, while in the broad oval asci of *Erysiphe* the two to three spores are more evenly distributed from the first. These irregularities, however, make still more clear the relatively independent activity of the aster-rays, for, however the nucleus may be placed, they succeed in enclosing in every instance practically equivalent volumes of the spore-plasma, as is seen by comparing the size of the spores after they are completely outlined. The orientation of the nuclear figures, though tending in earlier stages of spore-formation to be radial to the surface of the ascus, is extremely varied, as seen in Fig. 46. This figure shows also that, though the nuclei are in general rather evenly distributed in the spore-plasma, the process can be carried out just as perfectly when several nuclei are in close proximity to each other, as are the three, *b*, *c*, *d*, in this section. In some

cases the young spore is decidedly lobed as in *b*, Fig. 45. *d*, in the same section, shows the plasma-membrane of the young spore indented, as a result of some disturbance in fixation which also produced the vacuole-like space lying opposite the indentation. In Fig. 47 we have a section showing seven of the eight ascospores completely enclosed in plasma-membranes isolating them from the adjacent spore-plasm. They have come to lie now in a more nearly median row in the ascus, though *a* and *b* are still overlapping, and the eighth at the lower end of the series was almost wholly in the next section and was hence omitted from the drawing. The astral rays have disappeared, though in *a*, *b*, *c*, and *f*, the beak and central body have not yet been drawn back to the nucleus. The nuclei have increased considerably in diameter and the chromatin forms a regularly distributed network. The spores are flattened upon each other and maintain their individuality and rounded outlines, indicating the existence of surface tension in their plasma-membranes. No cell-wall is present as yet.

As I have pointed out in my former paper the existence of surface tension in the spores at this stage is most conclusive evidence for the view that the plasma-membrane is a specially differentiated layer. As we have seen, the protoplasm of the ascus is entirely homogeneous before the spores are cut out by the metamorphosis of the aster, and to prevent the plasma in the spore from mixing and becoming continuous with the epiplasm outside, the assumption of the differentiation of a membrane-layer is essential. It is not necessarily implied that this differentiation is chemical; it may consist perhaps in an increased density and absence of coarser granules. I am inclined however to consider a very decided differentiation as necessary, since it can hardly be seen how a merely denser, more homogeneous colloidal layer could fail to lose its identity and diffuse into a chemically similar compound on both of its surfaces. If it be assumed, as claimed by Rhumbler for *Amoeba*, that the ectosarc is formed from the endosarc as a result of contact with water, and that in the ascospore, as

a result of metabolic changes beginning at the nucleus, the spore-plasma becomes chemically altered and different from the adjoining epiplasm as soon as it is cut off by the fused rays, we might imagine that the interaction of the thus differentiated spore-plasma and epiplasm could be maintained and a bounding layer produced, as it is supposed to be formed by the action of water on the surface of the *Amoeba*. That such differences could be so soon produced, and that the epiplasm could produce on the spore-plasma the same effect which so foreign a substance as water produces on the surface of an *Amoeba*, seems difficult to imagine. Bütschli's theory of a permanently differentiated outer layer (oily in its nature) fits much better with the phenomena here. The independence of motion of the rays through the ascus-plasma, comparable, as I have already pointed out, only to the vibratile activity of cilia, also seems to demand the assumption of a very considerable difference between their composition and that of the medium in which they move. In later stages the spores enlarge somewhat, and come to lie in a single row in the axis of the ascus. In Fig. 48, owing to a sharp bend in the upper end of the ascus, the spore-row seems to come nearer the apex of the ascus than is really the case. Spore-walls are still lacking at this stage. The spores are less flattened on each other, and are beginning to take on the elliptical outline of the fully ripened condition. Central bodies, such as are quite conspicuous on the spore-nuclei of *Erysiphe* at this stage, are hardly ever to be discovered in *Lachnea*.

In the above description I have not attempted to redescribe all the points in the process of spore-formation which are identical with those already described for *Erysiphe*. I have rather aimed merely at presenting those minor points in which the process varies in *Lachnea* from the condition in the other forms described.

The main point of difference between *Lachnea* and *Peziza Stevensoniana* and *Ascobolus furfuraceus* in this process is that in *Lachnea* the anaphases in the last division forming the

eight nuclei in the ascus proceed as far as the formation of a nuclear membrane and the appearance of the nucleolus, before the metamorphosis of the aster into the plasma-membrane of the spore is completed, whereas in the other forms the delimitation of the spore is accomplished while its nucleus is still in the densely aggregated stage with no nuclear membrane yet formed. In this *Lachnea* more nearly resembles *Erysiphe*. It will be seen, however, that *Lachnea* really occupies in this respect an intermediate position between the other two Discomycetes mentioned and *Erysiphe*, since in reality the daughter nuclei are not completely developed in *Lachnea* at the time when the spores are completely delimited, but increase in size later, as is seen by comparing Figs. 43 and 46. Such differences as this are doubtless adaptations to particular growth-conditions in the different genera. As was suggested before, the habit of producing successive series of asci in brief periods when external conditions are favourable may have led to the modification of the process in the Discomycetes as compared with *Erysiphe*.

SUMMARY.

If we compare now the methods of spore-formation in the ascus and in the sporangia studied, the differences in the two cases are at once apparent. In the ascus, as in the higher plants, the cutting out of the daughter cell from the mother cell is effected by the agency of the same fibrous kinoplasmic elements as were concerned in the division of the nucleus. In the higher plants the flat cell-plate is formed by the 'cone-principal' of the karyokinetic figure as named by Van Beneden, while in the ascus the daughter cell is cut out of the protoplasm of the mother cell by an ellipsoidal cell plate formed from the fibres of the antipodal cone. In this process the daughter cell is cut out of the interior of the protoplasm of the mother cell, so that it remains surrounded on all sides by the material of the mother cell. The daughter cells do not

contain all the protoplasm of the mother cell, a considerable mass remaining as the so-called epiplasm. This is typical free cell-formation, as I have pointed out before. In all the sporangia studied, the cleavage is from the surface of the protoplasm, or from the surface of vacuoles of the mother cell. The daughter cells are thus separated by cleavage-furrows, and the nature of the division from the surface inwards precludes the possibility of the formation of an epiplasm.

That cleavage-furrows may start either from the superficial surface of the mother cell or from the surface of vacuoles imbedded in its protoplasm, is evidence that the vacuolar membranes and the external plasma-membrane of the cell are closely related structures, as has already been maintained by De Vries and Pfeffer. In both ascus and sporangium the daughter cells first formed are naked bits of protoplasm bounded only by plasma-membranes and without cell-walls. In the sporangia studied, the cleavage does not form uninucleate cells at once, though this is probably the case in the sporangia of the Saprolegniaceae. In the large sporangia of *Synchitrium* and *Pilobolus* the cleavage is progressive, first dividing the mother cell into multinucleate masses, which are gradually split up into the uninucleate protospores. This progressive segmentation has no parallel in the asci, where from the start a single nucleus forms the centre for the formation of each daughter cell. This progressive cleavage is by no means a series of bipartitions of a multinucleate cell, such as was suggested by Büsgen's work on the sporangium of *Mucor*, but is entirely irregular, forming at the first segments of varied size which contain a varying number of nuclei. I know of no process which is analogous to it in this respect either in plant or animal cells. The growth of the protospores in *Synchitrium* and *Pilobolus* to form sporangia in the one case and by division the typically binucleated sporangiospores in the other, is perhaps analogous to the growth in certain asci whereby the typically one-celled uninucleate ascospore becomes several or many celled or in some cases multinucleated

while remaining one celled. My earlier studies of ascospores led me to the erroneous conclusion that the one-celled ascospore was regularly uninucleate. I have since found ascospores in which nuclear division had occurred without cell division, and such cases have also been pointed out by other investigators¹. These cases are interesting because of the possibility of a reduction of the number of chromosomes at this stage, and should be investigated further as to the phenomena of nuclear division.

That in *Synchytrium* the embryonic growth in the sporangium produces sporangia, while in *Pilobolus* and in all asci with multicellular spores the germination is by a germ-tube from each spore-cell, may be, as has been suggested for other similar cases, entirely a matter of adaptation in the one case to germination in water, and in the other to germination in air or solid media. The aim of the process is always to produce a larger number of reproductive bodies, and thus to use up advantageously any excess of nutritive materials accumulated in the sporangium or ascus. In the Exoasci an analogous embryonic growth takes the form of yeast-like budding, and the number of conidia produced is plainly dependent on the amount of nutriment available in the ascus.

A possible comparison might be made between the uninucleate stage of *Synchytrium* and the uninucleate stage of the ascus. Each is followed by a multinucleated stage and cleavage to form uninucleate spores, but the comparison is an artificial one. The divisions in *Synchytrium*, though they lead directly to spore-formation, are probably morphologically equivalent to the nuclear divisions that take place in the mycelium and bulb of *Pilobolus*. At any rate, some possible connecting types should be found to give the comparison any value.

As the mechanism which effects the division of the mother cell into daughter cells, we have in the ascus the kinoplasmic fibres, and in the sporangium constriction-furrows from the

¹ Dittrich, Zur Entwicklungsgeschichte der Helvellineen, Beiträge zur Biol. der Pflanze, 1899.

plasma-membrane of the surface or of vacuoles. This appears to be a very fundamental distinction. It may, however, be taken as indicating possibly a further resemblance between the plasma-membrane and the fibres of the karyokinetic figure.

If we consider now the bearing of the observations presented, on the doctrine that the ascus is a more highly developed and specialized modification of the sporangium of the Zygomycetes, it is plain that the very different methods of cleavage in the two cases are opposed to the assumption of any close relationship between them. In fact, it seems rather difficult to imagine any intermediate stages which could connect the process of cleavage by surface-furrows, as seen in the sporangium, with the free cell-formation of the ascus. It must be noted too that Popta's work on *Ascoidea* and *Protomyces*, which Brefeld considers intermediate forms between the lower Fungi and the Ascomycetes, has failed in any way to bridge this gap. Until some transitional forms have been found between these widely separated methods of cell-division, their evidence must be considered as decidedly against the assumption of any morphological relationship between the sporangium and the ascus. The method of division in the sporangium by surface-furrows is easily connected with other cases of division by constriction, such as are seen in the cell-division of *Cladophora* or in the formation of the conidia in the Erysipheae as I have described it, but the free cell-formation in the ascus seems as yet entirely unique, and its occurrence in widely separated forms of the Ascomycetes is justification for considering it as perhaps the most important and specific feature by which to distinguish the ascus from other spore-producing cells. The presence of epiplasm has always been considered one of the most distinctive features of the ascus, and those who have contended for the relationship of the sporangium and ascus have been much concerned to discover a similarity between the epiplasm and the intersporal slime in the sporangium. It is, however, sufficiently apparent that these two

substances are entirely distinct in their origin and consistency. The presence of epiplasm is a necessary result of the peculiar method of cutting out the daughter cells in the ascus, and together with this, may be taken as a criterion for distinguishing asci in every case. From this standpoint the ascus appears so far as yet known as a new structure, characteristic of the group of the Ascomycetes. By this I would by no means say that the process of cutting out the spores by free cell-formation as I have described it, may not have been developed from some other method of cell-division. Investigation of the method of spore-formation in such forms as *Exoascus*, *Dipodascus*, and *Eremascus* may perhaps throw further light on the matter. For the present the peculiar phenomena observed by Strasburger¹ in the swarm-spore formation in *Oedogonium* may be regarded as possibly analogous to and indicating a possible origin of the process in the ascus. Strasburger found that in a cell of *Oedogonium* which is about to form a swarm-spore, a hyaloplasmic-mouthpiece appears on the side of the cell-body, and that the nucleus, which up to this time may have been in any part of the cell, migrates to a position just beneath this mouthpiece. Rays now appear in the protoplasm at the base of the mouthpiece, and extend back into the protoplasm of the cell. The cilia also begin to bud out around the base of the mouthpiece. The cell-body now rounds up more and more to form the swarm-spore, which may be enclosed in a thin vesicle, when it escapes from the mother cell. Strasburger believes that this vesicle here, and in other cases of swarm-spore-formation, represents the cast off plasma-membrane of the mother cell. How the new plasma-membrane of the swarm-spore is formed is not certain. It is probable that the hyaloplasmic mouthpiece is a centre of formative activity at this stage. The migration of the nucleus to this region reminds one of what Haberlandt has found as to the position of the nucleus in cells whose walls are being thickened on one side. Haberlandt concludes that the nucleus is directly concerned in the forma-

¹ Zellbildung u. Zelltheilung, 3. Aufl.

tion of cellulose, and an analogous conclusion might be drawn here as to the relation of the nucleus to the formation of cilia. This view would be further strengthened by Strasburger's¹ observation on the swarm-spores of *Vaucheria*, where he found the cilia arranged in pairs over the whole surface of the spore, and a nucleus just beneath each pair.

Strasburger believes that in *Oedogonium* a centrosome lies at the base of the mouthpiece as the centre about which the rays above mentioned are oriented. The material for the cilia is undoubtedly formed in this region at the base of the mouthpiece, and if it is also the centre from which materials for a new plasma-membrane are diffused outward along the rays of the aster which is also formed at this place, we might consider the process as analogous to that in the ascus. Further details of the process in *Oedogonium* are necessary before the value of such a comparison can be tested.

The total dissimilarity of the process of cleavage in the sporangia described and the ascus as I have shown it in the above account, makes it necessary to look for the ancestors of the Ascomycetes elsewhere than in the lower Fungi. Thaxter's² studies of the Laboulbeniaceae have emphasized greatly the resemblance of that group to the Florideae and the hypothesis of the multiple origin of the Fungi from the Algae has gained correspondingly in strength. From this standpoint an attempt to homologize the swarm-spore-formation in such forms as *Oedogonium* with the free cell-formation in the ascus is not without value. Strasburger has pointed out a difference between ordinary cell-division and swarm-spore-formation in *Cladophora*, and it may be that this difference has been further developed in higher forms. The cleavage in the sporangia of the Phycomycetes described corresponds to the method of cell division in *Cladophora*, and it may be that the type of division shown in the swarm-spores has been developed into that shown in *Oedogonium* and the ascus. It would be especially interesting to know in detail

¹ Schwärmsporen, Gameten u. pflanzliche Spermatozoiden. Hist. Beitr., iii.

² Cont. to Mon. of Laboulbeniaceae, p. 251.

how the formation of spermatozoids in *Oedogonium* compares with that of the zoospores. The spermatozoids are known to have the structure of the zoopores, but more than one are produced in each cell. I have not been able to obtain sufficient material for the investigation of this point. Where we are to seek the origin of the ascus-type of spore-formation remains uncertain except perhaps for the facts in the swarm-spore-formation just noted. Brefeld has treated as practically one the question as to the presence of sexuality in the Ascomycetes and the relationship between the ascus and sporangium of the Phycomycetes. It is plain, however, that the sexual origin of the ascus-fruit and the relationship of ascus and sporangium are entirely distinct problems. The ascogonium may be the outgrowth of a fertilized egg and still the asci be homologous with the sporangia of *Sporodinia*, for example, where regularly the sexually produced zygosporangium germinates into a branching sporangiophore without the intervention of a mycelium. The analogy between such a germinating zygosporangium of *Sporodinia* and the egg of *Erysiphe* with its ascogonium and asci is quite striking. In *Sporodinia* however sporangia are produced later from vegetative mycelia without the preliminary formation of zygo-spores. There is thus no obligatory alternation of fruit-forms, an indefinite number of sporangial fruitings being followed by zygosporangium-formation. From this type of reproduction, which is common among the lower Algae and Fungi, we could pass to the condition of things we find in the Ascomycetes in two ways. First by the suppression of all production of sporangia which does not follow immediately on the germination of the fertilized egg so that the sexual and asexual fruiting bodies should gradually assume the relation of obligatory alternation; or it could be assumed that the fertilized egg gradually develops an intermediate stage of cell-division and growth, by which it produces a large number of asexual reproductive bodies before the mycelium which produces the non-sexual sporangia or conidia is developed. It is generally assumed that the alternation of generations of the Liverworts

and Mosses has arisen in this latter fashion. For the Ascomycetes it is impossible as yet to construct an ancestral line of development which shall settle this question. We can say however, as noted above, that the unlikeness in the method of spore-formation in the ascus and the sporangia which I have studied makes it impossible to assume any very direct relationship between the Phycomycetes and Ascomycetes.

To keep clear this distinction in the method of cell-division in the sporangium and ascus I would propose that the term *free cell-formation* be restricted to the method of cell-division in the ascus. The method of division by which the sporangiospores and conidia are formed may be described as cleavage by constriction. In *Synchytrium decipiens* and *Pilobolus*, where the division proceeds to the ultimate separation of the energides as protospores, the process can be called *progressive and complete cleavage*. In *Sporodinia* we have a restricted cleavage. In cutting off the gametes in *Sporodinia* and the conidia of the mildews we have division by simple constriction. The process is probably the same in the cell division of *Cladophora*. In cases such as the cutting off of the columella in *Sporodinia* and *Pilobolus* we have the process more or less modified by the appearance of internal cleavage by flattening and fusion of vacuoles.

Doubtless further types and modifications of the cleavage process will be found in investigating other, even nearly related Fungi, and it is hardly necessary to attempt further applications to the general theories of cell-division until a larger number of cases have been worked out for comparison.

EXPLANATION OF FIGURES IN PLATES XXIV—XXVI.

Illustrating Professor Harper's paper on Cell-Division in Sporangia and Asci.

All figures were drawn with the aid of the Abbe *camera lucida*, and with the Zeiss apochromatic objectives 8 mm., or 2 mm., Num. Ap. 1. 40.

PLATE XXIV.

Figs. 1-8. *Synchitrium decipiens*.

Fig. 1. Uninucleate cell almost full grown. $\times 250$.

Fig. 2. Multinucleate stage showing progressive cleavage by furrows from the surface. Central mass of protoplasm not yet segmented. $\times 500$.

Fig. 3. Portion of cell showing two nuclei and two cleavage-furrows. $\times 1500$.

Fig. 4. Median section of cell showing cleavage farther advanced. $\times 250$.

Fig. 5. Section from surface of a cell in early stage of cleavage. $\times 500$.

Fig. 6. Cell after segmentation is completed, showing uninucleate protospores. $\times 250$.

Fig. 7. Section of sporangium with spores after they have become polygonal by mutual pressure and contain several nuclei. $\times 500$.

Fig. 8. Mature spores, multinucleated and with cell-walls. $\times 500$.

Figs. 9-33. *Pilobolus crystallinus*, except figs. 28-32.

Fig. 9. Median longit. section of tip of sporangiophore bearing young sporangium. Figs. 9-12 show the nuclei merely as dots.

Fig. 10. Tip of slightly older sporangiophore with sporangium; section a little to one side of the middle.

Fig. 11. Median section of older sporangium, showing course of the protoplasmic flow from the mouth of the sporangiophore. *a*, section of protoplasmic ridge around the mouth of the sporangiophore.

Fig. 12. Median section of sporangium at stage when columella is forming, showing cleavage-furrows at base and flattened vacuoles above.

Fig. 13. Section of spore-plasma and sporangial wall just before cleavage begins. $\times 1250$.

Fig. 14. Section of spore-plasma from base of sporangium, showing first appearance of superficial cleavage-furrows. *a*, collar; *b*, columella-cleft; *c*, section of protoplasmic lining of columella; *d*, angular vacuole in spore-plasma. $\times 750$.

Fig. 15. Same as Fig. 14, but cleavage further advanced; *a*, sporangial wall. $\times 750$.

PLATE XXV.

Fig. 16. Section through a portion of the upper part of a sporangium, showing irregular sausage-shaped bodies formed by cleavage of the spore-plasma; a little older than Fig. 15. $\times 750$.

Fig. 17. Similar section to Fig. 16, but older, showing uninucleate protospores formed by cleavage. $\times 750$.

Fig. 18. Protospores with dividing nuclei, young daughter nuclei connected by remains of spindle fibres. *a*, collar; *b*, protoplasm of columella. $\times 750$.

- Fig. 19. Four protospores, which have enlarged and become multinucleate. $\times 250$.
- Fig. 20. Embryonic cell, with nuclei arranging themselves in two groups with formation of hyaline equatorial zone. $\times 1250$.
- Fig. 21. Slightly older than the last. $\times 1250$.
- Fig. 22. Division of embryonic cell by constriction. $\times 1250$.
- Fig. 23. Mature binucleated sporangiospore with wall. $\times 1250$.
- Fig. 24. Outline drawing of a part of a median section through a sporangium with binucleated sporangiospores; *a*, sporangial wall; *b*, collar, showing its extension upward on the columella; *c*, intersporal homogeneous slime; *d*, columella-wall; , protoplasm of columella.
- Fig. 25. Spore swollen and germinating in nutrient medium. $\times 1250$, stained with 'carmalaun.'
- Fig. 26. Outline of germinating spore at later stage with seven nuclei. $\times 1250$.
- Fig. 27. Spore with germ tube and numerous nuclei. $\times 1250$.
- Fig. 28. Drawn from living spore germinating in water. Figs. 28-32 drawn with apoch. obj. 2 mm. and oc. 8.
- Fig. 29. Later stage of germination in water, which showed streaming protoplasm.
- Fig. 30. Living spore germinating in nutrient medium.
- Fig. 31-32. Later stages of germination.
- Figs. 33-37. *Sporodinia grandis*.
- Fig. 33. Median longit. section of young sporangium, showing the beginning of the differentiation of the spore-plasm. $\times 425$.
- Fig. 34. Median longit. section, showing the development of vacuoles which are to form the columella-cleft. $\times 425$.

PLATE XXVI.

- Fig. 35. Section showing dense spore-plasma and foamy protoplasm of columella and their separation by large and small vacuoles. $\times 1000$.
- Fig. 36. Section showing formation of columella-cleft and furrows passing upward to divide the spore-plasma. $\times 425$.
- Fig. 37. Median section of sporangium with ripe spores.
- Figs. 38-48. *Lachnea scutellata*. Figs. drawn with obj. 2 mm. and oc. 8.
- Fig. 38 enlarged. Figs. 42 and 46 reduced.
- Fig. 38. Ascus with nucleus dividing, daughter nuclei still connected by spindle fibres; *a*, nucleolus.
- Fig. 39. Ascus with the two nuclei in equatorial plate stage.
- Fig. 40. Later stage of division in binucleate ascus.
- Fig. 41. Ascus with four nuclei in equatorial plate stage.
- Fig. 42. Ascus in octinucleate stage. Five nuclei with asters shown in section.
- Fig. 43. Nuclei with beaks, at whose apex is the aster.
- Fig. 44. Formation of ascospores by free cell-formation.
- Fig. 45. Later stage of same process.
- Fig. 46. Ascus in section, showing five spores with their plasma-membranes just completed; interior aster rays still present.
- Fig. 47. Ascus in section, with seven spores; beaks still present on part of the nuclei.
- Fig. 48. Ascus with eight spores, still somewhat flattened upon each other.

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HARPER. — ON CELL-DIVISION IN SPORANGIA AND ASCI.

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University Press, Oxford.

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