On the Development of the Spores of Riccia glauca.

BY

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With Plates XXI and XXII.

THE principal facts in the development of the spores of *Riccia* have already been described by Leitgeb[1] and Strasburger[2]. In the seventeen years which have elapsed since Strasburger's paper was written botanical microtechnique has, however, made such immense progress that it was thought desirable to subject these spores to a renewed investigation. Quite recently Garber[3] has dealt with the life-history of *Ricciocarpus natans*, and Lewis[4] with the embryology and development of *Riccia lutescens* and *R. crystallina*, but neither of these authors has described the spore or the development of its membranes.

Lewis's observations show that *Riccia lutescens* is merely a terrestrial form of *Ricciocarpus natans*, and he believes that the differences which exist in the structure of the thallus and the arrangement of the sexual organs are not sufficiently marked to justify the genetic separation of *Ricciocarpus* from *Riccia*. He accordingly drops the genus *Ricciocarpus* altogether and includes *Ricciocarpus natans* among the Ricciae.

The material for the present study was fixed in strong and medium chrom-acetic mixtures (Chamberlain's formulae), strong Flemming's solution and in alcohol and acetic acid mixture. Of these the stronger chrom-acetic fluid proved the most satisfactory, but excellent results were also obtained with the alcohol and acetic mixture.

I will begin my account with the fertilization of the egg-cell. The general character of this process is shown in Pl. XXII, Fig. 28, from which it will be seen that it closely resembles the fertilization of *Riccia* (*Ricciocarpus*) *natans* as described by Garber. My photograph also shows that the shrinkage

[1] Leitgeb, Ueber Bau und Entwicklung der Sporenhäute. Graz, 1884, pp. 39–49.

[2] Strasburger, Ueber das Wachsthum vegetabilischer Zellhäute. Histologische Beiträge, Heft 2, 1889, pp. 104–11.

[3] Garber, The Life-history of *Ricciocarpus natans*. Bot. Gazette, vol. xxxvii, 1904, pp. 161–77.

[4] Lewis, The Embryology and Development of *Riccia lutescens* and *Riccia crystallina*. Bot. Gazette, vol. xli, 1906, pp. 109–38.

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of the egg-cell is far less than is usually figured, and in this point I am again in agreement with Garber's statements. The first division of the fertilized egg-cell is usually obliquely transverse (Fig. 32). The succeeding divisions have been so frequently described that I need not recapitulate them here. They result in a mass of sporogenous tissue surrounded by a single layer of sterile wall-cells, the whole being enclosed within a two-layered calyptra. The young spore-mother-cells are at first separated from one another by extremely delicate membranes (Fig. 29). These stain, often deeply, with bismarck brown, but I am unable to get a decided reaction in them with ruthenium red, whilst with calcium-chloride-iodine and chlor-zinc-iodine they colour yellow but show no signs of containing cellulose.

The cell contains a quantity of starch which is especially abundant round the nucleus. Upon the primary walls which separate the spore-mother-cells from one another a secondary and, later, a tertiary thickening layer is deposited (Pl. XXI, Fig. 5). Both layers give the reaction of cellulose as well as those of pectose, but the tertiary thickening layer (viz. the one in immediate juxtaposition with the protoplast) stains the more deeply with pectic reagents.

The protoplast surrounded by the tertiary thickening layer now rounds itself off and becomes separated from the primary mother-cell membrane. The secondary thickening layer, which has become more or less mucilaginous in consistency, sometimes separates completely from the primary walls and then forms a well defined layer surrounding the protoplast (Figs. 5, 9, 31). At other times, on the contrary, it remains partly adherent to the primary wall and in that case it becomes drawn out into a number of strands bridging over the gap between the rounded protoplast and the primary wall. The latter condition is represented in Fig. 6, and it will be observed that my drawing closely resembles Leitgeb's Fig. 3, Taf. II. Leitgeb, however, believed that the space between the primary wall and the protoplast was occupied by a homogeneous mucilage, and that the strands of material which both he and I have figured are composed of food-materials diffusing in from the outside. In my preparations it is quite certain that no homogeneous mucilage occupies the space between protoplast and primary wall; moreover the strands of material stretching across this space give cellulose and pectose reactions exactly corresponding with those obtained in the secondary thickening layer (e. g. of Fig. 5 where the cells are still partly united).

It should be mentioned that the condition represented in Fig. 6 is no doubt somewhat exaggerated by the reagents used. The measurement of the spore-mother-cells shown in Figs. 30 and 9 gave an average diameter of about 46 μ, whilst that of the cells in the sporangia from which Fig. 6 was drawn was only 40 μ. I believe, therefore, that the effect is somewhat heightened by the reagents employed, but I see no reason to conclude that

the condition is entirely due to them. During the isolation of the spore-mother-cells from one another the sporangial cavity enlarged considerably. Taking the measurements from the periphery of the outer of the two layers of the archegonial wall the diameter of the cavity at such a stage as that shown in Fig. 29 is about 240 μ, whilst when the mother-cells are rounding off (as in Fig. 30) it has increased to about 300 or 345 μ.

Garber and Lewis have both described a large amount of nutritive material which fills the space between the mother-cells and which is secreted by the surrounding cells. In my preparation of *Riccia glauca* I have seen nothing of this material [1].

The rounded mother-cells now proceed to divide. The following description is based upon the study of preparations which have been stained with Heidenhain's haematoxylin, either alone or with a light counter stain with bismarck brown. The large nucleus of the spore-mother-cell just before the commencement of division contains a conspicuous, deep-staining nucleolus and a number of delicate linin fibres which have little affinity for dyes (Figs. 1 and 2). In these features the resting nucleus of the sporogenous cells of *Riccia glauca* differs from the description given by Lewis for the two species which he has studied. He found the nuclear cavity to be occupied by a linin network upon which the scanty chromatin is irregularly scattered; moreover he states that no nucleolus was to be seen. I have examined a large series of sections of sporangia containing mother-cells at all stages up to their maiotic division, but I have never observed a nucleus which contained a reticulum such as Lewis figures, nor one which was without a nucleolus. In the case of *Riccia glauca*, therefore, we can be certain that no such non-nucleolated, reticular resting stage occurs.

It may also be added that the resting condition of the vegetative nuclei of the thallus of *Riccia glauca* agrees essentially with what I have described above in the case of the sporogenous cells.

The nucleolus in nearly all cases has a lobular outline (Fig. 1) and I believe that this is due to the fact that the nucleolus is not a homogeneous body, but is composed of a number of small chromatic masses or granules held together by a common matrix. These granules are usually so closely crowded together in the nuclei of the sporogenous cells that it is not easy to see their separate individuality. In a few cases, however, the nucleolus was actually seen to be composed of distinct granule-like bodies which had become rather more widely separated from one another than usual by the action of the reagents employed or through the pressure of the microtome knife (Fig. 2) [2]. In the vegetative nuclei at the growing apex of the thallus the constitution of the nucleolus can be much more readily determined when

[1] See Garber, l. c., Plate X, Fig. 37.

[2] Compare also Fig. 16, in which the separate chromatin granules of the earlier stages of division are massing together to form one body.

differentiation with the iron-alum has been carried to the right point. It can then be seen that these nucleoli consist of a faintly stained matrix in which are embedded a number of intensely black bodies (Fig. 3).

Compound nucleoli of a similar character occur in the nuclei of the Musci. In the spermatogenous cells of *Atrichum undulatum* the nucleoli can be quite clearly seen to consist of a lightly coloured matrix containing a number of chromatic particles [1].

In several sporangia I have found that the linin threads tend to become more or less massed towards one side of the nuclear cavity (Fig. 7). This may correspond to a synapsis stage, although I am not prepared to say how far reagents are responsible for its production in the present case.

Following this condition we find that a much more deeply staining and thicker thread has been developed, which traverses the nuclear cavity in a number of coils or loops (Fig. 8). This is unquestionably the spirem-thread, and it differs radically from the short thread described by Lewis in *Riccia crystallina* [2]. The thread can often be followed continuously for a considerable distance, and I believe that it forms an unbroken filament.

In well stained preparations the spirem-thread shows very beautifully an alternation of deeply coloured bodies (chromomeres) with lighter areas (Fig. 8).

It is very probable that the increased amount of chromatic material which the thread contains at the spirem stage has been derived from the nucleolus and most likely at the expense of the chromatic granules which this body encloses. I have unfortunately been unable to find the stages in the division of the sporogenous cells which lie between the establishment of the spirem and the arrangement of the chromosomes at the equator of the spindle (Fig. 10). In the spermatogenous cells of the antheridium, however, in which a spirem is also developed, the actual segmentation of this thread into the chromosomes could be followed, and it was clearly seen that during this process the nucleolus became more and more inconspicuous. By the time the chromosomes are fully established the nucleolus has been lost sight of altogether.

Both Garber and Lewis have recorded four chromosomes in *Riccia* (*Ricciocarpus*) *natans*, and Lewis found the same number in *Riccia crystallina*. In *Riccia glauca* the number of chromosomes is higher than this, and I have been able to determine with certainty that the reduced number is either seven or eight (Figs. 9 and 11). The distribution of the chromosomes to

[1] I should like to take this opportunity of correcting an error which I made in a previous note upon 'The chromosomes of *Funaria hygrometrica*' (New. Phyt., vol. ii, 1903, p. 166). I there stated that the number of chromosomes which appeared in the first division of the spore-mother-cells was four. Since this was written I have examined properly fixed material of several mosses (*Funaria hygrometrica, Atrichum undulatum, Mnium hornum, Polytrichum juniperum*) and I have found that in all cases the number of chromosomes is far higher than I formerly supposed.

[2] Compare Lewis's Plate VII, Fig. 35, with my drawing of this stage.

the daughter-nuclei is shown in Fig. 11. On first reaching the apex of the spindle the chromosomes are crowded closely together (Fig. 12). Soon, however, a nuclear membrane is formed and the chromosomes proceed to open out.

During the earlier stages of the telophase a number of chromatic bodies can be seen distributed upon the linin, and these bodies are no doubt the derivatives of the chromosomes (Figs. 13 and 14). At a later stage the scattered chromatin bodies have come together to form a single lobular nucleolus, whilst linin fibres, containing little or no stainable material, extend through the nuclear cavity (Figs. 15 and 16). The spindle during the metaphase and anaphase of the division is a comparatively narrow structure. A conspicuous cell-plate is developed at the equator of the spindle (Fig. 12). During the telophase of division the spindle shortens and broadens out very considerably and the cell-plate becomes correspondingly broader (Fig. 14).

A membrane is developed at the equator of the spindle, no doubt between the split halves of the cell-plate, although the splitting of the plate could not be followed here on account of its great delicacy. This membrane, which stains rather deeply with bismarck-brown, does not at first reach right across the cell (Fig. 15), but by the time that the nuclei are again dividing it has almost or quite reached the periphery of the cell (Fig. 17). After a short interval of rest the nuclei enter upon the second maiotic division.

At the conclusion of this division cell-membranes are formed which complete the division of the mother-cell into the four daughter-cells, which are conveniently, if incorrectly[1], called the special-mother-cells. These membranes, separating the special-mother-cells from one another, give both cellulose and pectose reactions, as do the secondary and tertiary thickening layers of the mother-cell which still form the peripheral envelope of the tetrad group.

It may be mentioned here that during the development of these septa a large proportion of the starch of the cell is seen to be aggregated in their neighbourhood (Figs. 17 and 18).

The special-mother-cell walls do not long remain in this condition, but secondary thickening layers are soon deposited upon the inner surfaces of the thin pectose-cellulose membranes (Fig. 19).

These thickening layers have an uneven outline, forming the papillate projections into the interior of the cell, which previous writers have fully described. Their reactions show that they consist of callose apparently unaccompanied by any other substance. They colour deeply in corallin-soda, in aniline-blue, in congo-red and in naphthol-black[2]. On the other

[1] Miss Benson, New Phytologist, vol. iv, 1905, p. 96.

[2] I have elsewhere dealt with the specific staining properties of naphthol-black (see Beihefte zum Bot. Centralblatt, Bd. XIX, Abt. I, Heft 2, 1905, p. 289).

hand, calcium-chloride-iodine and chlor-zinc-iodine do not stain these layers. In ruthenium-red and bismarck-brown they assume a faint colour, but I do not think that this is sufficiently marked to indicate the presence of pectose in these layers.

The membranes which first limited the cells of the tetrad from one another before the thickening layers were deposited can now be seen as middle lamellae running through the midst of the callose layers, and as a peripheral covering to the entire tetrad. These primary membranes continue to colour distinctly pink-violet with calcium-chloride-iodine solution and deeply red with ruthenium-red, whilst callose reagents leave them unstained. They still possess, therefore, the unchanged pectose-cellulose constitution which they had in the first place.

It may be mentioned here that Leitgeb[1] reached very different results on these points. During the earlier stages following the thickening of the special-mother-cell walls he could distinguish no middle lamella, and only after the first spore-wall has made its appearance 'differenzirt sich in den Scheidewänden die Mittellamelle.' This is certainly incorrect, for the middle lamellae are nothing but the original pectose-cellulose septa of the unthickened special-mother-cell wall which maintain their individuality throughout.

Leitgeb further states, on the same page, that in aniline-blue an outer layer of the special-mother-cell wall colours deep blue whilst the inner parts, corresponding to our secondary thickening layers, only assume a yellowish tinge in this solution. If this were the case we should have to conclude that the composition of the special-mother-cell wall is very different from that which I have described above, but I have so repeatedly assured myself that the behaviour of this wall towards aniline-blue is precisely the opposite to what Leitgeb found and, moreover, this has been so thoroughly supported by the reactions of the wall with other reagents, that there cannot be the slightest doubt of the correctness of my conclusions.

The occurrence of callose in the special-mother-cell walls of one of the Hepaticae is of interest since in no other member of the Archegoniate series which I have examined is that substance to be found in this position; and it is only when we reach the Gymnosperms and Angiosperms that callose can again be seen surrounding the pollen-tetrads[2].

It is very probable that callose occurs constantly in the special-mother-cell walls of the liverworts since some observations which I am making upon the spore-development of *Anthoceros laevis* have shown that callose is also present in the tetrad walls of this plant.

In the Musci the special-mother-cell walls contain pectose and cellulose,

[1] Leitgeb, l. c., p. 43.

[2] In all Gymnosperms and Angiosperms which I have examined callose formed the only demonstrable constituent of the special-mother-cell wall.

whilst in the leptosporangiate ferns, the Ophioglossaceae and in the ligulate and eligulate Lycopodiums these walls contain either cellulose and pectose or pectose alone, but no trace of callose[1]. On another occasion[2] I have referred to the manner of origin of callose in the special-mother-cell walls of *Oenothera*, and the same remarks apply with equal force to the callose of *Riccia*. In the present case it is impossible to suppose that the callose can have arisen from the transformation of cellulose, since none of this substance precedes the callose nor can any cellulose be seen to disappear from other neighbouring membranes. We must conclude, therefore, that the callose in the tetrad walls of *Riccia* is formed directly as such by the protoplast.

The tetrad-group grows in size from about 60 μ to about 75 to 85 μ in diameter and then the first spore-wall[3] is formed round each of the four cells. The question of the origin of this layer constitutes one of the chief points of difference between the accounts of Leitgeb and Strasburger. The former writer was convinced that this wall is a transformation product of the innermost layers of the special-mother-cell wall, whilst Strasburger is equally positive that it is a new formation of the protoplast which has no relation to the special-mother-cell wall.

I have spent no little time upon this question and the only conclusion which I am able to reach is a negative one. After carefully considering the grounds upon which Leitgeb and Strasburger based their respective views I am forced to conclude that these were insufficient to prove the case either one way or the other. Leitgeb lays the greatest weight upon the firm adhesion which exists between the special-mother-cell wall and the first spore-wall. After treating the spores with various reagents (chlor-zinc-iodine or a not too strong mixture of chromic and sulphuric acids) he found that the first spore-wall remained firmly fixed to the special-mother-cell wall, although this was greatly swollen.

An argument based upon the adhesion of two layers to one another cannot, however, be accepted as proof of their common origin. Cases are known in which two layers are firmly united but which have unquestionably been separately deposited by the protoplast. Thus Fitting[4] has referred to the special-mother-cell wall and the secondary thickening layer of the spore-mother-cell wall of *Isoetes* which adhere closely together but which are independently developed. The special-mother-cell wall and the very young pollen-wall of the Phanerogams furnish another example of two layers which

[1] I have, unfortunately, had no opportunity of examining the special-mother-cell walls of *Equisetum* as yet.

[2] Beer, l. c., p. 290.

[3] In the following account I have avoided the terms exospore or perispore and speak only of first and second spore-membranes. Since, however, the innermost layer of the spore is certainly homologous with the endospore of other plants I have used that name for it.

[4] Fitting, H., Bot. Zeit., Bd. 58, 1900, p. 126.

are often at first inseparably united, but which nevertheless have a distinct origin. In his examination of *Riccia* Strasburger found that by bursting the special-mother-cell wall by means of pressure he was able to separate the spore-protoplast, surrounded by the first spore-wall, from the special-mother-cell wall. Moreover, he states that the folds of the first spore-wall never quite reach to the summits of the indentations in the special-mother-cell wall [1]. If this were actually the case it would, as Strasburger clearly saw, form strong evidence in favour of the independent origin of the two layers. My own preparations, however, do not confirm Strasburger in this respect. Wherever the section is accurately longitudinal (as regards the fold of membrane and the indentation) it can be distinctly seen that the first spore-wall lines the indentation in the special-mother-cell wall to its very apex. Where, however, the section has cut the fold of the first spore-wall somewhat obliquely the true relations of this fold to the indentation are not always at once clear.

From what has been said above it will be seen that the evidence which we possess is entirely inconclusive and that neither Leitgeb nor Strasburger were justified by the facts in assuming a definite position.

A study of the microchemical reactions of this wall furnishes no assistance in deciding this question. The whole behaviour of the wall indicates that it is cuticularized from a very early period; whether there is a basis of cellulose, callose, or pectose preceding or underlying the cuticularization was not apparent from any of my experiments.

It may be mentioned here that after treating sections of older spores with a mixture of chromic and sulphuric acids and then (after washing) adding chlor-zinc-iodine it can be seen that the first spore-wall has a rather densely laminated structure (Figs. 24 and 25).

Not long after the first spore-wall has been formed a deposit of mucilaginous substance can be seen at the equatorial rim [2] of the spore (Fig. 20). As Leitgeb correctly stated, this mucilage has the same chemical and physical properties as the secondary thickening layers of the special-mother-cell wall; that is to say it is composed of callose. Leitgeb further supposed that this mucilage was derived from the special-mother-cell wall, a portion of which wandered through the first spore-wall at the equatorial region. The first spore-wall is, however, always continuous and never interrupted at any spot, as Leitgeb thought was sometimes the case (Figs. 20, 21). Strasburger considered the mucilage to be a part of the first spore-wall; his chief reason for doing so was that he found that this wall and the mucilage were not sharply marked off from one another at the equatorial rim. I find that the mucilage is formed later than the first spore-wall, that the callose

[1] Strasburger, l. c., p. 109.

[2] Leitgeb called this equatorial rim a 'Saum,' whilst Strasburger wrote of 'einem äquatorialen Flügel, das heisst einem an der Grenze von Rücken- und Bauchfläche verlaufenden Saum.'

mucilage is always sharply defined from the delicate spore-wall which has quite different staining properties, and that it is not difficult to separate the one from the other by means of reagents. I regard the mucilage, therefore, as a new formation which has no relation either to the special-mother-cell wall or to the first spore-wall.

The older writers believed that this mucilage formed a continuous layer over the inner face of the first spore-wall. I do not find this to be the case.

Over the ventral surfaces of older spores, in which the second spore-wall has been developed, it can be seen that the two membranes lie closely against one another without any mucilage between them (Fig. 21). Over the dorsal surface of the spore the two layers of the wall are indeed frequently separated from one another and then the space between them appears to be, at least partly, occupied by a mucilage. How far this separation between the two walls of the spore over the dorsal surface is a normal feature of their structure is, however, difficult to say. In older spores the mucilage at the equatorial rim has again become absorbed and can no longer be seen.

At first this mucilage is limited internally only by the plasma membrane of the protoplast, which is pushed inwards at the equatorial seam by the plug of mucilage (Fig. 20). Before long, however, the spore-protoplast develops a new wall within the first one, and this then forms a flattened internal boundary to the mucilage at the rim of the spore (Fig. 21). This second spore-wall is a cuticularized structure almost from the commencement. At the very first, however, it is probably composed of uncuticularized pectose-cellulose, for when it is just discernible it stains more deeply with bismarck-brown than is usual with cuticularized membranes, and, moreover, the lamellae which are subsequently added to its thickness unquestionably have, at first, a pectose-cellulose constitution.

During the earliest stages of its existence the second spore-wall appears, even under high magnifications, as a perfectly homogeneous layer, but in spores which are a little older it has become considerably thicker and then a dark line can be seen traversing the middle of this wall and dividing it into an inner and an outer part (Figs. 22, 35). This dark line grows in thickness with the age of the spore until it not infrequently becomes a thick layer which stains intensely black with Heidenhain's haematoxylin and forms the most conspicuous feature in the spore-wall (Fig. 26). It is not easy to assure oneself of the real nature of this dark layer, but after comparing together a large number of spores I am led to the conclusion that this layer most probably only represents a gap which is formed between two sets of lamellae and which becomes occupied by some dark-coloured, stainable material. This view seems to me to be supported by the fact that the dark layer varies in the time and in the position of its appearance. Some-

times it is discernible at a very early stage in the history of the second spore-wall. Sometimes the spore has become much older and the second spore-wall has become quite a thick structure before any signs of this layer can be seen[1]. Again its appearance is sometimes nearer, sometimes further away from the outer periphery of the spore-wall. In preparations which have been treated with a mixture of chromic and sulphuric acids a separation of two sets of lamellae can often be seen in the second spore-wall with an evident gap between them. Further evidence is given by a study of older spores in which a similar but much narrower black band can usually be seen between the inner boundary of the second spore-wall and the endospore (Fig. 26). What the nature and origin of the substance which occupies this space may be is quite unknown. It does not seem unlikely, however, although I can bring forward no proof for the view, that the dark substance which collects in the gap of the spore-wall is the material which colours brown the older spore-walls throughout their thickness.

Most instructive preparations of the spore-wall at the middle periods of development may be obtained by treating sections with a mixture of chromic and sulphuric acids, washing and then examining them in chlor-zinc-iodine or calcium-chloride-iodine. The first spore-wall is coloured yellow and shows a densely laminated structure. Within this wall lies the second spore-wall. The outer portion of this wall is seen to be composed of loosely arranged lamellae which sometimes lie closely against the first spore-wall but which have usually become separated from it by the action of the reagent (Figs. 24, 25). The inner portion of the wall, which is generally separated from the loose lamellae by a gap, has a homogeneous appearance and no lamellae can be distinguished in it. If the action of the acids has been carried to the right point it can be seen that the inner part of this internal layer of the second spore-wall gives beautiful cellulose reactions with the iodine reagents. The band of cellulose is considerably thicker on the dorsal surface of the spore, whilst cuticularization had become more complete over the ventral surfaces. Congo-red in dilute solution also colours the cellulose areas a deep red.

Although the inner portion of the second spore-wall has a homogeneous appearance, even after the action of reagents, it is nevertheless really built up of successive lamellae.

During the development of the spore this inner region of the second spore-wall grows very considerably in thickness, and the sections show with unmistakable clearness that this growth is due to the deposition of a series of lamellae upon one another. The newest lamella is formed by the protoplast of the spore in the most intimate attachment with the plasmatic membrane. By plasmolysing the protoplast this lamella is usually at the same time separated from the walls of the spore and the contracted proto-

[1] The late appearance of this dark layer is shown in Fig. 23.

plast can then be seen to be surrounded by a thin layer which gives cellulose-pectose reactions (Fig. 36). It may be said, therefore, that in the formation of the second spore-wall the first lamellæ which are deposited are comparatively loosely arranged together (outer region of second spore-wall), whilst the later ones become so firmly united that the lamellose nature of this portion of the wall becomes obscured (inner region of second spore-wall). It is these two regions of the wall—the loosely and the densely laminated areas respectively—which become more or less separated from one another by the interpolation of the dark material which was mentioned above.

The endospore is formed comparatively late in the development of the spore. It gives the reactions of cellulose and pectose, and it is usually separated from the second spore-wall by a narrow space occupied by dark material similar to that which occurs in the more conspicuous gap between the two layers of the second spore-wall (Fig. 26). In spores which are nearly or quite mature the walls are deeply coloured brown and have become much denser and in consequence thinner. The lamellated structure of the wall is now obscured. Heated to redness on platinum foil with a drop of concentrated sulphuric acid the entire spore dissolves without leaving a silica skeleton behind.

Nothing has been said above of the nutrition of the spore or of the sources of the material for the growth of its membranes, so that a few words upon this subject must be added here. That the protoplast of the spore is itself actively concerned in the growth of the membranes which surround it can scarcely be doubted. The new lamellæ which are added to the second spore-wall are formed, as we have seen, in the most intimate union with the plasmatic membrane; the nucleus of the spore also presents an appearance which strongly suggests that it is participating in metabolic activities.

Unlike the usual resting nucleus of *Riccia glauca*, to which I have already referred, the chromatin is not confined to the nucleolus, but is also distributed along a rather thick filament which strongly recalls the spirem-thread of the dividing nucleus (Fig. 27 *a*, 27 *b*). It is difficult to decide whether this thread forms one continuous structure or not.

As the spore grows older and its walls become thickened we find that, although the thread long maintains its spirem-like arrangement, it gradually stains less and less deeply with the haematoxylin (Fig. 27 *c*). Somewhat similar spirem-nuclei have been described in various animal cells. Thus the well-known case of the salivary glands of *Chironomus* larvae, studied by Balbiani, may be recalled, or the ovarian eggs of *Triton taeniatus*, in which Born found a spirem-stage to precede a more diffuse arrangement of the chromatin. In most of the cases already known in which the chromatin is distributed through the nucleus in this manner, we are dealing with cells

which exhibit considerable metabolic activity, and we may probably infer that some relation exists between the spirem-arrangement and the cell activity. A similar relation between the peculiar nuclear structure and cell-metabolism no doubt also occurs in the developing spores of *Riccia glauca*. The actual material which is used by the protoplast in forming and adding to the spore-walls must be derived from without the spore. The reserve material and cytaplasm of the spore suffer very little diminution during development, and if these are drawn upon to furnish material for the growth of the membranes, this loss is at once fully compensated by the arrival of new material from without. The starch-contents of a spore at about the middle period of its development is shown in Fig. 34, and almost precisely the same appearance is presented by the spore-protoplast in the preceding and succeeding stages.

Both the sterile parietal layers of the sporangium and the inner layer of the calyptra[1] degenerate and yield some material which is no doubt employed in the growth of the spore-walls. Neither of these layers is, however, rich in substance (see Figs. 4, 29), and I scarcely think it is possible that their degeneration can furnish all the material required for the very considerable growth undergone by the membranes. Most probably this source is supplemented by material which is assimilated by the vegetative cells of the thallus, and which diffuses into the sporophyte in a state of solution. After the first spore-wall has been formed, and during all the earlier periods of the growth of the second spore-wall, a mucilage is constantly present in the sporangium between the spore-tetrads (Fig. 33). The origin and significance of this mucilage are, however, somewhat obscure. It is certainly not the material secreted by the surrounding cells of the thallus, since not a trace of mucilage can be detected in any of these. The callose special-mother-cell walls, which for some time continue to surround the spore-tetrads, gradually disappear, but there is no evidence to show that their substance makes any contribution to the sporangial mucilage which, moreover, gives none of the reactions of callose. Two sources remain, both or either of which may be responsible for the sporangial mucilage. In the first place the degeneration of the parietal cells of the sporangium and of the inner archegonial layer may contribute to the formation of the mucilage. In that case it still remains to be explained how it is that the parietal cells have degenerated some time before the mucilage can be seen, whilst some remains of the inner archegonial cells can frequently still be detected after the mucilage has again become absorbed. Secondly, the degeneration of the primary mother-cell walls and of their thickening layers, which are both lost sight of about this time, may give rise to part or all of the sporangial mucilage. A difficulty in the way of at once accepting this view of the origin of the

[1] The outer layer of the calyptra persists to a very late stage.

mucilage lies in the fact that whilst the secondary thickening layer of the mother-cell walls gives both cellulose and pectose reactions, I have been unable to demonstrate the presence of cellulose in the mucilage.

It must, therefore, remain uncertain for the present to what extent the mucilage in the sporangium possesses a nutritive value.

By the time that the second spore-wall has become differentiated into the two sets of lamellae (with the dark layer separating them from one another) the mucilage has become very scanty, and soon after it entirely disappears.

Summary.

1. Fertilization of the egg-cell of *Riccia glauca* corresponds essentially with Garber's description of this process in *Riccia* (*Ricciocarpus*) *natans*.

2. The spore-mother-cells are at first separated from one another by extremely delicate membranes, which stain deeply with bismarck-brown but in which no cellulose could be demonstrated.

3. Upon these primary spore-mother-cell walls secondary and, later, tertiary thickening layers are deposited. Both these layers give pectose-cellulose reactions.

4. The protoplast, surrounded by the tertiary thickening layer, now rounds itself off. The secondary thickening layer, which becomes more or less mucilaginous, sometimes separates completely from the primary wall and then forms an external envelope to the mother-cell, whilst at other times it still remains partly adherent to the primary wall, and in that case it becomes drawn out into strands of mucilage bridging over the space between primary wall and tertiary thickening layer. The latter condition corresponds to Leitgeb's description of this stage, but he erroneously interpreted the mucilage-strands as nutritive material passing into the mother-cell.

5. In *Riccia glauca* no demonstrable nutritive material was found between the isolated mother-cells such as Garber and Lewis have described in the case of *Riccia* (*Ricciocarpus*) *natans*.

6. The resting nucleus of the spore-mother-cell contains a large, deep-staining nucleolus and a number of delicate linin fibres. No non-nucleolated, reticular resting nucleus, as described by Lewis in *Riccia* (*Ricciocarpus*) *natans* and *R. crystallina*, was found in *Riccia glauca*.

7. The nucleolus of the nucleus of *Riccia glauca* appears to be a compound structure consisting of a number of deeply chromatic masses or granules embedded in a matrix which stains only faintly.

8. A long and well-marked spirem-thread occurs in the prophase of the division of the spore-mother-cell. This differs radically from the short thread described and figured by Lewis in *Riccia crystallina*.

9. The reduced number of chromosomes in *Riccia glauca* is either seven or eight, but it could not be decided with certainty between these two numbers.

It will be noted that this number is considerably higher than that (four) recorded by Garber and Lewis in *Riccia* (*Ricciocarpus*) *natans* and *R. crystallina.*

10. In the telophase of the division a number of chromatic bodies, which are no doubt derivatives of the chromosomes, are distributed upon the linin fibres. Subsequently these scattered bodies aggregate together to form the lobular nucleolus of the resting nucleus.

11. The membrane, which is formed between the daughter-cells resulting from the first division of the mother-cell, does not at first extend to the periphery of the cell.

12. At the conclusion of the second maiotic division the special-mother-cells are separated from one another by delicate membranes which have a pectose-cellulose composition.

13. Upon these primary special-mother-cell walls secondary thickening layers are deposited which give the reactions of callose. This callose is directly deposited as such by the protoplast, and is not a transformation product of cellulose.

14. The first spore-wall is a cuticularized structure from a very early period. No decisive data could be found to determine whether this wall is derived from the transformation of the innermost lamellae of the special-mother-cell wall or whether it is a new formation directly due to the secretory activity of the protoplast. It was shown that the arguments brought forward by previous writers are insufficient to prove the case either one way or the other.

15. Within the first spore-wall at the equatorial rim a plug of mucilage is deposited. This mucilage, which gives the reactions of callose, has no direct relation either to the thickening layers of the special-mother-cell wall (as Leitgeb supposed) or to the first spore-wall (as Strasburger supposed). It is a new and independent formation.

16. The second spore-wall is next formed within the first wall. It is cuticularized from a very early time, but there is reason to believe that previous to its cuticularization it reacts as a pectose-cellulose membrane.

17. At first the second spore-wall appears to be quite homogeneous, but subsequently it can be seen to be composed of three parts:—

(i) an external loosely laminated region;
(ii) a layer of dark-coloured material; and
(iii) an internal densely laminated region.

The dark-coloured material appears to have been interpolated into a space which forms between the two laminated regions of the wall.

18. The endospore, which is formed late in the development of the

spore, gives the reactions of pectose and cellulose. It is often separated from the second spore-wall by a very thin band of dark-coloured material similar to that which occurs in the gap between the two regions of the second spore-wall.

19. No silica could be detected in the spore-membranes.

20. The protoplast of the spore is actively concerned in the growth of the membranes which surround it. The intimate union which exists between each new lamella, which is being added to the wall, and the protoplast in itself indicates this fact, whilst the curious spirem-like structure of the spore-nucleus, resembling that of certain animal cells, also suggests the occurrence of active metabolic processes in the cell.

21. The actual material necessary for the growth of the spore-walls is partly derived from the breaking down of the parietal cells of the sporangium and of the inner layer of the calyptra. This source is most probably supplemented by material which is assimilated by the vegetative cells of the thallus, and which diffuses into the sporophyte in a state of solution.

22. After the first spore-wall has been formed, and during all the earlier periods of the growth of the second spore-wall, a quantity of mucilage is constantly present in the sporangium between the spore-tetrads. The origin and significance of this mucilage are, however, still somewhat obscure. The various possibilities of the case are discussed in the body of the paper.

In conclusion I should like to express my thanks to Professor J. B. Farmer, F.R.S., for kindly examining my sections and for offering several valuable suggestions.

EXPLANATION OF FIGURES IN PLATES XXI AND XXII.

Illustrating Mr. Beer's paper on the Spores of *Riccia*.

All Figures refer to *Riccia glauca*.

PLATE XXI.

A. DRAWINGS.

Fig. 1. Spore-mother-cell. Resting nucleus showing lobular nucleolus.

Fig. 2. Spore-mother-cell. Nucleolus of resting nucleus broken up into distinct granules.

Fig. 3. Nucleoli of nuclei from the apex of the thallus showing chromatic granules embedded in a matrix.

Fig. 4. Sterile parietal cells of sporangium in which the mother-cells were becoming rounded off.

Fig. 5. Spore-mother-cells which are separating from one another. Where they are still attached a middle lamella (the primary wall) and secondary and tertiary thickening layers can be recognized. × about 1100.

Fig. 6. Spore-mother-cell which is becoming rounded off. Secondary thickening layer forms strands between primary wall and tertiary thickening layer. × about 1100.

Fig. 7. Spore-mother-cell. Synapsis (?).

Fig. 8. Spirem-stage of the first division of the spore-mother-cell. × about 1100.

Fig. 9. Chromosomes at equator of spindle. Secondary and tertiary thickening layers of the wall surround the spore-mother-cell.

Fig. 10. Chromosomes at the equator of the spindle in first division of the spore-mother-cell. × about 1100.

Fig. 11. Anaphase of first division of the spore-mother-cell. × about 1100.

Fig. 12. Chromosomes crowded together at the poles of the spindle. × about 1100.

Fig. 13. Telophase of first division of the spore-mother-cell. × about 1100.

Fig. 14. Later stage of telophase.

Fig. 15. Daughter-nuclei in resting condition before commencement of second division. Septum between cells not yet complete.

Fig. 16. Daughter-nucleus from similar cell as in Fig. 14. Chromatin granules are aggregating together to form the compound nucleolus.

Fig. 17. Second division of the spore-mother-cells. The septum between the cells now nearly or quite reaches the periphery. × about 1100.

Fig. 18. Young tetrad with delicate septa between the cells and the periphery still clothed with secondary and tertiary thickening layers of mother-cell-wall.

Fig. 19. Special-mother-cells. Examined in glycerine and congo-red. Thickening layers red; middle lamellae and periphery colourless.

Fig. 20. Spore with first spore-wall (drawn black) at the equatorial rim of the spore. Examined in glycerine and congo-red. Secondary thickening layers of special-mother-cell and mucilage at the rim of spores (both left white in the figure) stained red; spore-wall yellowish. Equatorial mucilage limited internally by the plasma membrane alone. × about 1100.

Fig. 21. Spore with first and second spore-walls in the neighbourhood of the equatorial rim. The second spore-wall still appears homogeneous. Examined in glycerine containing a very little fuchsin.

Fig. 22. Spore at the equatorial rim. Early stage in the differentiation of the second spore-wall.

Fig. 23. Older spore. Examined in calcium-chloride iodine. Second spore-wall differentiated into an outer distinctly laminated region and an inner apparently homogeneous region. In the present case the darkly coloured layer is only just appearing between the two regions at this comparatively late stage.

Fig. 24. Portion of spore-wall after warming in a mixture of chromic and sulphuric acids, washing and examining in calcium-chloride-iodine solution. Laminated structure of first spore-wall and outer region of second spore-wall is well shown. Intimate union exists between remains of thickening layers of special-mother-cell walls and first spore-wall. Both first and second spore-walls colour yellow. × about 1100.

Fig. 25. Somewhat older spore treated similarly to the one drawn in Fig. 24. All the membranes colour yellow. × about 1100.

Fig. 26. Older spore from microtome section stained with Heidenhain's haematoxylin and bismarck-brown. Dark-coloured layer between inner and outer regions of the second spore-wall is very conspicuous. A similar, but narrow, dark layer lies between the second spore-wall and the endospore. × about 1100.

Fig. 27. Nuclei from developing spores:

(*a*) from spore with first spore-wall only;

(*b*) from spore with two spore-walls but second wall still homogeneous;

(*c*) from older spore with thick, differentiated second spore-wall.

PLATE XXII.

B. PHOTOGRAPHS.

Fig. 28. Archegonium showing fertilization of the egg-cell.

Fig. 29. Sporangium containing spore-mother-cells; calyptra two-layered.

Fig. 30. Spore-mother-cells becoming rounded off and separated from one another.

Fig. 31. Second division of the mother-cells and young tetrad. Secondary and tertiary thickening layers of the mother-cell wall still surround the periphery of the cells.

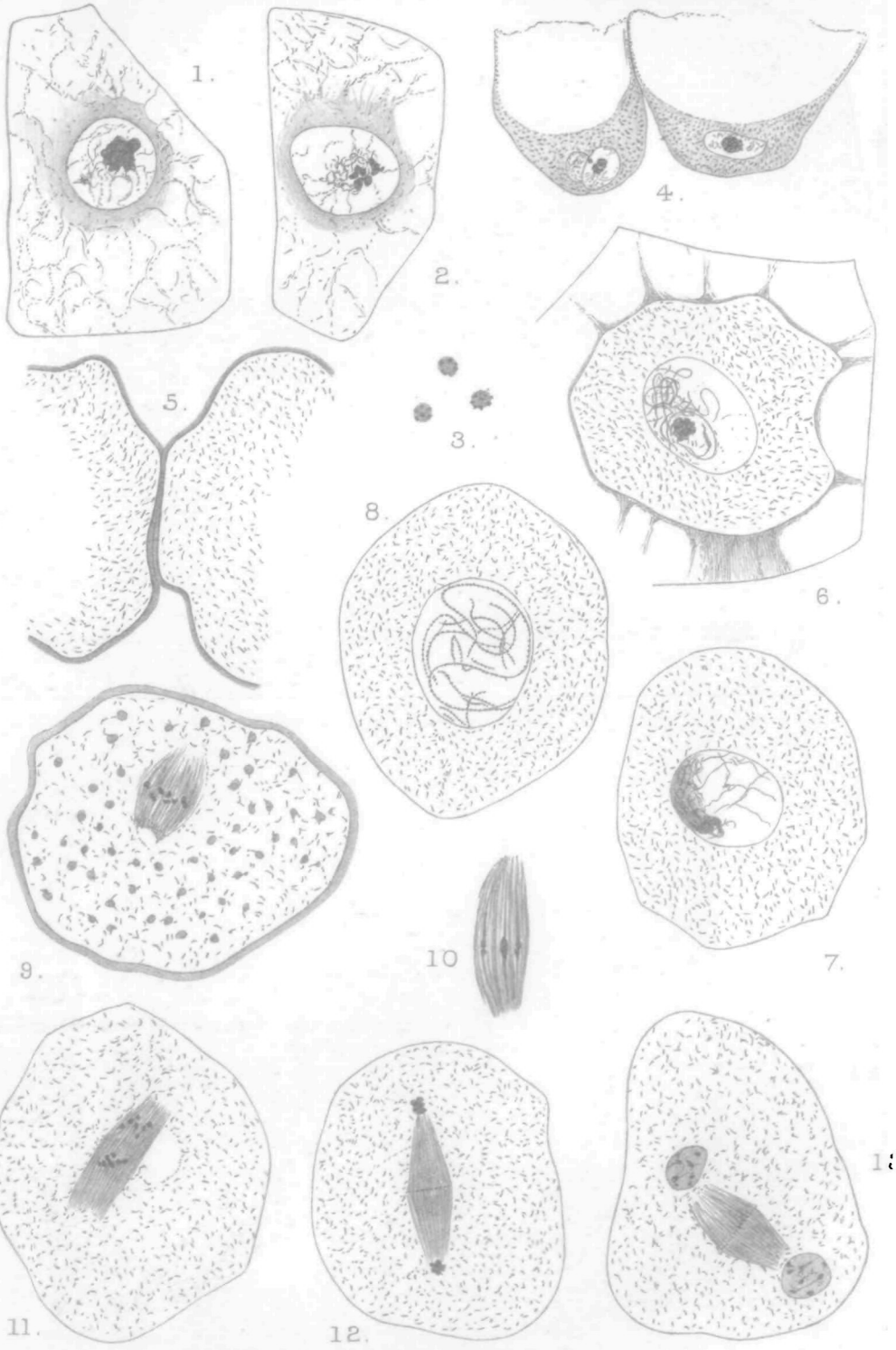
Fig. 32. First division of the egg-cell.

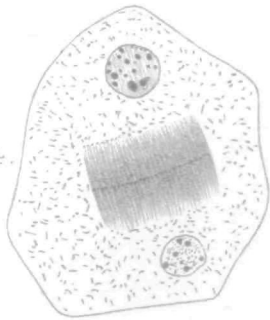
Fig. 33. Sporangium containing spores with two-layered coats. Note mucilage between the spore-tetrads.

Fig. 34. Spore at about middle period of its development treated with iodine solution to show distribution of starch.

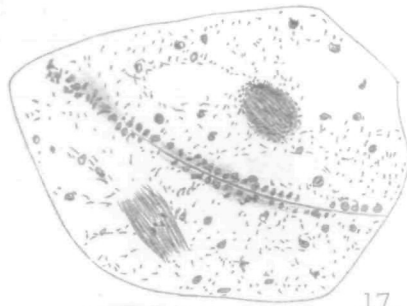
Fig. 35. Section of spore showing layers of its wall. Darkly coloured layer is particularly noticeable.

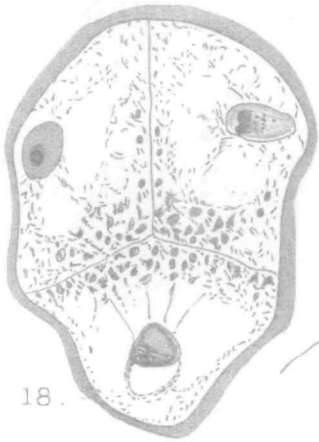
Fig. 36. Section of spores to show the new lamellae which are to be added to the thickness of the second spore-wall plasmolysed away from that wall together with the protoplast to which they are firmly fixed. These lamellae gave cellulose-pectose reactions.


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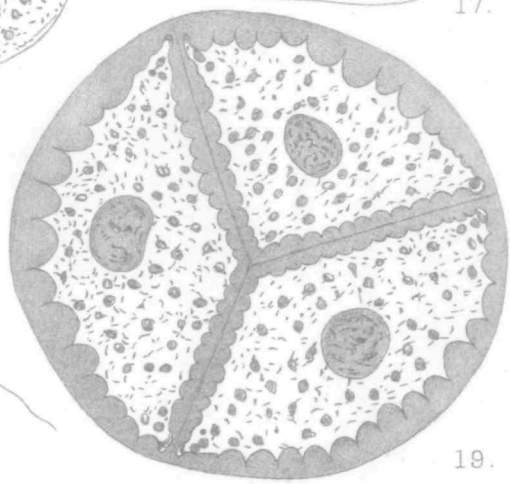

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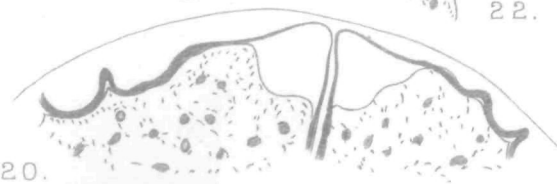

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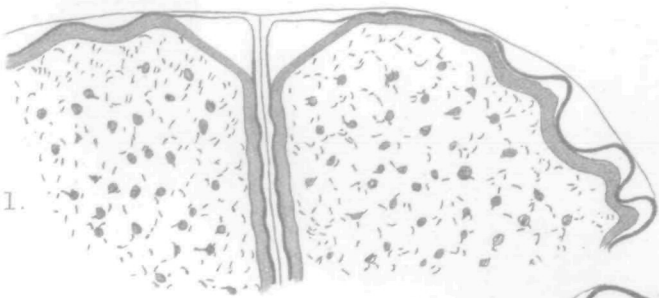

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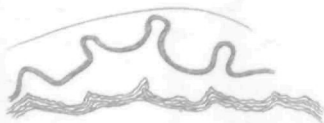

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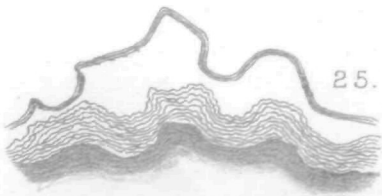

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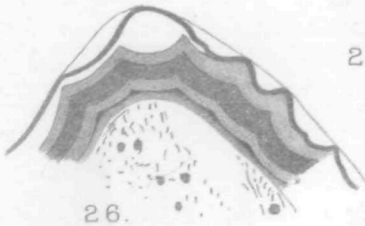

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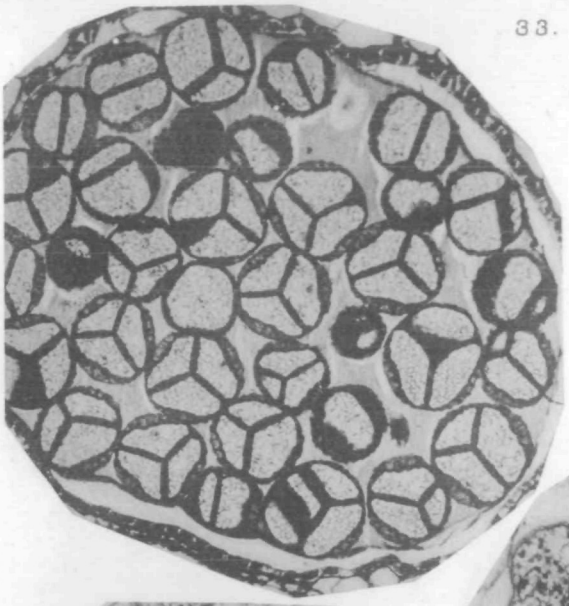

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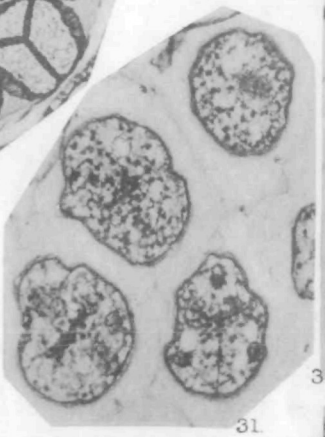
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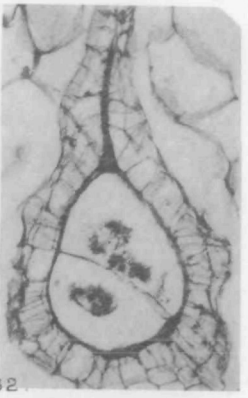
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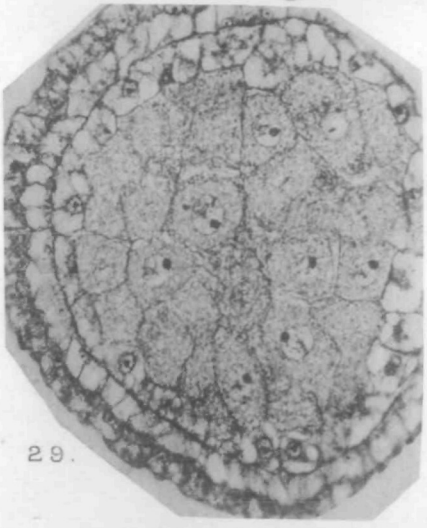
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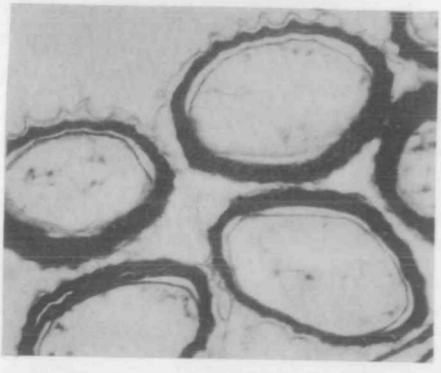
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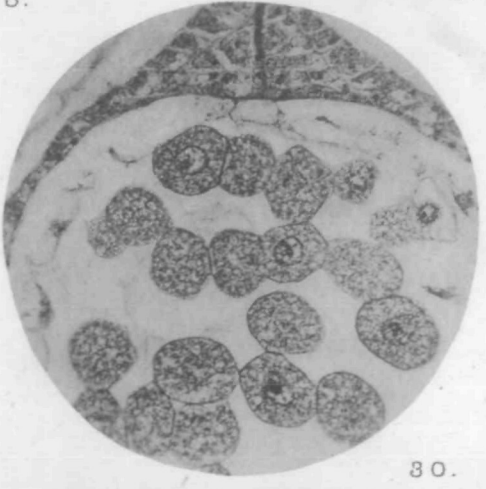
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