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EDITED BY

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KING'S BOTANIST IN SCOTLAND, PROFESSOR OF BOTANY IN THE UNIVERSITY AND KEEPER OF THE ROYAL BOTANIC GARDEN, EDINBURGH

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ASSISTED BY OTHER BOTANISTS

VOLUME XVIII

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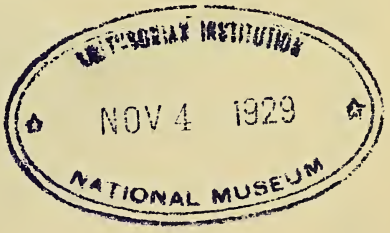

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The Gametophytes, Archegonia, Fertilization, and Embryo of Sequoia sempervirens.

BY

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With Plates I–IV.

INTRODUCTION.

ALTHOUGH investigations among the Gymnosperms, especially among the Cycadales and Ginkgoales, have, in the last few years resulted in most important and startling discoveries, this field of research has not received the attention it deserves. The literature on the Coniferales is gradually accumulating and revealing much that is of interest and of importance. These contributions are, however, too fragmentary to deserve the appreciation which they might otherwise receive.

Representatives of every family of the Coniferales have been investigated, especially in regard to the gametophyte generation. Of the numerous types selected by the various investigators many phases in the life-history have been revealed which are of great morphological interest and importance. In spite of the large number of forms that have been worked upon, however, *Pinus* is the only Conifer in which a connected account of the important events completing the life-cycle has been compiled. The works on the other Conifers are nevertheless of great importance, and although they are at present but disconnected chapters in the life-history, their true value will no doubt be fully appreciated as soon as the missing chapters have been written.

The interesting genus *Sequoia* is represented by two living species, *Sequoia gigantea* and *Sequoia sempervirens*. The former species is confined to very narrow limits in California, while the latter extends along the coast ranges of middle and northern California and for about twelve miles into the State of Oregon. Of the latter species there are at present over one hundred trees growing on the campus of Stanford University. As the majority of these trees are healthy and vigorous, and although young, produce cones every year, and especially as many of them grow in the immediate vicinity of the Botanical Laboratory, excellent opportunity for the daily collections of material was afforded. Taking advantage of these exceptional facilities, I have thought it worth while to work out the morphology of the gametophytes, with the hope of filling in the gaps left

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by Arnoldi, Shaw, and Strasburger, the only writers who have contributed to our knowledge of the gametophytes of this interesting Conifer.

It is therefore the object of the present work to give a connected account of the events leading to the development of the gametophytes, sexual organs, fertilization, and embryo, thus completing as far as possible the life-history of *Sequoia sempervirens*.

While the observations recorded by Shaw and Arnoldi are of great interest, they are by no means complete, and as we shall see later, some are even inaccurate.

Shaw (1896) has given a description of how the male and female flowers develop in *Sequoia sempervirens*. When very young he finds that the macrosporangium is about as long as it is broad. The integument at this time consists of an epidermis and two layers of hypodermal cells. The integument develops rapidly and soon comes to be about twice as long as the nucellus. Soon after pollination the upper and inner layers of epidermal cells enlarge and by their elongation finally close the micropyle. By this means the pollen-grains are completely enclosed in a subconical cavity at the apex of the nucellus. Within the nucellus several sporogenous cells now make their appearance. Shaw reports that these cells divide twice, each one giving rise to four macrospores. Upon germination the macrospores develop a number of female prothallia. As the embryo-sacs increase in size they contain several nuclei. The further development of the prothallia was not observed. The archegonia were found to be very numerous and distributed over the upper portion of the prothallium and each one has but a single neck-cell. At the time of pollination each microspore consists of two cells, a large central cell with a large nucleus and a much smaller cell. As the pollen-tube develops the nucleus of the larger cell moves forward and enters the tube. The tube extends down between the nucellus and the integument and as often as not it branches. The further course of the tubes was not followed. Later a number of long suspensors bearing the young embryos on their tips were formed in the endosperm.

Arnoldi (1900) has given a description of the manner of endosperm-formation in *Sequoia sempervirens*. According to this description the form of the embryo-sac may vary considerably. When young it consists of a very large central vacuole surrounded by a parietal layer of cytoplasm in which numerous nuclei are found. As the sac develops the parietal layer increases and the protoplasm accumulates in great abundance in the lower end and at the same time the free nuclei divide repeatedly. In the central region a portion of the vacuole remains, and here the cellular endosperm is formed by means of 'Alveolen' as Sokolowa describes for other Conifers, while the remaining endosperm is formed by ordinary free cell-formation. The development of the archegonia is confined to the tissue derived from the 'Alveolen,' so that the prothallium has a distinct generative region.

In the development of the endosperm in *Sequoia*, Arnoldi sees a striking similarity to that which occurs in *Gnetum*.

In the same year (1900) Arnoldi published some observations on the archegonia and pollen-tubes in *Sequoia sempervirens*. He finds that the archegonia arise from peripheral endosperm-cells and are present in large numbers and may appear singly or in groups. Each archegonium has two neck-cells but none contain a ventral canal cell. The position taken by the pollen-tubes is between the nucellus and the endosperm and they eventually lie opposite the archegonia.

In his more recent work, Arnoldi (1901) touches upon fertilization and the development of the embryo. In addition to *Sequoia* this short paper also discusses these phases in the life-history of *Taxodium*, *Cryptomeria*, *Cunninghamia*, *Glyptostrobus*, and *Sciadopitys*. In *Sequoia sempervirens*, which concerns us more particularly, he finds that the pollen-tube eventually contains two male cells and two free nuclei, of which one is the tube nucleus. At the time of fertilization the male cell becomes elongated or even spirally twisted. The male and female nuclei fuse in the middle of the egg and then move to the base of the archegonium, where the first segmentation-spindle is developed. Following this division two cells are organized, one behind the other. The lower of these divides again so that the embryo now consists of a row of three cells. The lower cell of the first division functions no further and soon becomes disorganized.

On the sporophyte of *Sequoia*, Peirce (1901) has contributed some interesting and important observations on fasciation, albinism and vegetative reproduction.

Methods.

There are few groups of plants that offer more difficulties in the way of cytological research than the Coniferales. The structures that are of greatest cytological interest are usually buried deep in the other tissues, thus requiring very careful dissection before being placed in the killing fluids. Then, again, if resin is present, as is usually the case, a rapid penetration of the fluid is impossible.

These and many other difficulties probably account for the fragmentary nature of the work that has been done. A brief statement of the methods adopted in the following work on *Sequoia* may be useful to others working in this field. The fixing fluids experimented with were as follows:—

1. Flemming's weak solution—
   25 c.c. of 1 % chromic acid
   10 c.c. of 1 % acetic acid
   10 c.c. of 1 % osmic acid
   55 c.c. distilled water.

2. Flemming's strong solution.
3. Chrom-Acetic mixture.
4. Chromic Acid—1 % sol.
5. Alcohol Acetic.

Of these Flemming's weak solution probably gave the best results although equally satisfactory fixation was generally obtained by the Chrom-Acetic and one per cent Chromic. The Alcohol Acetic solution proved to be a failure.

The fixing fluids were always taken into the field and the material deposited in them immediately. In the very early stages of the ovules and also of the pollen no dissection was necessary. On account of the air present in them these structures had a tendency to float. This difficulty was, however, overcome by sinking the material in the fluid by means of cotton plugs.

In the very early stages the entire ovules were removed and immediately killed without further dissection, but in all the later stages it was found necessary to remove the integument. This, however, was not resorted to until after the pollen-tube had penetrated the nucellus. To insure rapid fixation most of the dissections were made with the material immersed in the fluid. The ovules were removed one by one, placed in a watch-glass containing the fixing reagent and while in the fluid the integument was immediately removed by means of a sharply pointed scalpel and forceps. With a little experience this may be accomplished very rapidly.

The material was allowed to remain in the fixing fluid from ten to twenty-four hours and then washed in running water from four to six hours. Care was now taken in transferring the material to alcohol. For this purpose Schleicher and Schüll's diffusion shells were used. The shells were cut to the height of small beakers and the material placed in the bottom, and 95% alcohol placed in the beakers. Water was now poured in the shell in sufficient quantity to make the combined solutions about 70% alcohol. By placing the shell, containing the material and water, in the beaker containing the 95% alcohol, a gradual diffusion took place which was not sufficiently rapid to cause shrinkage. In two or three hours the material was transferred directly to 95% alcohol. I found the shells much more convenient than ordinary parchment paper.

In preparation for imbedding the material was thoroughly dehydrated in absolute alcohol. Bergamot oil was used to precede the infiltration of paraffin. After dehydration the material was placed in a mixture of 1 part absolute alcohol and 1 part bergamot oil; then into pure bergamot oil; then into a mixture of 1 part Bergamot oil and 1 part melted paraffin; and finally into pure paraffin.

Minot's wheel microtome was employed for cutting and the sections varied from 2 μ to 8 μ in thickness according to the detail desired.

It was found very desirable to use albumen instead of alcohol as a fixative. When the staining was not satisfactory, the sections fixed on the slide with albumen were bleached and restained without trouble. With the alcohol method, however, restaining was impossible, as the sections were invariably washed off the slide. By restaining, many valuable demonstrations were restored.

The triple stain safranin, gentian, and orange G., was found to be the most satisfactory in differentiating the various cell-structures.

The Male Gametophyte.

The reduction-division of the microspore mother-cell leading to the formation of the tetrads takes place during the first week in December. The first division is rapidly followed by the second, and within a week or ten days after the first division the tetrads have separated and the pollen-grains formed.

The microspores remain within the sporangium at least three weeks before pollination takes place. During this period they become larger, spherical in form, and surround themselves with a hard thick wall. The cytoplasm is very granular and contains a small amount of starch. The nucleus is comparatively small and is always centrally situated (Pl. I, Fig. 1). While yet in the sporangium and about a week before pollination the nucleus of the microspore enlarges and divides ; so that at the time the pollen is shed there are two nuclei in each grain. Sections made before and after pollination showed a considerable difference in the size of the nuclei, the one being about twice the size of the other. The larger one was centrally situated, while the smaller one was invariably found near the spore wall. The smaller nucleus was surrounded by a sharply differentiated zone of very granular cytoplasm, which suggested the presence of a membrane between the two nuclei as shown in Fig. 2. The chromatin of the smaller nucleus was in the form of small granules closely packed together ; it consequently stained more deeply than the larger nucleus, where the meshes of the chromatin network appeared to be much more loosely arranged. A study of the further history of these nuclei has convinced me that the larger nucleus is the so-called tube-nucleus and the smaller one represents the generative cell.

A very careful search was made with the hope of finding a vestige of the vegetative tissue of the gametophyte. One or more vegetative cells have been reported for the Cycads *Ginkgo* and *Pinus*, but a most searching examination failed to reveal even a vestige of such a cell or nucleus in *Sequoia*. I am strongly inclined to believe that the development of these evanescent structures has been entirely suppressed.

Observations of two years indicated that pollination takes place during the first week in January, just about the time the female flowers

make their appearance. During this time the trees are constantly enveloped in a cloud of pollen, so that it would be almost impossible for any of the exposed ovules to escape the reception of at least a few of the grains. At this time the integument of the ovule is about on a level or a little above the apex of the nucellus, and from four to six pollen-grains are here deposited. The grains remain in this position for three or four weeks without further germination, when the integument grows over them and closes the micropyle in the manner described by Shaw (1896).

The first indication of the further germination of the pollen-grains was the splitting off of the hard thick wall. (If some ripe grains are examined in water under the microscope, it will be seen that the casting off of the outer wall takes place suddenly and with considerable force, leaving a thin delicate membrane underneath.) The pollen-tubes now push out over the tops of the nucellus, and one or two of them may grow down between the nucellus and the integument, as shown in Fig. 3. Material collected during the first week in March frequently showed the pollen-tubes extending more than halfway down the side of the nucellus. In such cases the tube-nucleus was invariably near the tip of the tube, with the generative nucleus considerably in the rear. Both nuclei were found in the central axis of the tube, suspended in a broad strand of cytoplasm which contained an abundance of starch grains.

While one or two of the tubes may follow the course between the nucellus and the integument, others may penetrate the nucellus immediately at the top, as shown in Fig. 4. The penetration of the tube is accompanied by a breaking down and a probable absorption of the cells of the nucellar tissue through which the tube forces its way. From a study of longitudinal and cross-sections in series it became quite evident that the course taken by the pollen-tubes may vary considerably. At a later stage cross-sections of the endosperm showed the tubes in various positions. Usually three or four tubes develop and become functional. In the majority of cases they are found situated between the female prothallium and the remaining tissue of the nucellus. Many were found partially surrounded by endosperm, while others were completely surrounded. In no case was I able to find any evidence of branching of the tube, although Shaw (1896) reports that 'quite as often as not it branches.'

Just about the time the tube penetrates the wall of the nucellus, the generative nucleus, having increased to quite the size of the tube-nucleus, divides. As shown in Fig. 4, there are now three nuclei in the tube, one large one and the two smaller ones. The largest of these, situated nearer the tip of the tube, is no doubt the tube-nucleus, while the other two are the stalk- and body-nuclei. Of these latter two there is a slight difference in size. As the larger one appears to be preparing for further activity, I regard it as the body-nucleus and the smaller one as the stalk-

nucleus. During the further development of the pollen-tube, the three nuclei remain in close proximity to each other. During these changes the size of the tube-nucleus remained the same, while that of the stalk-nucleus increased slightly. The body-nucleus, however, increased to at least three or four times the size of the stalk-nucleus, as shown in Fig. 5. During its development the body-nucleus surrounds itself with a dense zone of granular cytoplasm. This zone increases until it is about half the diameter of the nucleus in thickness, when it becomes shut off from the rest of the cytoplasm in the tube by a distinct membrane. The tube now contains one large cell and two free nuclei (Fig. 6).

Previous to the formation of the body-cell, the free nuclei lie close together suspended in the same strand of cytoplasm, which contains an abundance of starch. The most of the starch was present in the vicinity of the body-nucleus, and it later becomes confined within the cytoplasm of the body-cell. The stalk-nucleus remains close to the body-cell (Fig. 5), even up to the time the male cells are formed.

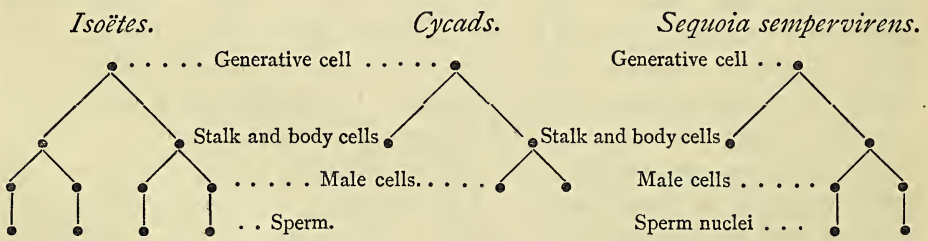
The changes resulting in the organization of the body-cell showed considerable variation as to the time of their occurrence. In some cases the mature body-cell was found in material collected early in May, while others were found as late as the middle of June. This irregularity as to the time of the changes was also noticeable in all later changes in the development of the male prothallium, even in the matter of fertilization.

Soon after the body-cell has been fully organized, its nucleus enlarges and prepares for division. By extreme good fortune the spindle of this division was found. As this is the division which results in the formation of the two male cells, it demands a careful examination. It was during the division of the body-cells in *Cycas*, *Zamia*, and *Ginkgo* (Hirase, 1898; Ikeno, 1896–8; Webber, 1897–1901) that the centrosome-like bodies known as blepharoplasts were discovered. It was thought probable that a vestige of such an organ might be found in *Sequoia*, but an examination of the cytoplasm surrounding the spindle failed to reveal a trace of any body that might be interpreted as a blepharoplast. It must be remembered, however, that the blepharoplasts are only concerned with the development of the cilia, and as these latter structures have never been found in connexion with the male cells in Conifers, it is not surprising that the organs responsible for their formation should also be missing. Fig. 7 shows the spindle dividing the body-nucleus with the daughter-nuclei at the poles. The formation of the cell-plate that separates the nuclei and divides the body-cell into two was not actually observed. But an examination of Fig. 7 where the connective fibrils curve out laterally, and of Fig. 8 where the two daughter-cells are lying side by side, makes it obvious that the cell-plate is developed in the usual way. After the wall separating the male cells has been formed, the latter remain close together for some

time, and a section of the two together has the outline of an ellipse that has been cut in half. They are rounded on one side and flat on the other. They are of equal size, and, as we shall see later, are both functional. Just before the male cells separate from each other, the nucleus in each has increased to about twice its original size. The chromatin is in the form of a network which contains a large irregularly shaped nucleolus.

At the time of fertilization the male cells become almost spherical, and are perfectly similar in regard to their size and structure. Arnoldi (1901) has reported that the male cells may become elongated, and he figures one that has a spirally twisted form. I was unable to find such conditions, and feel confident that they are abnormal or due to shrinkage by poor fixation. In all the cases I have examined the male cell was spherical, and, as we shall see later, its spherical form may persist for some time after its nucleus has been injected into the egg.

As we shall point out later, under the head of fertilization, only the nucleus of the male cell enters the archegonium, the rest of the male cell remaining outside in the pollen-tube. The nucleus is first liberated and, with but a small film of protoplasm surrounding it, passes between the neck-cells of the archegonium and immediately fuses with the egg-nucleus. In all other Gymnosperms in which observations have been recorded, at least one male cell enters the archegonium, so that in this respect the spermatogenesis of *Sequoia* is unique. According to the following diagram, Coulter and Chamberlain have compared the spermatogenesis of the Cycads with that of Isoëtes.



It will be observed that the male gametophyte is complete with the organization of the male cells, and this is true for all other gymnosperms where spermatogenesis has been worked out. If, however, we construct a similar diagram for *Sequoia*, the additional step of the discharge of the male cell-nucleus suggests more strongly the spermatogenesis of the Pteridophytes than even that of the Cycads, although the male cells of the later are ciliated.

The Female Gametophyte.

Shaw (1896) has given an accurate account of the development of the macrosporangium and the integument, but has, however, given a very meagre description of the sporogenous cells and the macrospores. There may be as many as five or six macrospore mother-cells organized from the hypodermal cells of the sporangium. Many preparations were made of this stage in the development of the sporangium, and a special study was made of the mother-cells as soon as they became differentiated from the surrounding sterile cells. They first become recognized as mother-cells by their large deeply staining nuclei. In the beginning they are not much larger than the other hypodermal cells, but they very soon enlarge, and their cytoplasm becomes very dense and granular and stains very readily with orange G. They are further characterized by the absence of large vacuoles. They are situated just about the centre of the sporangium; about five or six layers of cells lying between the uppermost of them and the epidermis at the apex. A careful study was made of the number of mother-cells formed, and there seems to be a slight variation in this respect. Six was the largest number found. In all the sporangia studied five or six was the prevailing number, but in no case were fewer than four found.

Shaw (1895) has reported that these sporogenous cells divide twice, each cell giving rise to four spores. As there are nearly always five or six of these sporogenous cells developed, and if each one gave rise to four spores, this would result in the formation of twenty or twenty-four macrospores. We shall show later that no such large number of macrospores were formed, but that, on the contrary, ten or twelve were the prevailing numbers met with. Owing to Shaw's statement, it was thought probable that some of the sporogenous cells had failed to divide and that others had divided twice. Accordingly a very vigorous search was made for the stages showing a second division. Although the spindles of the first division were found frequently, some with the chromosomes at the equator of the spindle (Fig. 10), and others with the daughter-nuclei formed, in no case was there any evidence of four spores having been formed from a single mother-cell. From these observations I feel tolerably certain that the five or six sporogenous cells (Fig. 9) differentiated from the hypodermal cells of the sporangium are the macrospore mother-cells, and after dividing twice give rise to the ten or twelve macrospores; one cell of the second division fails to develop.

Soon after their organization, about the first of March, the mother-cells divide, each producing two macrospores. As this division of the mother-cell is the reduction-division, which marks the beginning of the gametophyte, the character and number of the chromosomes demanded considerable attention. As far as the writer is aware, the reduction of the chromosomes

in the division of the macrospore mother-cells has never been actually observed in any of the Conifers. It was hoped that, as the spindles were found, some light might be thrown upon this most important step in the life-history of *Sequoia*, but after the examination of the chromatin, all such hope was lost. The spindle of the reduction-division was found in several preparations, but the chromosomes were too many to allow of an accurate estimate of their number. To add to the difficulty, the chromosomes were in the form of large V-shaped structures. The arms of the V were very long, and when at the equator they extended almost to the poles of the spindle. It also invariably happened that several of the chromosomes overlapped their neighbours, making it almost impossible to observe the method of splitting and separation. It was interesting to note, however, that as the daughter-chromosomes approached the poles of the spindle they were very much smaller than the mother-chromosomes at the equator; this condition, however, was also observed in the divisions during the formation of the prothallium and also in the young sporophyte. Although the actual reduction could not be observed in the division of the macrospore mother-cell, we shall point out later that the number of chromosomes in the prothallium and in the development of the archegonium is obviously half that in the embryo. Reduction-spindles are shown in Fig. 10.

Soon after the macrospores are formed, they surround themselves with distinct walls and almost immediately begin to germinate. The germination is first noticeable by a slight increase in the size of all of the spores. This increase in size is always accompanied by a division of the nucleus, so that each of the ten or twelve germinating spores or female prothallia has two free nuclei in its cytoplasm. Their further growth is apparently at the expense of the sterile tissue in which they lie imbedded. The cells in this tissue, in contact with the young growing prothallia, show every sign of disorganization; the nuclei appearing as homogeneous deeply staining masses lying in the distorted and more or less fragmented cells. At this stage (Fig. 11) there are about eight or ten layers of sterile cells between the uppermost macrospore and the apex of the sporangium. The majority of the young prothallia show no further development after the first nuclear division, but two or three of them continue to elongate, and their increase in length is always directed toward the chalaza. Such a condition is shown in Fig. 11. Here eight young prothallia are represented, five of which have grown but very little, while the remaining three are many times the size of the original spores. The smaller prothallia function no farther, and are probably absorbed by the growth of the larger ones. Of the two or three larger prothallia represented at this stage, and which continue their growth down through the nucellar tissue, one grows more rapidly than the others, and for convenience we will speak of it as the primary prothallium. The one or two remaining prothallia persist in their further development,

and since (as we shall point out later) they have a considerable influence on the ultimate form of the primary prothallium, we will designate them as the secondary prothallia.

The growth of the primary and secondary prothallia is always accompanied by a rapid division of their free nuclei. Stages were found showing two, four, eight, sixteen, and thirty-two free nuclei. It was too difficult to count the numbers greater than this in sections, but this was sufficient to indicate that there is a regular and successive division of all the free nuclei, at least in these early stages. By the time the prothallia have reached the two or four nuclei stage the sporangium has increased to over twice its length. As Shaw (1895) has pointed out, the growth resulting from the elongation of the nucellus is very much greater in the region between the spores and the chalaza.

At the time when there are eight or sixteen nuclei in the prothallium, as shown in Fig. 12, there is a layer of cytoplasm lining the walls, and there may be several vacuoles separated by strands of cytoplasm which contain many starch-granules. The nuclei at this time are suspended in a broad strand of cytoplasm which runs the length of the young prothallium and are usually arranged in a row, as shown in Fig. 12.

The further increase in the size of the primary prothallium is always accompanied by an increase in the size of the vacuoles as well as an increase in the number of free nuclei. Although the primary prothallium is at this time somewhat larger than the secondary, the two present much the same condition. From now on, however, the rate of their development is very different. The development of the primary prothallium proceeds very rapidly, and is followed very slowly by that of the secondary, which, as we shall see later, never produces true cellular prothallial tissue.

The initial stages in the development of the prothallium agree in general with the conditions found in *Taxus* by Jäger (1899) and Campbell (1902), and in *Pinus* by Coulter and Chamberlain (1901), with this difference, that more than one prothallium in *Sequoia* reaches an advanced stage of development. The following events, however, which lead to the formation of the cellular prothallium differ in many interesting respects from any of the Conifers in which the endosperm-formation has been worked out. As Arnoldi (1899–1900) has pointed out, the events are strikingly similar to those found by Lotsy (1899) in the formation of the endosperm in *Gnetum*.

As the vacuoles in the primary prothallium grow, they eventually fuse together to form an enormous single vacuole which forces the cytoplasm and the nuclei to the walls. As represented in Fig. 13, the prothallium now consists of a large central vacuole, surrounded by a comparatively thin layer of cytoplasm in which a large number of free nuclei are distributed along the wall which surrounds the whole structure. The amount of cytoplasm

now increases considerably, and free nuclear division proceeds at a rapid rate. The cytoplasm and nuclei, as they increase, accumulate in greater abundance at the lower end, and as this accumulation goes on, the large vacuole becomes correspondingly smaller and is confined to the upper end of the prothallium. Up to this time the shape of the prothallium has been more or less irregular in outline, the upper region remaining more or less constricted as compared with the broad middle and lower portions, and there has been no trace whatever of any cell-plate formation between the free nuclei. A portion of the prothallium in this condition is shown in Fig. 14.

In his recent work on the formation of the endosperm in *Sequoia sempervirens*, Arnoldi (1900) finds that as the parietal layer of cytoplasm thickens it accumulates in great abundance at the lower end of the prothallium. Free cell-formation now proceeds in the lower and upper regions, but in the region surrounding the vacuole, structures ('Alveolen') similar to those described by Mlle. Sokolowa (1891) in the formation of the endosperm of other Conifers are organized. The 'Alveolen' ultimately give rise to ordinary cellular tissue. He also reports that the archegonia are developed only in the tissue derived from the 'Alveolen.'

My own observations on the thickening of the parietal layer and the accumulation of the cytoplasm and nuclei in the lower and upper regions agree very closely with those of Arnoldi. I was, however, unable to observe the formation of 'Alveolen' as he describes. As the long narrow vacuole continues to decrease in size, the cells surrounding it become larger. And these are no doubt the structures Arnoldi interprets as 'Alveolen.' As we shall point out later, the archegonial initials are not confined to this region.

A careful study of this stage in the development of the prothallium has convinced me that the final division of all the free nuclei which immediately precedes cell-plate formation is nearly simultaneous. In a single section over two hundred and fifty mitotic figures were counted. Many of these spindles showed the chromosomes at the equator. Some of them, even at the lower and the upper regions, showed the daughter-nuclei formed with the connective fibrils between them (Fig. 15). The nuclei in the vicinity of the vacuole divided in the same way and at the same time as the other nuclei, and I therefore could not confirm Arnoldi's observations on the formation of 'Alveolen' in this region. That this was the final division of the free nuclei was shown quite conclusively by the fact that cell-plate formation had already begun between some of the daughter-nuclei in various regions of the prothallium.

With so many nuclei undergoing division just about the same time they presented every possible phase of mitosis. Some of the nuclei were enlarging and just preparing to divide, many showed the chromosomes at the equator, others showed the chromosomes on the way to the poles, others again

showed the daughter-nuclei well organized, with the kinoplasmic fibrils stretching between them. As near as could be estimated, the number of chromosomes appeared to be sixteen. These various stages of the dividing nuclei had an influence upon the rate at which the cell-plates were formed, and consequently the various regions of the prothallium showed all stages in the development of the plates.

While the daughter-nuclei resulting from this division are being organized, the continuous fibrils of the spindle persist and increase in number; the result is that each daughter-nucleus is surrounded by a system of kinoplasmic radiations. These radiating fibrils not only join the sister-nuclei but they connect with the fibrils radiating from the other neighbouring nuclei. The result is that certain regions of the prothallium show large numbers of nuclei all joined together by systems of radiating fibrils, as shown in Fig. 15. Each nucleus at this time is enveloped in a dense granular zone of cytoplasm, from which the system of kinoplasmic fibrils radiates. The radiations show all the characters of ordinary spindle-fibrils, and they apparently have the same origin, that is, they are differentiated out of the cytoplasm. The first indication of the plate appears in the form of small granules or thickenings on the fibrils. These thickenings occur about midway between the nuclei, and as they increase in size the fibrils become less numerous in the vicinity of the nuclei. This would suggest that the fibrils were transformed into plate-forming substance, in much the same way as Timberlake (1899) has indicated. As illustrated in Fig. 16, the thickenings occur on all the fibrils stretching between the nuclei, so that each nucleus becomes completely boxed in by the developing plates. Different regions of the prothallium showed various stages in the formation of the plates. In a single section we may have conditions represented in Fig. 15 and Fig. 16, or where the daughter-nuclei are just being organized.

In the upper portion of the prothallium some of the cells may be very large and elongated. These are no doubt the structures which Arnoldi (1901) has described as 'Alveolen.' I find, however, that the archegonial initials may develop much below this region, and I therefore cannot endorse Arnoldi's view that there is a distinct generative tissue in the prothallium of *Sequoia sempervirens*.

It is interesting to note that Arnoldi has reported a very different method of endosperm-formation in *Sequoia gigantea*. Here the entire endosperm is formed in much the same way as in other Conifers described by Sokolowa (1891), that is, by means of 'Alveolen.' When such great difference exists between two species of the same genus, it tends to eliminate the endosperm-formation as a means of establishing relationships among the Coniferales.

During the development of the primary prothallium as described

above, one or two of the secondary prothallia have continued their growth, but at a much slower rate. Previous to the formation of the cell-plates, it is difficult to distinguish between the primary and the secondary prothallia, but after free cell-formation has ceased in the primary prothallium, the form of the secondary prothallium becomes well defined. Its course is long and tortuous, and in many cases winding in and out through the tissue of the primary prothallium, as illustrated in Fig. 18. Its shape is greatly modified, as its growth is limited to the spaces left by the more rapidly growing primary prothallium. Instead of having a single large vacuole and a parietal layer of cytoplasm, several long narrow winding vacuoles are present, separated by strands of cytoplasm. In several cases the spindles were found, showing that nuclear-division is carried on in the same manner as in the primary prothallium. In no case, however, was there any trace of cell-plate formation observed, so that I am inclined to believe that the secondary prothallia never develop sufficiently to produce cellular prothallial tissue.

A very interesting relationship between the primary and secondary prothallia was noticed, which no doubt explains the sluggish development of the latter. After the cell-plates were formed in the primary prothallium, the parietal protoplasm of the secondary prothallium clings very closely to the cells of the former. These cells grow out into the free protoplasm and evidently act as absorbing organs. This intimate relationship is shown in Fig. 19. Here the newly formed cells are seen projecting into the protoplasm of the secondary prothallium in a dovetail fashion. The nuclei of these cells are very much larger than the others, and have the appearance of being engaged in very active metabolism. There is no doubt that these projecting cells absorb the protoplasmic substance of the secondary prothallium, and thus retard its development.

The primary prothallium of *Sequoia* takes just about three months to mature. The macrospores are formed during the first week in March, and the first archegonial initials were observed in material collected June 8. At this latter date the nucellar tissue had been almost completely absorbed, little more than the epidermal layer of cells remaining. Within the integument we now have a most confusing complex of structures, for in addition to the primary cellular prothallium there are usually present one or two secondary prothallia in an advanced stage of development and three or four pollen-tubes.

The Archegonia.

Very soon after the nuclei of the endosperm have been shut off from each other by the cell-plates, and after true cellular prothallial tissue has been organized, certain cells in the upper half of the prothallium become differentiated into the archegonial initials. These cells are quite numerous, and occupy a position not at the periphery, but near the central axis of the

prothallium. In cross-sections of this region, as shown in Fig. 26, they may be easily distinguished from the surrounding vegetative cells. When first differentiated, their distinguishing characters are their large size, highly granular cytoplasm, which stains readily with orange G., and each one has a large, centrally situated, deeply staining nucleus. When first distinguishable, the archegonial initials are not much larger than the ordinary vegetative cells by which they are surrounded. They rapidly increase in size, however, and are soon several times their original dimensions. As they do not all enlarge simultaneously, they present various shapes and sizes, and sections very frequently showed the matured archegonia and the young initials in the same plane. As the initial grows in size, the cytoplasm appears more conspicuously granular, and the nucleus becomes much larger as it prepares for the division which cuts off the primary neck-cell. This first mitosis of the archegonial initial takes place during the latter part of June. Sections of material collected June 25 showed an abundance of the various stages of the spindle. Fig. 20 shows one of them with the chromosomes on the way to the poles. These various stages of mitosis afforded an excellent opportunity of confirming the observation made during the endosperm-formation, that the number of chromosomes in the gametophyte is half that of the sporophyte. A comparison of Fig. 20, which represents the last spindle but one in the life-history of the gametophyte, with Fig. 32, which represents the first mitosis in the history of the sporophyte, shows without much question the difference in the number of the chromosomes in the two generations.

In addition to the archegonial initials numerous jacket-cells are also differentiated. These are distributed very irregularly among the archegonia. Some archegonia may be almost surrounded by the jacket-cells, while others may be completely devoid of them. In the early stages of differentiation it is impossible to distinguish between the jacket-cells and the archegonial initials. They are structurally identical in regard to their cytoplasm and nuclei. This fact, accompanied with their irregular distribution over the prothallium, suggests very strongly that the jacket-cells are archegonial initials which have become sterile.

As soon as the first mitosis is completed, the archegonial initial is divided into two by a distinct cell-wall. The two cells thus formed are the central cell and the primary neck-cell. The central cell now grows very rapidly, and, as shown in Fig. 21, comes to be many times the size of the primary neck-cell. It was noticeable that the elongation of the central cell always took place in the direction of the neck-cell, so that the latter was forced forward toward the periphery of the prothallium. As it is pushed forward by the elongation of the central cell, the neck-cell divides, so that before it reaches the periphery of the prothallium the archegonium has two distinct neck-cells. It may be mentioned that Shaw (1895) reported the

presence of but a single neck-cell in *Sequoia sempervirens*, but this error has recently been corrected by Arnoldi (1900–1), who has found two. It was noticeable in examining my preparation that the two neck-cells were not always seen in longitudinal sections, but in cross-sections they were very distinctly made out. The neck-cells are not only to be distinguished by their position and shape, but their contents differ from those of other prothallial cells. The cytoplasm is devoid of large vacuoles, and, being highly dense and granular, stains more readily with the orange G. than do the other cells.

My observations agree with those of Arnoldi (1901), both in regard to distribution of the archegonia over the upper half of the prothallium and that two is the typical number of neck-cells. I have, however, found four distinct neck-cells in a considerable number of archegonia (Fig. 24). This larger number is, however, exceptional. A somewhat similar variation in the number of neck-cells has been reported by Murrill (1900) for *Tsuga*, and according to Coker (1901) the number of cells in the neck of the archegonium in *Podocarpus* may vary from two to twenty-five.

It was interesting to observe the position that many of the archegonia take in relation to the pollen-tubes. Long before the archegonia are formed, the pollen-tubes have grown down and their courses are well established, so that their growth is not directed toward the archegonia. On the other hand, the growth of the archegonia is invariably directed towards one or the other of the pollen-tubes. It has been pointed out (p. 6) that the course of the pollen-tube varies considerably. Some may grow down alongside the prothallium, others are partially surrounded by prothallial tissue, and others completely surrounded. In every case there were found numbers of archegonia pointing towards one or the other of the tubes, and their necks in contact with the tube-wall (Fig. 27).

For some time after the neck-cells have been organized, the central cell is completely filled with a very dense granular cytoplasm, in the centre of which is suspended a very large nucleus. The next step in the development of the egg-cell is important and interesting, because it bears directly on the question of the general occurrence of the ventral canal-cell in the Conifers. Arnoldi (1900), in his careful investigations, failed to find any vestige of such a cell in *Sequoia*. He also denies its existence in *Cryptomeria*, *Cunninghamia*, and *Taxodium*. On the other hand, the ventral canal-cell or nucleus has been found by Strasburger (1879) and Belajeff (1893) in *Juniperus*; by Blackman (1898), Chamberlain (1899), and Ferguson (1901) in *Pinus*; by Coker (1900–2) in *Taxodium* and *Podocarpus*; by Murrill (1900) in *Tsuga*; and by Land (1902) in *Thuja*. In *Juniperus*, *Pinus*, and *Tsuga* the spindle dividing the central nucleus into the egg and ventral nuclei has been described and figured, so that there can be little doubt of its presence in these forms at least.

After a very careful search I was unable to find the spindle that gives rise to the ventral canal-cell nucleus in *Sequoia*, but enough evidence was found to convince me that such a nucleus is cut off from the central nucleus. At a stage soon after the neck-cells have been organized, several archegonia showed two distinct nuclei in the cytoplasm. As shown in Fig. 22, the nuclei are of the same shape and size, and are situated at opposite ends of the archegonium. The nucleus at the base of the archegonium becomes the egg-nucleus, and there is little doubt that the one nearer the neck-cells represents a vestige of the ventral canal-cell. In *Pinus* and *Tsuga* a distinct cell-plate is formed, which separates the ventral canal-cell from the egg-cell. In the majority of the Conifers investigated, however, the ventral canal-cell is only represented by a nucleus, no cell-plate being formed. This is evidently the case in *Sequoia*, for the ventral canal-cell nucleus functions no farther, and very soon becomes disorganized. In one or two archegonia it was found more or less flattened against the neck-cells, and in others it was more or less fragmented. That this is not the nucleus of the male cell was shown quite conclusively by the fact that the neck-cells were in no way disturbed. It apparently breaks down soon after it is cut off from the central nucleus. By the time the archegonium is ready for fertilization the ventral canal-cell nucleus has entirely disappeared. This very short period of its existence probably accounts for its having been overlooked by Shaw and Arnoldi.

Soon after the disappearance of the ventral canal-cell nucleus, the egg-nucleus moves forward and occupies a position about halfway between the centre of the archegonium and the neck-cells. Meanwhile a large vacuole is developed in the lower part of the archegonium, as shown in Fig. 23. This figure shows a typical mature archegonium ready for fertilization.

Fertilization.

It has already been pointed out that the courses taken by the pollen-tubes have been well established long before the archegonia have been organized. It is therefore obvious that the direction taken by the tubes is not at all influenced by the female organ. On the other hand, however, it would seem that the pollen-tube had an influence upon the direction taken by the developing archegonia, for the growth of these latter structures is almost invariably directed towards the nearest tube. Fig. 27 shows how the archegonia appear in a cross-section of the prothallium. In this section there are four archegonia arranged in a semicircle, and with their neck-cells in contact with the wall of the pollen-tube. A longitudinal section would show ten to fifteen archegonia in a row, with the neck-cells directed toward the tube. The archegonia do not all point toward one tube, but each tube —and there are usually three or four present—has a number of archegonia directed toward it. Considering the diverse positions occupied by the

tubes, this position is peculiar, as it suggests a case of the female organ seeking the male.

Owing to the large number of archegonia present, and their peculiar arrangement around the pollen-tubes, fertilization is easily accomplished. When the archegonia are mature, that portion of the wall of the tube opposite the opening between the two neck-cells is the only structure intervening between the egg and the male cells. At this time the two male cells have become spherical and lie one behind the other in the tube, and take up a position near the wall which touches the neck of the archegonia. The nucleus of the male cell is very large, containing one or two large nucleoli, and a very regular network of chromatin. The latter is in the form of small spherical granules suspended on the threads of linin. The egg-nucleus is slightly different in this respect. The chromatin here is much more finely granular, and consequently does not stain as deeply as the male nucleus.

The wall of the tube opposite the archegonium about to be fertilized apparently dissolves, for a small portion of the male cell now penetrates the archegonium by forcing the two neck-cells asunder, as shown in Fig. 29. Through the narrow communication thus established the male nucleus finds its way into the archegonium. As it squeezes through the narrow passage between the neck-cells, it becomes constricted and much elongated, as shown in Fig. 31, and immediately fuses with the egg-nucleus. It is a remarkable fact, as shown in Fig. 29, that only a very small amount of the cytoplasm of the male cell enters the archegonium. After the discharge of the nucleus into the archegonium, the greater part of the male cell remains outside and is discarded. As far as I am aware, such a condition has never before been reported for any of the Conifers, and it goes to establish the generally accepted view that the essential thing in fertilization is the fusion of the nuclei. As shown in Fig. 29, the denucleated male cell retains its spherical form, but has a vacuolated appearance in the central region once occupied by the nucleus. The section immediately following the one from which this figure was drawn showed the nucleus of this male cell in the act of fusing with the egg-cell in the archegonium. An examination of a large number of preparations has convinced me that this is the typical method of fertilization in *Sequoia sempervirens*. For some time after fertilization has been affected, the denucleated male cell is invariably found in the tube outside of the archegonia. Even as late as the early stages of development of the embryo, shrunken fragments of it were found. According to Coulter and Chamberlain (1901), nearly the whole of the contents of the tube in *Pinus* is injected into the cytoplasm of the egg. Goroschankin (1883) reports that both male cells pass into the archegonium, and Strasburger (1884) finds a similar occurrence in *Picea vulgaris*. In *Pinus silvestris*, Dixon (1894) and Blackman (1898) find that all the structures present in the pollen-tube pass into the egg. This has also been observed by Ferguson (1901) in

Pinus Strobus, in *Taxodium* by Coker (1900), and in *Cephalotaxus* by Arnoldi (1900). It therefore happens that at the time of fusion of the male and female nuclei in many Conifers the archegonium contains both male cells as well as the stalk- and tube-nuclei. In his recent work on *Thuja*, Land (1902) finds that the tube- and stalk-nuclei may sometimes enter the egg, but in the majority of cases they do not enter at all, but become disorganized in the space above the archegonium complex. Compared with these other Conifers, *Sequoia sempervirens* stands unique, in that only the nucleus and a very small amount of cytoplasm of the male cell enters the egg. Only the two sex-nuclei are present in the egg at the time of fusion.

As the male cell passes through the narrow canal of the neck, it immediately advances toward the egg-nucleus. As shown in Fig. 31, it has a long-drawn-out appearance. As it advances, the forward portion becomes rounded and very much wider than the long tapering hinder portion. While in this condition it first flattens against and then pushes in the membrane of the egg-nucleus. The long tapering end now draws in, and the male nucleus is more or less spherical. At the time of their fusion the male and female nuclei are of equal size, and in this respect differ from those in *Tsuga* and *Pinus* (Murrill, 1900; Blackman, 1898), where the male is much smaller than the female. Each cell has a large nucleolus, and the chromatin is in the form of small granules suspended in a regular network of linin threads. The chromatin in the female nucleus appears to be more finely granular than in the male, and consequently the male stained more deeply with safranin.

In *Pinus* the conjugation of the sexual nuclei has been worked out with considerable detail by Blackman (1898), Chamberlain (1899), and Ferguson (1901). According to these writers, the membranes of the sexual nuclei remain intact for some time after the penetration of the male into the female. Blackman further finds that the first segmentation-spindle begins its formation before the male and female nuclei lose their identity. This has been partially confirmed by Chamberlain (1899), and Ferguson (1901), in *Pinus*, and by Woycicki (1899) in *Larix*, who were able to distinguish the male and the female chromatin as distinct groups inside the walls of the female nucleus.

In *Sequoia sempervirens* the behaviour of the conjugating nuclei differ slightly from that described above for *Pinus* and *Larix*. As the male nucleus pushes into the female it becomes only partially surrounded by the membrane of the latter. This is no doubt due to the fact that the conjugating nuclei in *Sequoia* are of equal size. The membrane separating the two nuclei apparently breaks down much earlier than in *Pinus*. The chromatic contents of the two nuclei flow together, forming a common network in which the male and female elements can no longer be distinguished.

The fusion-nucleus thus formed is very large and occupies a central

position, and in the few cases observed the chromatin was in the spireme condition. From the fact that the first cleavage-spindle was frequently met with, and the fusion-nucleus in but a few cases, I conclude that a very short time intervenes between the conjugation of the sexual nuclei and the formation of the first cleavage-spindle.

Before passing on to the events that follow fertilization, we have yet to explain what becomes of the second male cell in *Sequoia.* Being functional, but unlike the other Conifers mentioned above, it does not fertilize the same archegonium. In *Pinus* (Blackman, 1898) only one male nucleus is functional. This is also true for *Cephalotaxus* (Arnoldi, 1900), and probably for other Conifers. In these forms both male cells enter the same female organ, so that one pollen-tube can accomplish the fertilization of but one archegonium. In *Sequoia sempervirens* this is clearly not the case, for the two male cells of a single pollen-tube were found in the process of fertilizing two separate archegonia. The behaviour of the second male cell is a duplicate of that described for the first male cell. They discharge their nuclei into two neighbouring archegonia just about the same time. As each male cell functions in separate archegonia, we have in *Sequoia* a case where each pollen-tube may bring about the fertilization of two archegonia. As there are usually three or four pollen-tubes surrounded by a larger number of archegonia, and since each male cell fertilizes a separate archegonium, there may be as many as six or eight embryos developed. We shall see below that this generally happens.

The Embryo.

As stated above, very little time elapses between the fusion of the male and female nuclei and the development of the first cleavage-spindle. This spindle was frequently met with, and always found with its poles in the long axis of the archegonium. In some cases observed the chromosomes were at the equator, or on the way to the poles; others again showed the daughter-nuclei already organized with the continuous fibrils connecting them. One of the most noticeable features of the spindle is the large number of chromosomes as compared with the number present in the gametophyte. As in the gametophyte, the chromosomes are very long V-shaped structures, and after they have split and moved toward the poles they appear to be about half the size of the mother-chromosomes when at the equator. As near as could be estimated, there are about thirty-two chromosomes in the sporophyte and sixteen in the gametophyte. Fig. 32 shows the first cleavage-spindle of the sporophyte with the large V-shaped chromosomes at the equator.

The events following the first cleavage-spindle proved to be very interesting, because they differ so widely from those of other Conifers in which the early stages of the embryo have been investigated. In most

Conifers the fusion-nucleus gives rise to a number of free nuclei, which take up a position in the plane at the base of the oospore. These free nuclei, by dividing, give rise to three tiers of cells with complete walls. The uppermost of the three tiers remains in the oospore, the middle tier develops the suspensors, and the lowest tier the embryo. This is no doubt true for *Pinus* and many other Conifers, but in *Sequoia* the embryo develops in quite another way.

Soon after the daughter-nuclei of the first cleavage have been organized, a cell-plate is formed between them, and this results in the development of two distinct cells, each surrounded by its own cell-wall. The first cleavage, therefore, does not result in the formation of free nuclei. Fig. 33 shows the two daughter-nuclei of the first cleavage. It also shows the cell-plate developing on the kinoplasmic fibrils which extend between the nuclei. The two cells thus organized from the first division occupy almost the entire cavity of the oospore, and lie one behind the other. These two cells now divide in the same plane as the first division, and the pro-embryo now consists of a single row of four large cells, as shown in Fig. 34. An examination of this figure makes it apparent that the division of the first two cells of the pro-embryo is nearly or quite simultaneous. In this connexion it is interesting to note that Arnoldi reports the presence of but three cells in the embryo at this time. He states that the uppermost cell of the first cleavage functions no further, but that the lower one divides and organizes two cells which become the suspensor and the embryo proper respectively. As shown in Fig. 34, there can be no doubt as to the division of both daughter-nuclei of the first cleavage.

The next stage observed showed five cells in the oospore, and, judging from the position of them, the fifth one arises by a division of the lowest cell in the row of four. The five cells take up a position as shown in Fig. 35. The fifth or lowest cell in the row now enlarges and divides. Of the last two cells thus organized, the end one becomes the embryo proper and the other one develops into the suspensor. As soon as the embryo-cell and the suspensor-cell are organized the latter enlarges very rapidly and becomes very much elongated. As it increases in length it bends downward and carries the embryo-cell, at its apex, down through the endosperm. As the embryo forces its way through the prothallial tissue the cells of the latter show every sign of disorganization, and are no doubt absorbed by the developing embryo. Fig. 37 shows a suspensor with its large single nucleus and a two-celled embryo at its apex.

Summary.

For some time after the separation of the tetrads, the microspore contains a single nucleus. Before leaving the sporangium this nucleus divides, so that at the time of pollination there are two nuclei present.

The larger of these nuclei is the tube-nucleus, and the smaller, which is situated near the wall of the spore, is the generative nucleus. No trace of nuclei or cells representing the vegetative tissue of the male gametophyte were found.

No further division of the nuclei in the pollen-grain takes place until the pollen-tubes are partially developed. There are usually three or four pollen-tubes present, and they pursue different courses. One or two of them may grow down between the nucellus and the integument for a considerable distance, while others penetrate the nucellus immediately at the apex.

Just about the time the tube penetrates the nucellus the generative nucleus divides, giving rise to the stalk- and body-nuclei.

The body-nucleus becomes very large, and surrounds itself with a zone of dense cytoplasm, which in turn is surrounded by a membrane. The tube now contains one large cell and two free nuclei.

During the latter part of June the body-cell divides and gives rise to two male cells. At first the male cells are flat on one side but soon become spherical. They are of equal size and both are functional.

There are from four to six macrospore mother-cells organized from centrally situated hypodermal cells of the macrosporangium. Each mother-cell divides twice, but one cell fails to develop into a spore, so that there are from eight to twelve macrospores formed. The first is the reduction-division which marks the beginning of the gametophyte. It takes place about the first of March.

Each macrospore begins to germinate. Some of the spores enlarge more rapidly than others, but the enlargement is always accompanied by a nuclear division. Although of various shapes and sizes, each macrospore now contains two free nuclei. The majority of the sacs or young prothallia show no further development, but two or three of them grow very rapidly and extend down through the tissue of the nucellus in the direction of the chalaza.

Of the two or three sacs that continue their growth, one develops much more rapidly than the other one or two. In the early stages of the prothallium there is a progressive and simultaneous division of the free nuclei. These are at first distributed throughout the length of a long central strand of cytoplasm. By the development of large vacuoles which eventually fuse together, the cytoplasm and the free nuclei are forced to the wall. The prothallium at this time consists of a very large central vacuole, surrounded by a parietal layer of cytoplasm in which the numerous free nuclei are distributed.

As the prothallium increases, the nuclei divide rapidly and frequently. The cytoplasm and nuclei now accumulate in great abundance in the lower, and less so in the upper portions, and the vacuole is reduced

to a comparatively small narrow area in the upper half of the prothallium.

The final division of all the free nuclei which immediately precedes the formation of the cellular endosperm is almost simultaneous. The spindle-fibrils which connect the daughter-nuclei in this division persist and increase in numbers, so that each nucleus is surrounded by a system of delicate radiating kinoplasmic fibrils. These fibrils not only connect with the sister-nuclei but reach out and join the fibrils of the neighbouring nuclei. The result is that large numbers of nuclei become joined together by radiating systems of kinoplasmic fibrils. The cell-plates are formed in the usual way between the nuclei. The formation of the endosperm in *Sequoia sempervirens* therefore does not follow the method described by Sokolowa (1891) for other Coniferales.

As the vacuole in the upper region disappears, the cells in this region are very large and elongated, and these structures are no doubt the 'Alveolen' described by Arnoldi. The archegonia are, however, not confined to this tissue in the prothallium.

During the development of the primary prothallium one or two secondary prothallia advance much more slowly. Their growth is confined to the narrow limits left by the primary prothallium. Their form is therefore very irregular, and they never develop cellular tissue. The cells of the primary prothallium which are in contact with the protoplasm of the secondary prothallia act as absorbing organs. This absorption of the protoplasmic substance of the secondary prothallium by the cells of the primary prothallium no doubt retards the development of the former and prevents it from forming tissue.

Soon after the endosperm is formed, certain cells deep within the upper portion of the prothallium become differentiated into archegonial initials. When quite small the primary neck-cell is cut off, and the central cell enlarges rapidly and carries the primary neck-cell forward towards a pollen-tube. During the enlargement of the central cell the primary neck-cell divides once. Occasionally four neck-cells were formed in the archegonium, but two seemed to be the typical number. Just before the archegonium has reached its full size the central nucleus evidently divides, for we now have two large nuclei present. These nuclei are of equal size, and are situated at opposite ends of the archegonium. The one near the neck represents the ventral canal-cell, and the lower one is the egg-nucleus. The ventral canal-nucleus very soon becomes disorganized and disappears entirely. The lower portion of the archegonium develops a large vacuole, and at the time of fertilization the egg-nucleus is centrally situated. As the archegonia develop, their elongation is always directed towards a pollen-tube, so that each tube becomes partially surrounded by the necks of several archegonia.

As soon as the archegonia are mature and ready for fertilization, the two male cells move toward the wall of the pollen-tube and take up positions immediately opposite the necks of two neighbouring archegonia. The wall of the male cell and of the tube in the region opposite the neck-cells evidently become dissolved, for the nucleus of the male cell, with a very small amount of cytoplasm surrounding it, squeezes through the narrow canal between the neck-cells and immediately advances toward the egg-nucleus. During its passage through the canal the male nucleus becomes very much constricted and elongated, but as it approaches the egg-nucleus it soon resumes its spherical form. The denucleated male cell remains outside of the archegonium and retains its spherical form for some time after fertilization has been effected, when it becomes disorganized. The nuclei of both male cells are functional, but they fertilize two neighbouring archegonia.

At the time of fusion the sex-nuclei are of equal size, and as the male pushes against the female it becomes partially surrounded by the membrane of the latter. The chromatin of both nuclei are in the spireme stage, and when the membrane between the two breaks down, a common chromatin network is formed in which the male and female elements can no longer be distinguished.

Very soon after the complete fusion of the sex-nuclei the first cleavage-spindle is developed. There are no free nuclei formed in the pro-embryo. The first cleavage results in the formation of two distinct cells, each surrounded by a complete cell-wall. Both of these cells divide, so that the embryo now consists of a row of four cells. The lowest of these enlarges and divides again. The two cells resulting from this last division give rise to the suspensor and embryo proper. Each fertilized archegonium gives rise to but a single embryo.

As near as could be estimated, there are sixteen chromosomes in the gametophyte and thirty-two in the sporophyte.

In conclusion, I wish to express my sincere thanks to my friend Capt. C. B. Hudson, who assisted me in collecting material.

Note.

Since the above went to press Dr. Coker's valuable work (1903) on *Taxodium* has appeared. I regret that it is too late to make a detailed comparison of *Sequoia* and *Taxodium* as worked out by Dr. Coker, as the two forms differ so widely in every essential detail. They should certainly be placed in different families.

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EXPLANATION OF FIGURES IN PLATES I–IV.

Illustrating Dr. Lawson's Paper on *Sequoia sempervirens*.

All the figures refer to *Sequoia sempervirens*, and were drawn with the aid of the Camera lucida. For the finer cytological details, Zeiss's homogeneous immersion obj. $\frac{1}{12}$ apert. 1·25 with compensating ocular No. 6, and for the lower magnifications obj. $\frac{1}{3}$ and $\frac{1}{6}$ and ocular No. 4, were used.

Fig. 1. A microspore as it appeared about two weeks before pollination, showing the single centrally situated nucleus. Material collected Dec. 15, 1901. × 900.

Fig. 2. A microspore at the time of pollination, showing the large centrally situated tube-nucleus (*t. n.*) and the smaller laterally situated generative nucleus (*g. n.*). Jan. 1, 1902. × 900.

Fig. 3. A longitudinal section of the ovule, showing the relative height of the macrosporangium and the integument. The micropyle is nearly closed, and the pollen-tubes are shown growing down between the sporangium and the integument. Within the sporangium six macrospore mother-cells are represented. Material collected March 12, 1902. × 125.

Fig. 4. A pollen-tube penetrating the nucellus near the top. The generative nucleus has divided, so that there are now three free nuclei in the tube. The large tube-nucleus (*t. n.*) is in advance of the stalk- (*S. n.*), and the body- (*B. n.*) nuclei. × 750.

Fig. 5. The lower part of the pollen-tube, showing the body-cell (*b. c.*) fully organized and the stalk-nucleus lying close beside it. Material collected June 12, 1902. × 750.

Fig. 6. The tip of the pollen-tube containing the tube-nucleus (*t. n.*), the stalk-nucleus (*S. n.*), and the large body-cell (*b. c.*) in the rear. Material collected June 15, 1902. × 750.

Fig. 7. The body-cell undergoing division. The daughter-nuclei are organized and are connected by a series of kinoplasmic fibrils. The fibrils curve out toward the cell-wall in preparation for cell-plate formation. Material collected June 29, 1902. × 750.

Fig. 8. Two male cells as they appear immediately after the division of the body-cell. At this time they are flat on one side, and each contains a large centrally situated nucleus. Material collected June 29, 1902. × 750.

Fig. 9. A longitudinal section through the central portion of the macrosporangium, showing six macrospore mother-cells. The cytoplasm of these cells is very dense and granular. The nuclei are comparatively large, and judging from the condition of the chromatin they are preparing for mitosis. Material collected March 2, 1902. × 800.

Fig. 10. Section same as Fig. 9. The macrospore mother-cells are undergoing division. Two spindles of the reduction-division are shown. March 12, 1902. × 800.

Fig. 11. A longitudinal section through the macrosporangium, showing eight germinating macrospores or young prothallia. Three of the prothallia are larger than the others, and each contains two nuclei. Their growth is directed towards the chalaza. April 25, 1902. × 175.

Fig. 12. A longitudinal section, showing two young prothallia after repeated nuclear division. The free nuclei are distributed along a central strand of cytoplasm which extends from end to end of the prothallium. April 25, 1902. × 175.

Fig. 13. A longitudinal section of a primary prothallium at a later stage, showing the very large central vacuole and parietal layer of cytoplasm in which the free nuclei are distributed. June 8, 1902. × 175.

Fig. 14. A longitudinal section of the middle and lower portion of the prothallium, showing the accumulation of cytoplasm and free nuclei, and the corresponding decrease in the size of the vacuole. The free nuclei have increased enormously in numbers, and are distributed uniformly throughout the cytoplasm. June 8, 1902. × 175.

Fig. 15. From a longitudinal section of the prothallium immediately after the final division of the free nuclei. Each nucleus is surrounded by a system of kinoplasmic radiations in such a way that a large number of nuclei are connected together by fibrils. This is the first step in preparation for cell-plate formation. June 12, 1902. × 1000.

Fig. 16. A section of endosperm, showing a stage immediately following that shown in Fig. 15. The cell-plate has developed from the kinoplasmic fibrils stretching between the nuclei. Between some of the nuclei the cell-plate is already fully formed, while in others the granules are seen on the fibrils which eventually become the cell-plate. June 12, 1902. × 1000.

Fig. 17. A longitudinal section of the upper portion of the prothallium, showing the distribution of the archegonia. The archegonia are shown in various stages of development. Archegonial initials, jacket-cells, and mature archegonia are shown in the same region. The growth of the older archegonia is directed toward the periphery of the prothallium. July 8, 1902. × about 150.

Fig. 18. A longitudinal section, showing a secondary prothallium in an advanced stage of development. The primary prothallium has already developed cellular tissue, and the course of the secondary appears to wind in and out through this. June 29, 1902. × 150.

Fig. 19. A cross-section, showing the close relationship between the primary and secondary prothallia. The cells of the primary prothallium in contact with the secondary extend outward and act as absorbing organs. These cells extend into the protoplasm of the secondary prothallium in a dovetail fashion, and their nuclei are very large and have the appearance of being engaged in very active metabolism. June 27, 1902. × 100.

Fig. 20. An archegonial initial in process of division. The spindle shows the large V-shaped chromosomes on the way to the poles. The cells resulting from this division are the central cell and the primary neck-cell. In order to bring out more clearly the character of the chromosomes, the figure was drawn at a somewhat higher magnification than the other figure of the archegonium. June 25, 1902. × 850.

Fig. 21. A young archegonium, showing the large central cell and the small primary neck-cell. June 25, 1902. × 750.

Fig. 22. A later stage of the archegonium, showing two neck-cells. The archegonium contains two large nuclei of equal size. The nucleus nearer the neck is the ventral-canal nucleus, and the one at the opposite end is the egg-nucleus. June 25, 1902. × 750.

Fig. 23. A typical mature archegonium ready for fertilization. There are two neck-cells present. The ventral-canal nucleus has disappeared, and the egg-nucleus is centrally situated. A large vacuole occupies the lower portion of the archegonium. June 29, 1902. × 750.

Fig. 24. A mature archegonium, showing four distinct neck-cells. June 29, 1902. × 750.

Fig. 25. A cross-section of a pollen-tube completely surrounded by female prothallial tissue. The tube contains the body-cell and the tube- and stalk-nuclei. June 25, 1902. × 150.

Fig. 26. A cross-section of the upper portion of the female prothallium, showing the position of the archegonial initials and jacket-cells when they first become differentiated. June 25, 1902. × 100.

Fig. 27. A cross-section of the upper portion of the female prothallium. The prothallial tissue partially surrounds a pollen-tube. Four mature archegonia are shown with their necks in contact with the wall of the tube. This position of the mature archegonia in relation to the pollen-tubes is very characteristic. June 27, 1902. × 100.

Fig. 28. A cross-section of the female prothallium, showing sections of their pollen-tubes. As shown in this figure, the outline of the prothallium is always modified to the shape and position of the pollen-tubes. Two of the tubes show the male cell. June 25, 1902. × 100.

Fig. 29. A denucleated male cell as it appears after the nucleus has been injected into the egg. It retains its spherical form, but the central region once occupied by the nucleus is distinctly vacuolated. The figure shows quite clearly that only a very small amount of cytoplasm from the male cell accompanies the nucleus through the narrow canal between the neck-cells into the egg. June 25, 1902. × 750.

Fig. 30. An archegonium immediately after the entrance of the male nucleus. The male nucleus is shown pushing in the wall of the female. June 25, 1902. × 750.

Fig. 31. Another stage in the fusion of the male and female nuclei. The rear portion of the male nucleus has a long-drawn-out appearance, due to its passage through the narrow canal between the neck-cells. June 27, 1902. × 750.

Fig. 32. The first cleavage-spindle with the large V-shaped chromosomes at the equator. June 25, 1902. × 750.

Fig. 33. A later stage of the first cleavage-spindle with the daughter-nuclei organized at the poles, and the cell-plate developing from the continuous fibrils. June 25, 1902. × 750.

Fig. 34. Each daughter-nucleus of the first cleavage has divided, so that the pro-embryo now consists of four cells placed one behind the other, and each surrounded by a distinct cell-wall. June 25, 1902. × 750.

Fig. 35. A later stage of the embryo, showing five distinct cells. June 25, 1902. × 750.

Fig. 36. An early stage in the development of the suspensor with the first cell of the embryo proper at its apex. June 25, 1902. × 750.

Fig. 37. A later stage of the suspensor, showing its single large nucleus and two cells of the embryo proper as it is carried forward down through the endosperm. July 8, 1902. × 750.

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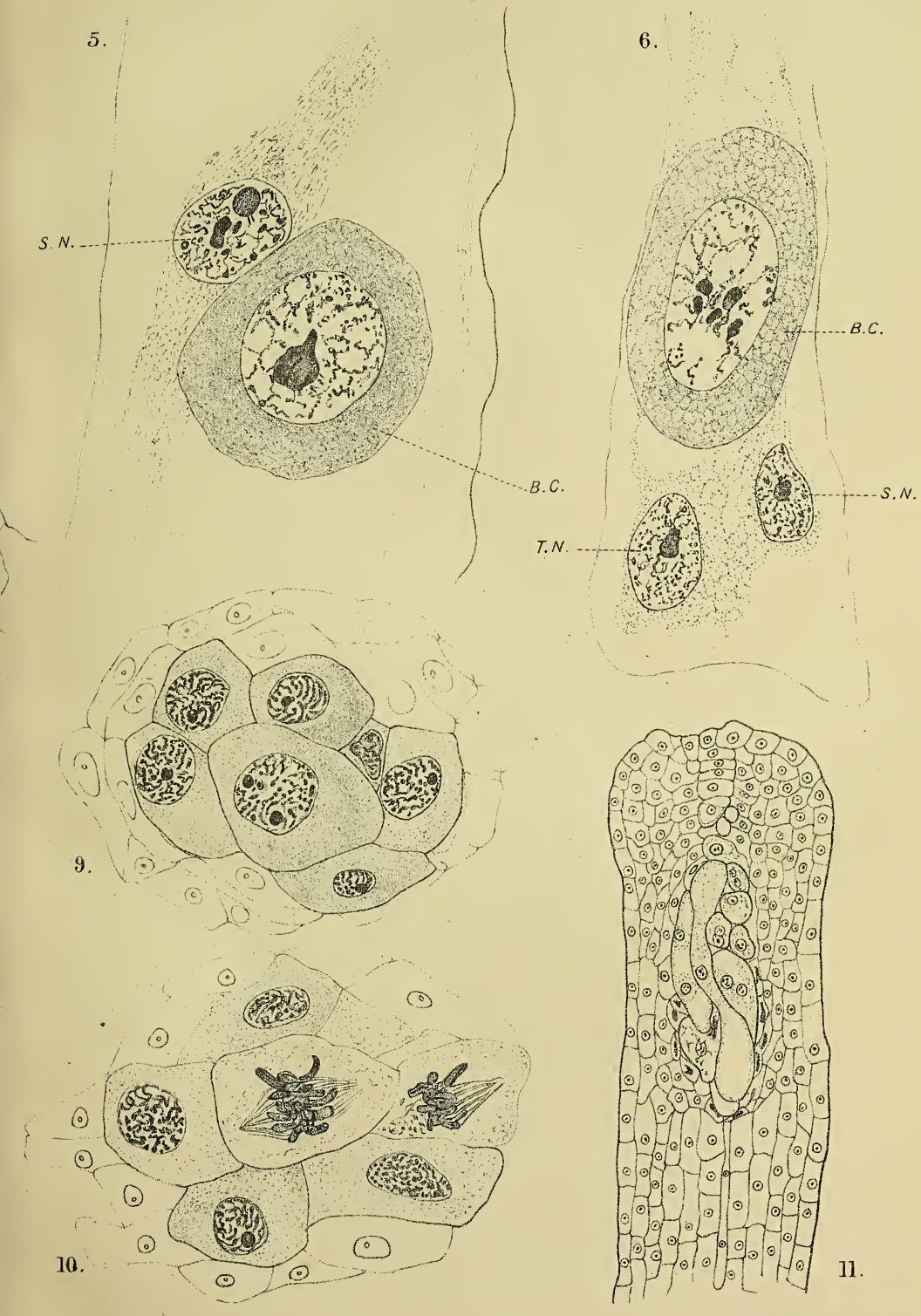

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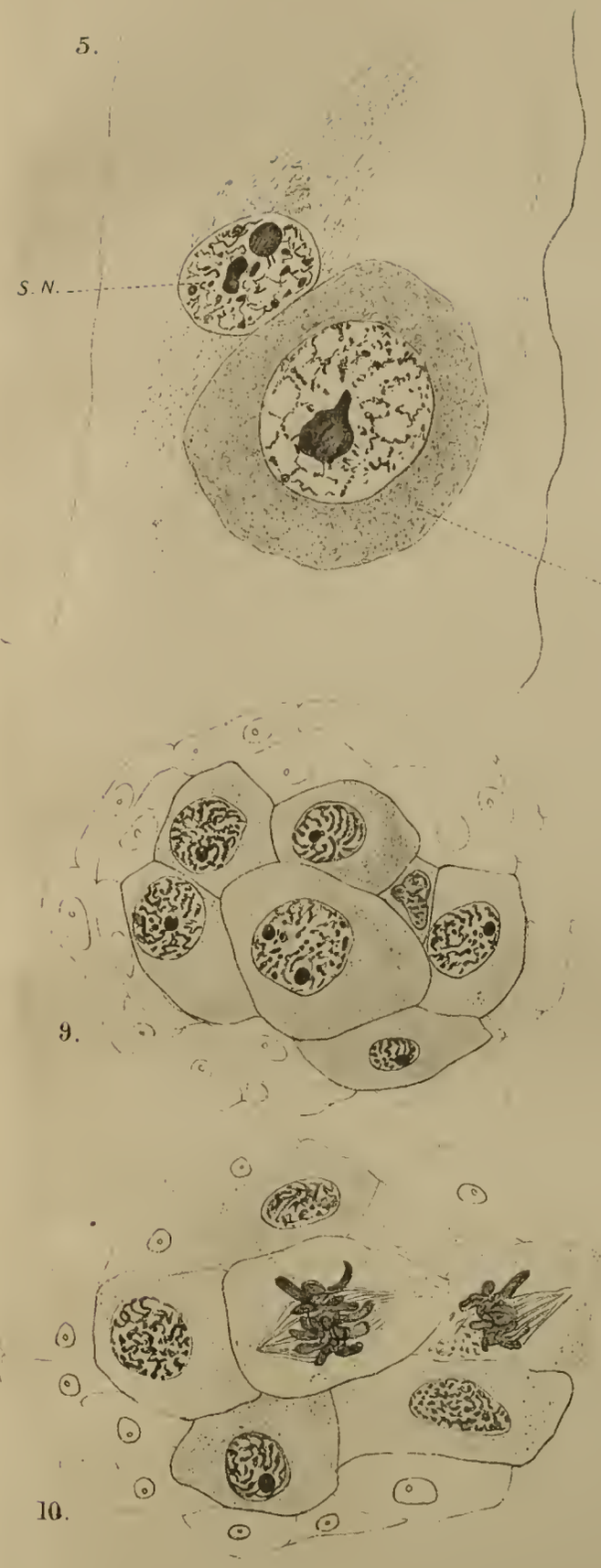

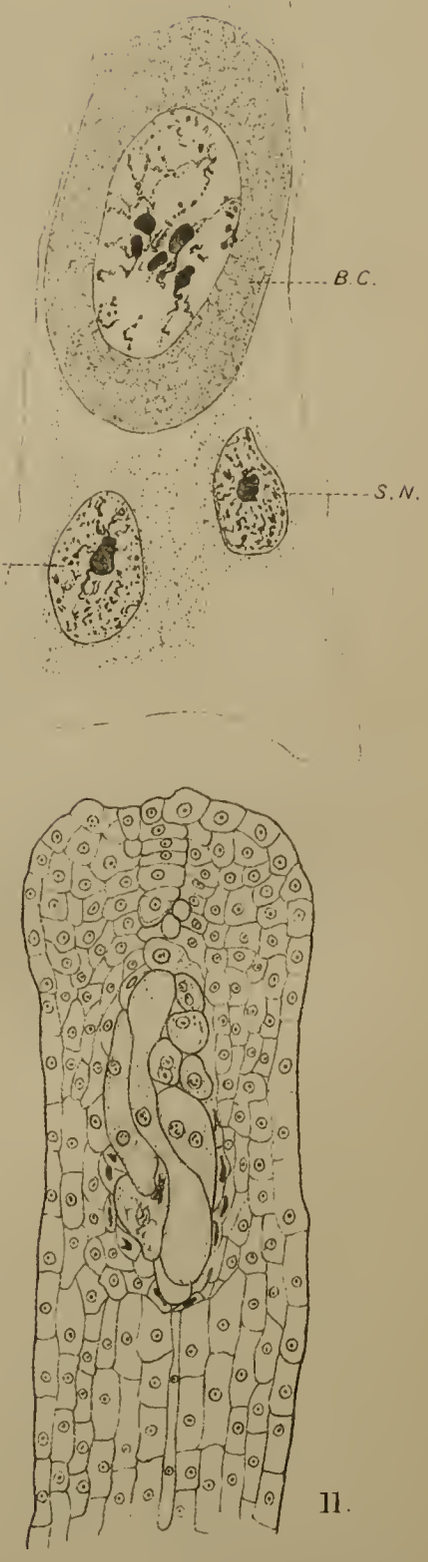

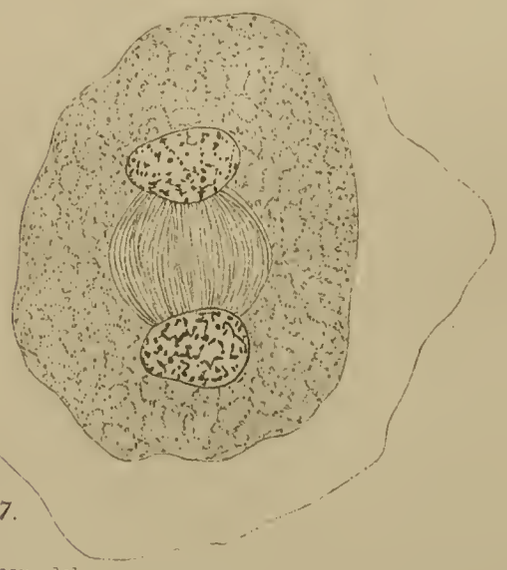

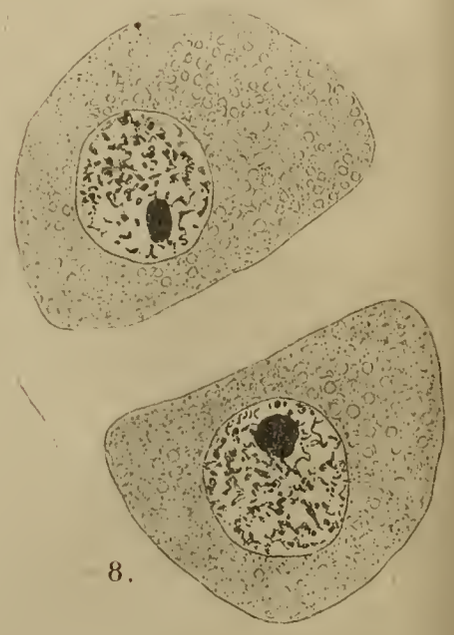

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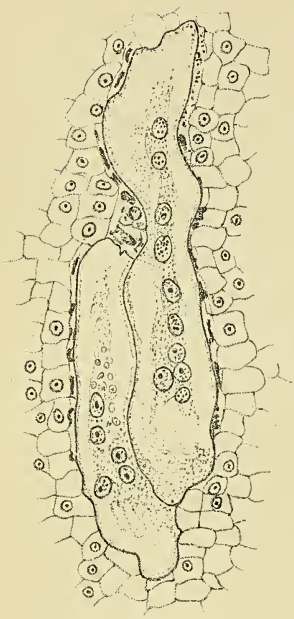

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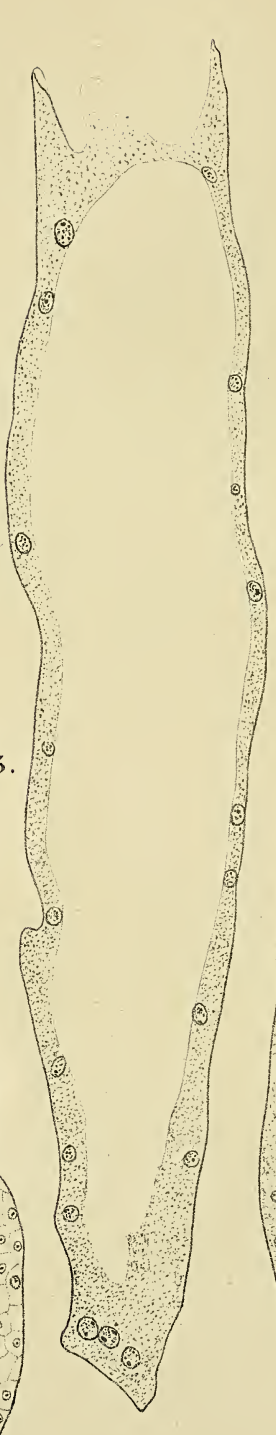

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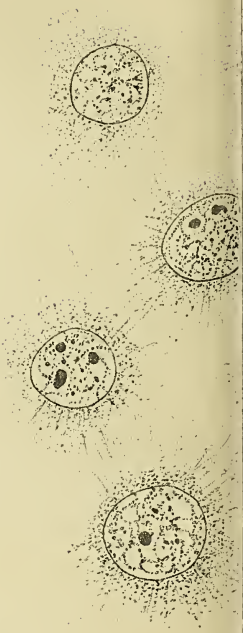

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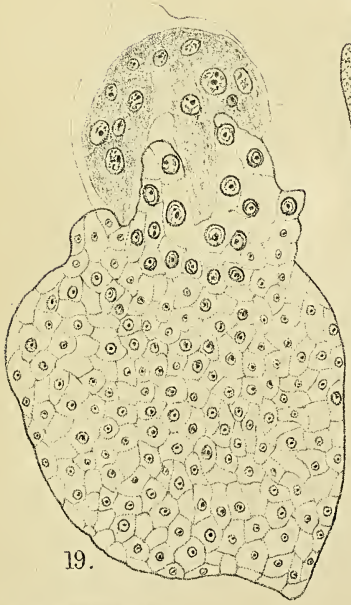

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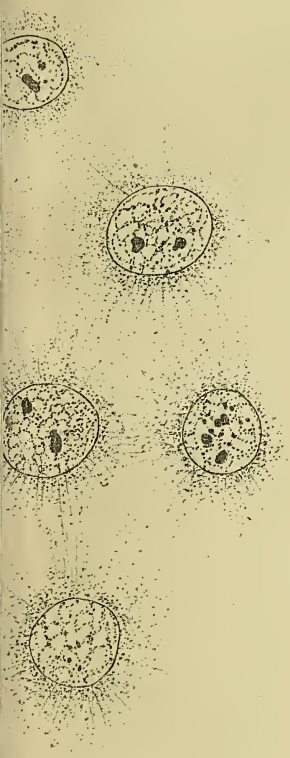

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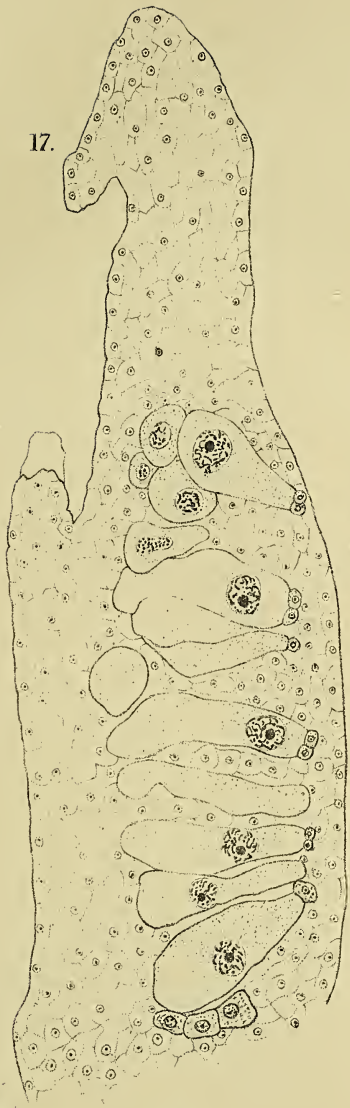

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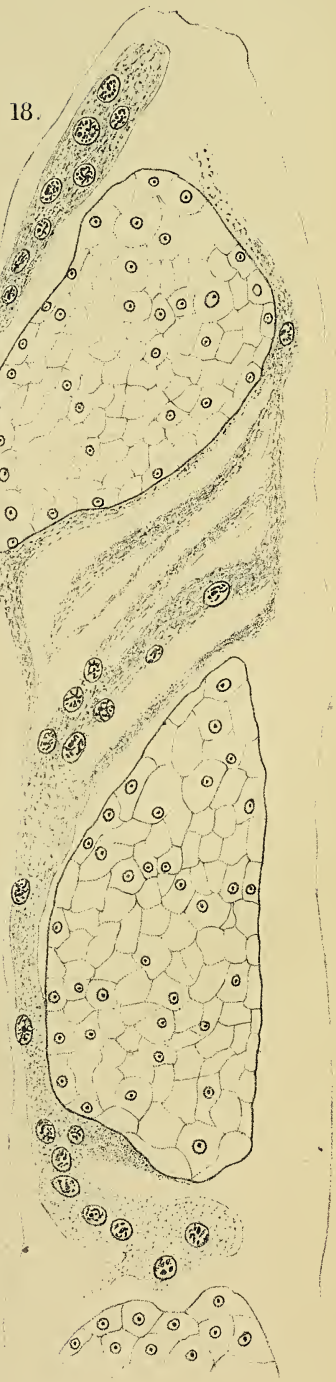

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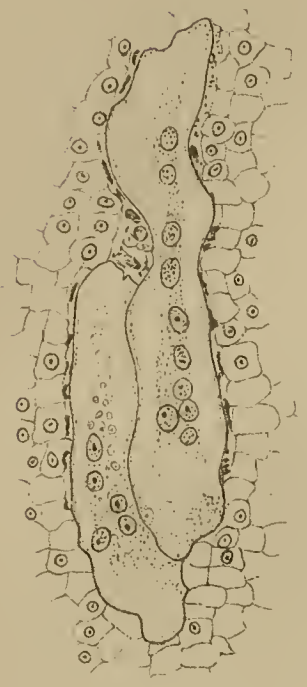

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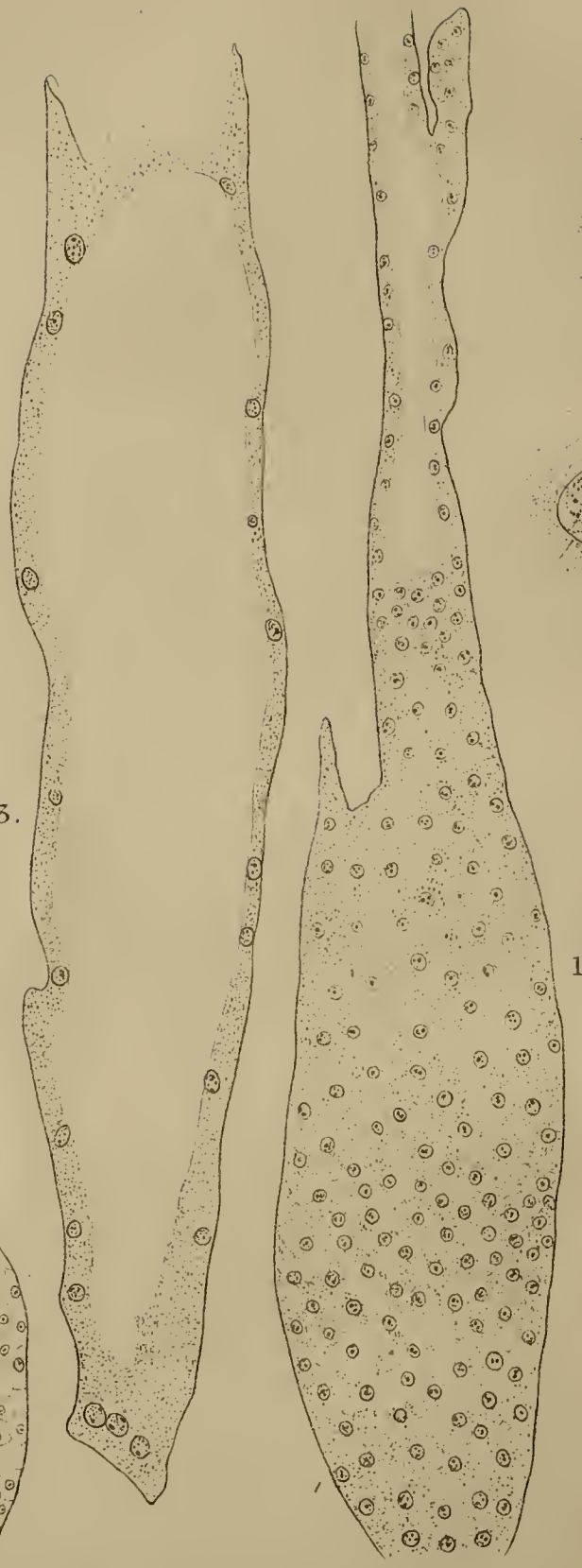

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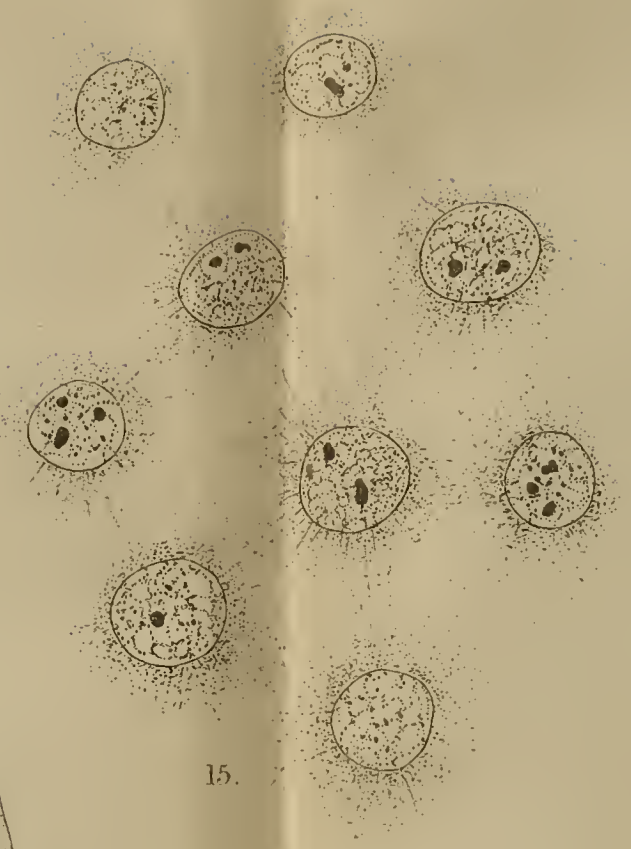

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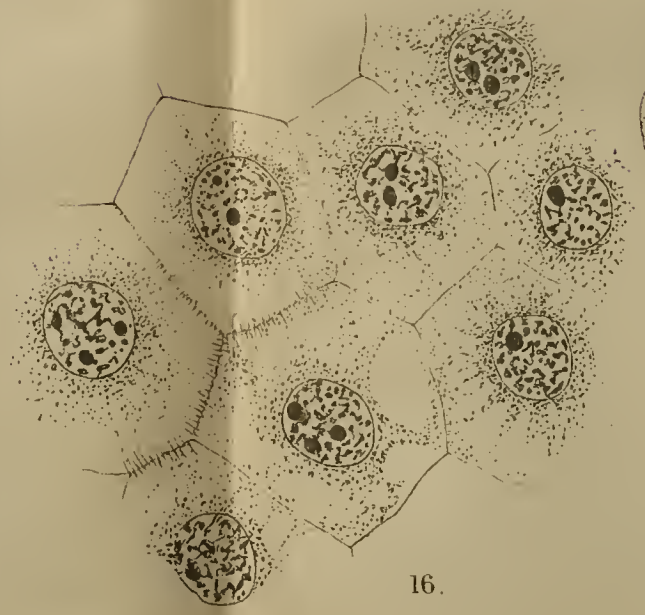

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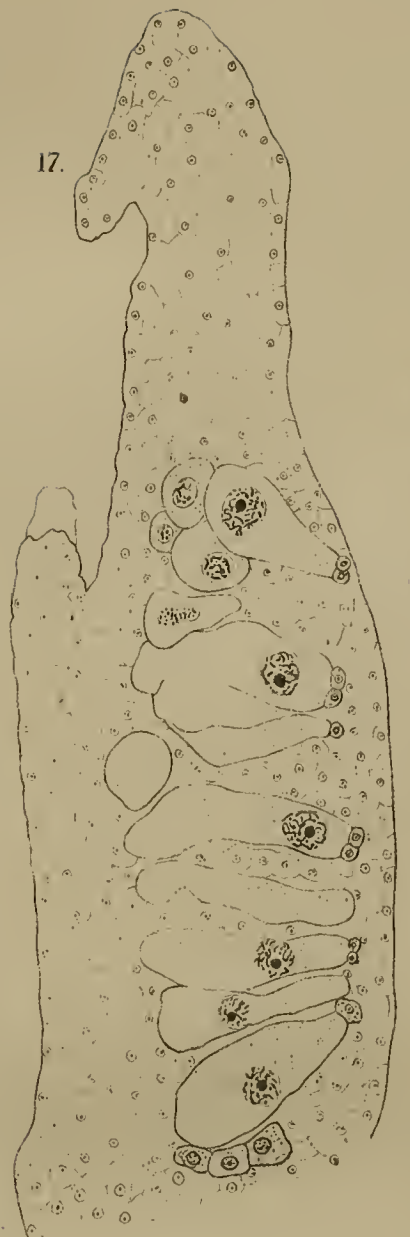

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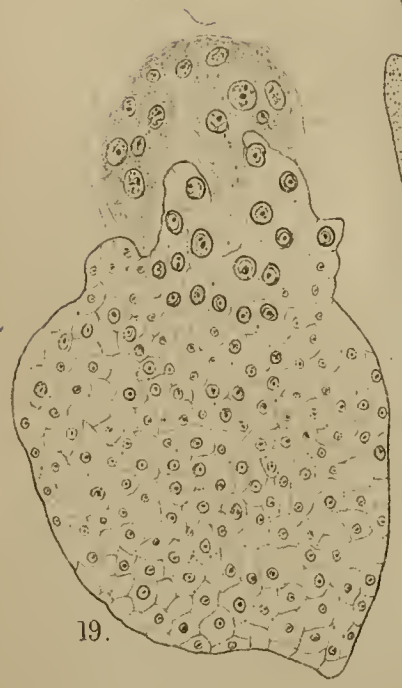

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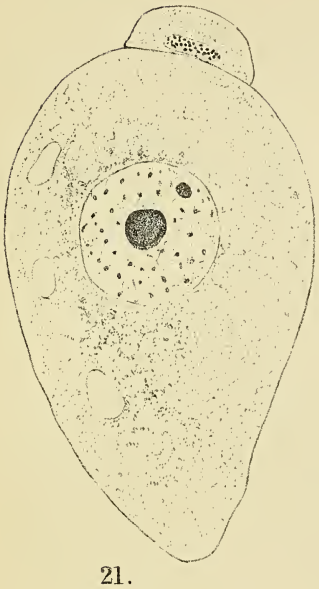

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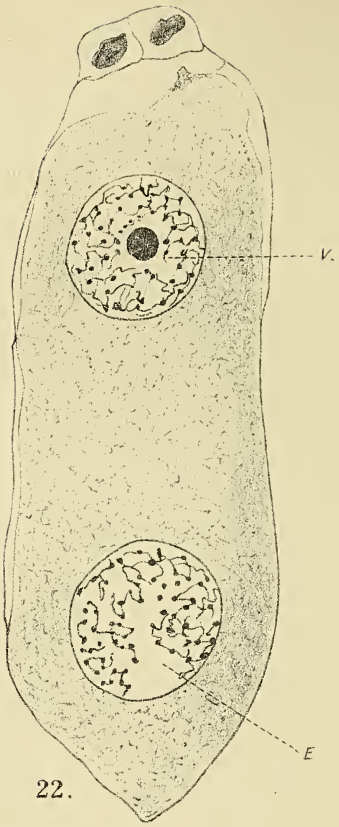

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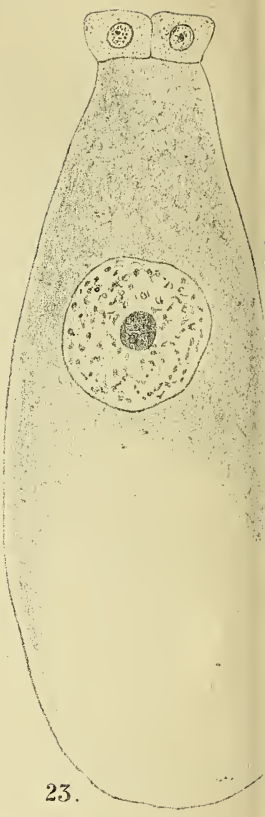

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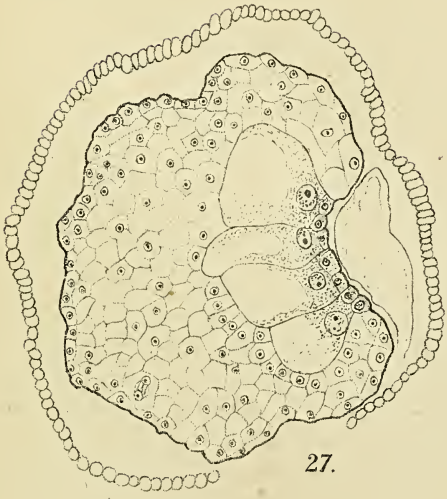

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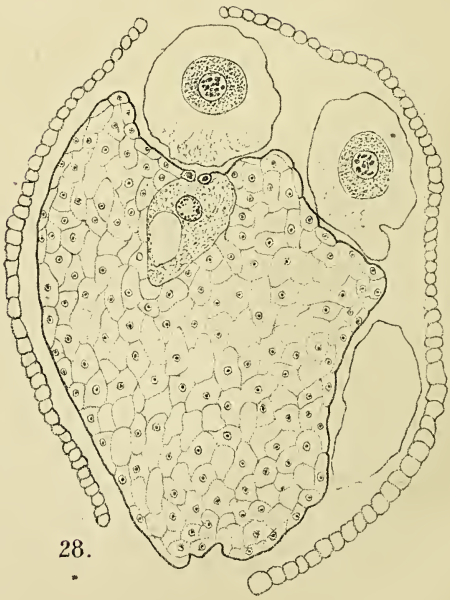

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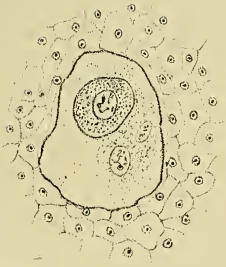

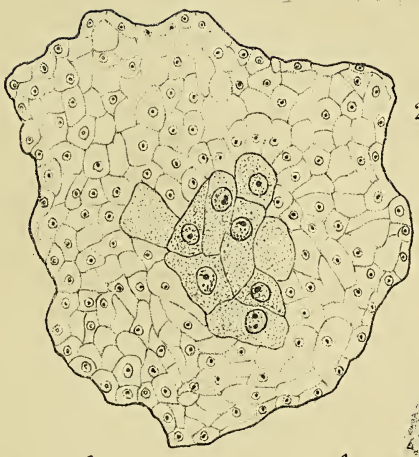

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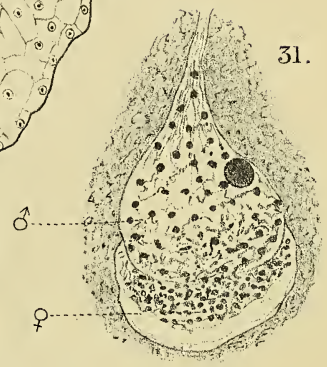

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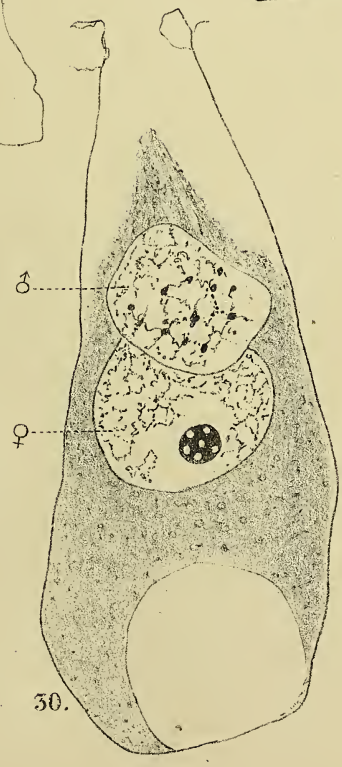

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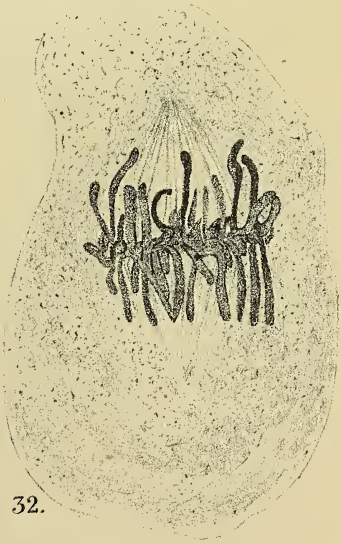

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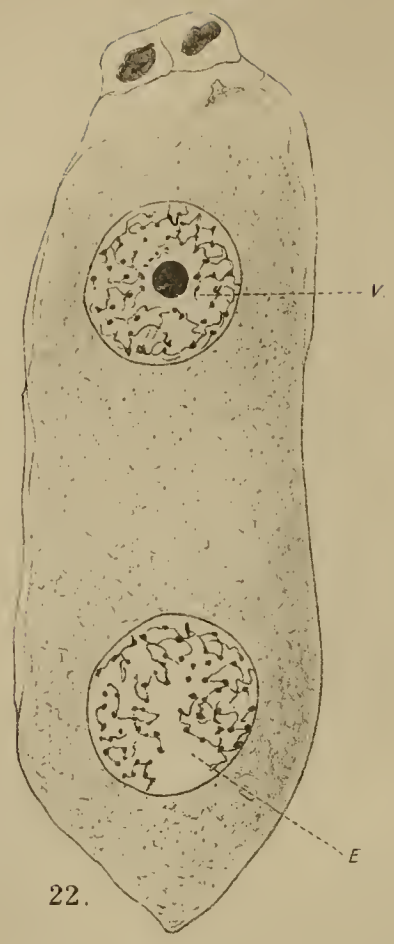

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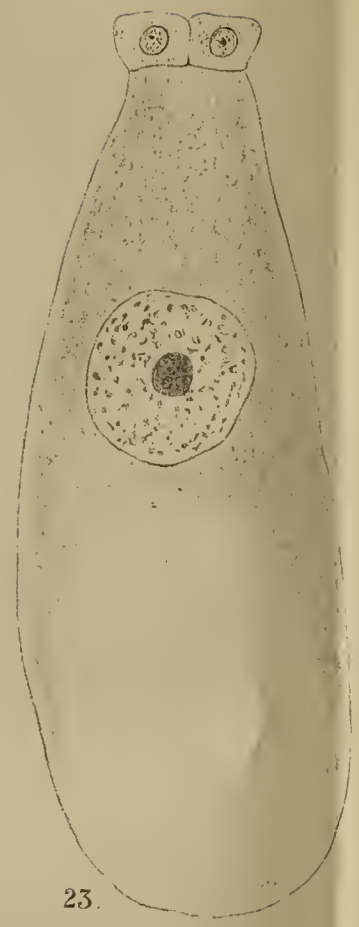

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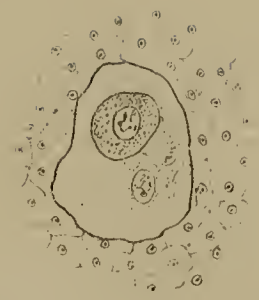

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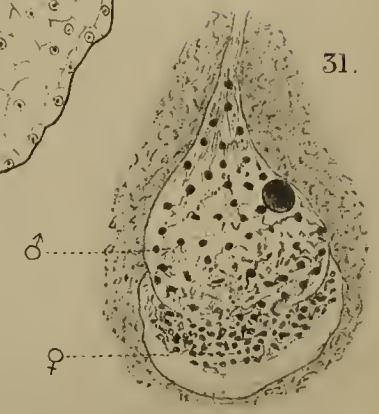

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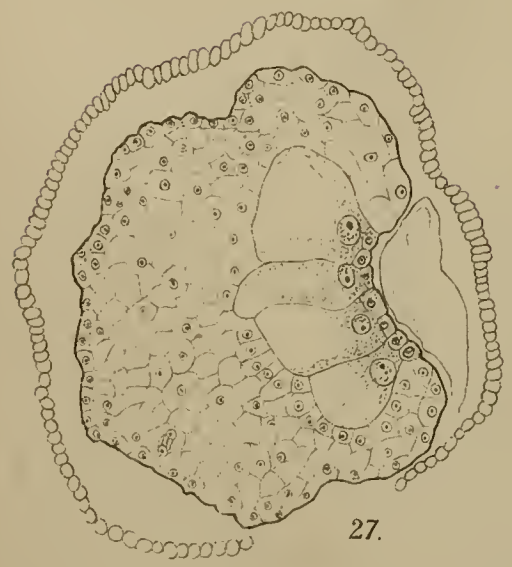

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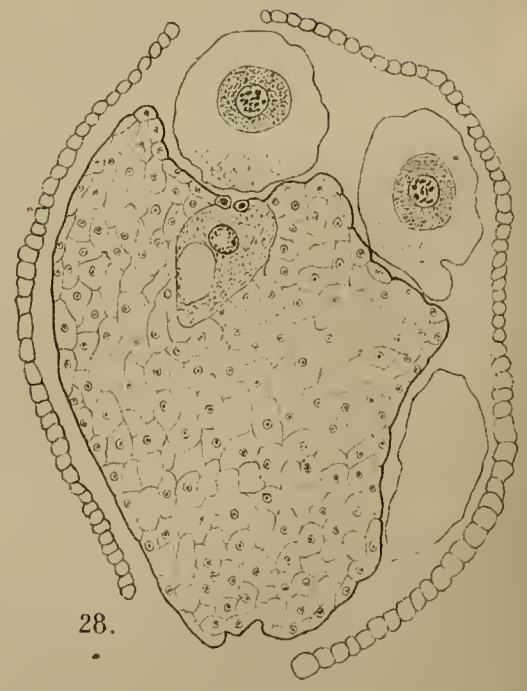

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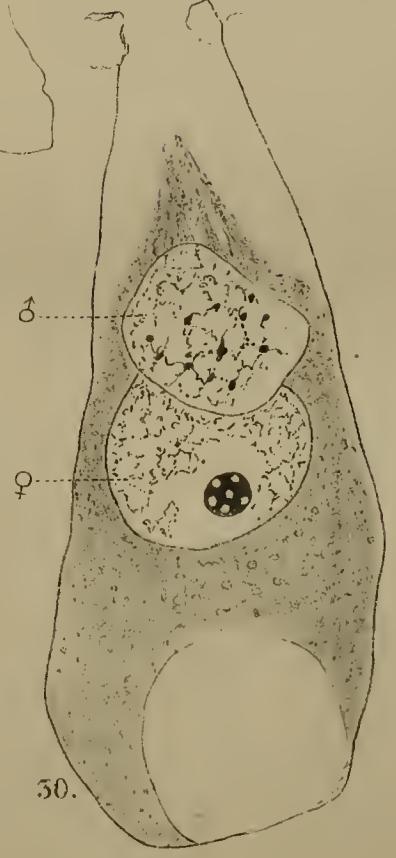

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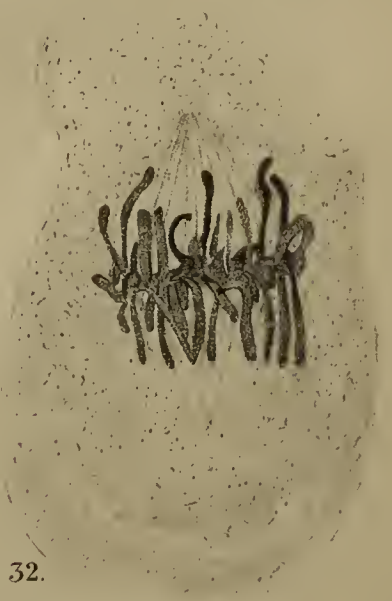

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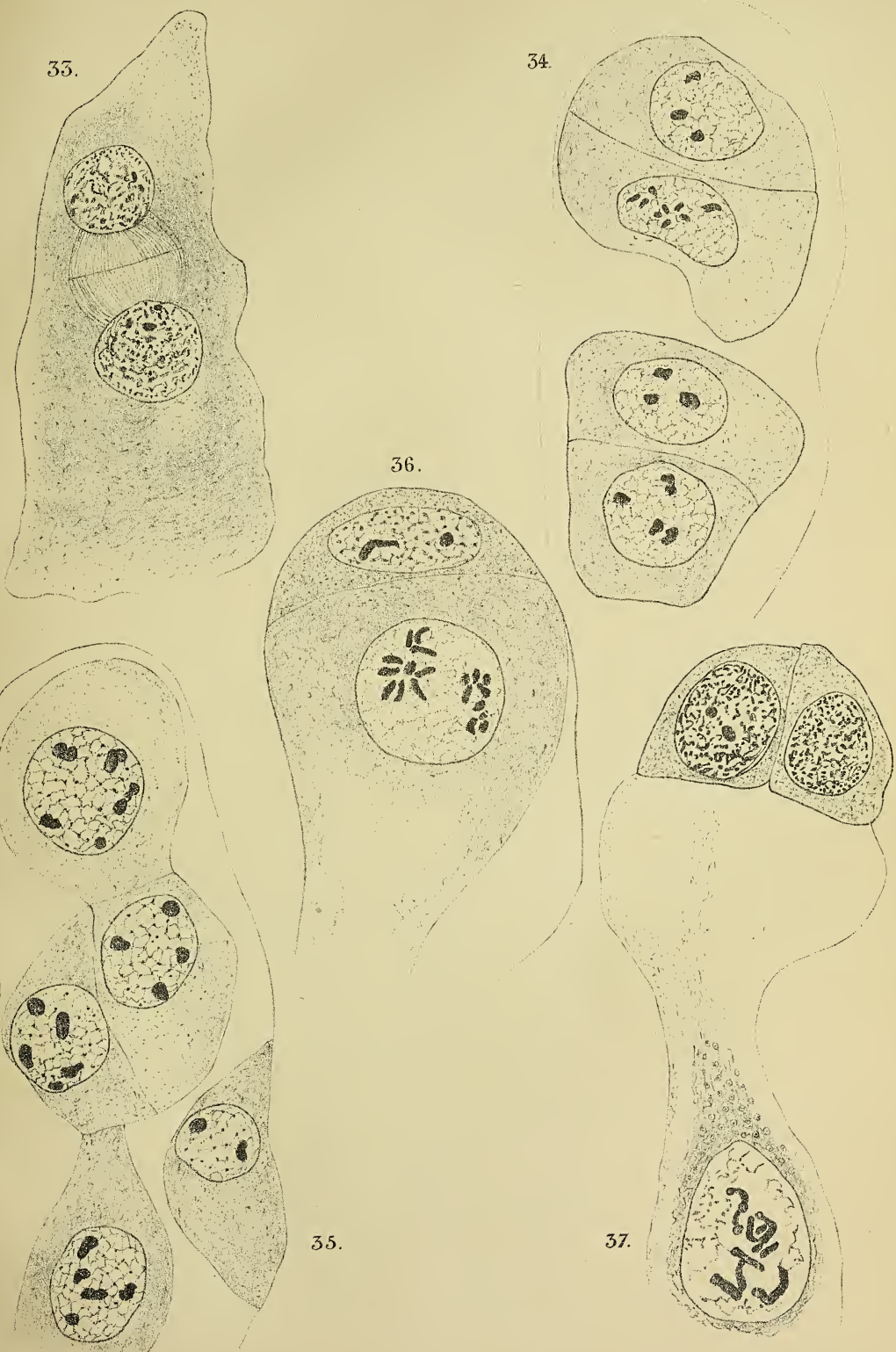

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LAWSON, —— GAMETOPHYTES OF SEQUOIA.

The Nucleolus and Nuclear Division in the Root-Apex of Phaseolus.

BY

HAROLD WAGER.

With Plate V

THE great prominence of the nucleolus in nearly all cell-nuclei, its definite form, its avidity for aniline dyes, and its behaviour during nuclear division led, more than twenty years ago, to the conclusion of Strasburger, Flemming, Guignard, and others that it is in some way connected with the growth and increased stainability of the chromosomes during the division of the nucleus. This view is still maintained by many cytologists, although the micro-chemical researches of Zacharias, and the later investigations of Strasburger are opposed to it.

From a series of observations which I have made upon the changes which take place in the nucleolus during the process of nuclear division in the root-cells of *Phaseolus*, it appears to me that not only is the nucleolus concerned in the formation of the chromosomes, but that there is a definite morphological connexion between them. Briefly stated, it is found that the nucleolus is intimately connected with the nuclear reticulum; that it contains nearly all the chromatin of the nucleus; that this is transferred, previous to division, into the nuclear thread, which is then segmented into chromosomes; and that, in the reconstitution of the daughter-nuclei, the chromosomes become fused into a number of more or less spherical or irregular masses which unite to form the daughter-nucleoli [1].

Although there are numerous observations which indicate a close relationship between nucleoli and chromosomes, the existence of such a definite morphological connexion between them had only been previously observed in a few cases, of which *Spirogyra* among plants and *Actinosphaerium* among animals are the most prominent examples. More recently a somewhat similar conclusion has been arrived at in the case of the root-cells of *Vicia*.

[1] These results were stated in a Paper read before Section K at the Bradford meeting of the British Association in 1900. See Brit. Ass. Reports, p. 944.

[Annals of Botany, Vol. XVIII. No. LXIX. January, 1904.]

Literature.

In the following brief account of the literature, reference is only made to the more important papers in which the relations of the nucleolus to the chromatin and chromosomes are dealt with, and more especially to those papers published since 1897. An excellent summary of the whole of the literature on nucleoli, down to 1897, is given by Montgomery in his valuable memoir on the Morphology of the Nucleolus[1].

According to Flemming[2] the nucleolus is a special organ of the nucleus or cell for the collection or elaboration of chromatin. It does not actually consist of chromatin (or nuclein), but of a substance in which the chromatin is elaborated, or, it may be, of a homogeneous substance which may be a chemical modification of chromatin or a preliminary stage in its formation. It possesses a definite form, but with no membrane around it, and it may be vacuolate with fluid contents in the vacuoles.

Strasburger[3] regarded the nucleolus as an inert mass consisting of a reserve substance or substances, allied to chromatin, for the formation of the chromosomes. During division the nucleoli become dissolved in the nuclear sap and are then taken up into the nuclear thread, to reappear again in the chromatin-network of the daughter-nuclei.

Pfitzner[4], Guignard[5], Went[6], and others give expression to similar views.

Strasburger, in his more recent memoirs[7], adopts a different view from that just stated. He now believes that the chromatin is contained in the nuclear sap, and that one must look for its origin in the cytoplasm and not in the nucleolus. The nucleolar substance serves for the building up of the spindle. The evidence for this appears to be the complete or partial disappearance of the nucleolus immediately preceding the formation of the spindle; the active and quantitative condition of the kinoplasm rises or sinks as the nucleolus dissolves or reappears, and the solution of the nucleolus is followed by the highest point of spindle-development. He brings forward the observations of Němec, who states that the kinoplasm is directly transformed into nucleoli, and that granules which show the peculiarities of nucleoli arise at the poles of the division-figure through transformation of the spindle-threads. Their appearance is especially due

[1] Jour. of Morph. xv, 1899.

[2] Zellsubstanz, Kern- und Zelltheilung. Leipzig, 1882.

[3] Ueber den Theilungsvorgang der Zellkerne und das Verhältniss der Kerntheilung zur Zelltheilung, Arch. f. mikr. Anat., xxi, 1882. Die Controversen der indirekten Kerntheilung, Arch. f. mikr. Anat., xxiii, 1884.

[4] Beiträge zur Lehre vom Bau des Zellkerns und seinen Theilungserscheinungen, Arch. f. mikr. Anat., xxii, 1883.

[5] Nouvelles recherches, &c., Ann. Sci. Nat., Bot., Sér. 6, xx, 1885.

[6] Beobachtungen über Kern- und Zelltheilung, Ber. d. deutsch. Bot. Gesell., v, 1887.

[7] Karyokinetische Probleme, Jahr. f. wiss. Bot., xxviii, 1895. Ueber Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. Jena, 1900.

to a lower temperature, and Strasburger remarks that Hottes [1] has shown that a low temperature promotes the appearance of extranuclear nucleoli whilst a higher temperature promotes the formation of the spindle-figure.

Zacharias [2], in a series of important papers in which the structure and micro-chemical reactions of the nucleolus are dealt with, contends that by the action of digestive fluid nucleoli are clearly differentiated chemically from chromosomes, and that this difference is also brought out by staining in methyl green in which the chromosomes stain more deeply than the nucleoli. Nucleoli may be vesicular and may exhibit a differentiation of structure into a homogeneous peripheral portion and a more refringent, granular or vesicular central portion. They appear to consist of plastin with albuminoids, but contain no chromatin. By observations on the living cell, especially the rhizoids of *Chara*, he finds that the nucleoli disappear just as the nucleus is about to divide, and that, just before this happens, they undergo amoeboid changes of form. Several nucleoli appear in the daughter-nuclei, and these afterwards fuse together into one. No definite conclusions as to what becomes of the nucleolus during division, or its function can be stated. In the nucleolus of *Spirogyra*, which many observers maintain is morphologically connected with the formation of the chromosomes, he finds no chromatin; but he remarks in his most recent paper that could such a morphological connexion between nucleolus and chromosomes be established it would follow from the combination of morphological and chemical data that during the formation of the elements of the nuclear plate definite chemical changes would take place.

Carnoy's observations [3] led him to distinguish four kinds of nucleoli—(1) 'nucléoles nucléiniens,' (2) 'nucléoles-noyaux,' (3) 'nucléoles plasmatiques,' and (4) 'nucléoles mixtes,' a combination of (1) and (3). He thinks that the plasmatic nucleoli may be concerned in the formation of the spindle. The 'nucléoles nucléiniens' are part of the chromatin-network and may consist of an amorphous mass of chromatin or of a chromatin-thread.

Macfarlane, in his earlier contributions [4] and in his latest [5] paper, contends that the nucleolus is the most specialized portion of the cell and contains the main mass of the chromatin-substance.

[1] Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich, p. 127.

[2] Zacharias, E., Ueber den Zellkern, Bot. Zeit., xl, 1882. Ueber den Nucleolus, Bot. Zeit., xliii, 1885. Ueber das Verhalten des Zellkerns in wachsenden Zellen, Flora, lxxxi, 1895. Ueber einige mikrochemische Untersuchungsmethoden, Ber. d. deut. Bot. Gesell., xiv, 1896. Ueber Nachweis und Vorkommen von Nuclein, Ber. d. deut. Bot. Gesell., xvi, 1898. Ueber die achromatischen Bestandtheile des Zellkerns, Ber. d. deut. Bot. Gesell., xx, 1902.

[3] La biologie cellulaire. Étude comparée de la cellule dans les deux règnes. Lierre, 1884. La cytodiérèse chez les Arthropodes. La Cellule, i, 1885.

[4] Trans. Bot. Soc. Edinburgh, xiv, 1881. Trans. Royal Soc., Edinburgh, xxx, 1885, and xxxvii, 1892.

[5] Current Problems in Plant Cytology. Contributions from the Botanical Laboratory, University of Pennsylvania, ii, 1901.

Mann [1] considers it probable that the nucleus and nucleolus are concerned in the assimilation of food-material, and that the nuclear chromatin may be less highly elaborated material than the nucleolar chromatin. He suggests that the nucleolus may be either 'an organ for the further transformation of substances already elaborated by the nucleus, or simply a storehouse for food-material, which has been already transformed by the nucleus into substances directly available for the nourishment of the achromatic elements of the cell.'

In *Spirogyra*, according to Tangl [2], Meunier [3], Moll [4], Decagny [5], Henneguy [6], Mitzkewitsch [7], and Van Wisselingh [8], the nucleolus contains chromatin and the chromosomes are derived entirely or in part from it.

Moll points out that only the nucleoli retain gentian violet with obstinacy; from all other parts it is extracted without difficulty. The nucleoli are found in three different forms—(1) homogeneous, (2) vesicular, (3) exhibiting a skein structure. The last is the more common. Chromatic substance does not exist to an appreciable amount outside the nucleolus. The nuclear segments are formed by the transference of the chromatic substance from the nucleolus into the nuclear plasm. 'It seems as if the chromatic substance were squeezed from the nucleolus by an aperture' into the nuclear plasm in which it appears 'as small fragments, ranged in an intermediate, achromatic thread, like the beads of a necklace; and thus a skein, containing chromatic substance, is formed.'

Mitzkewitsch states that in the process of division the nucleolus increases in size, its membrane disappears and it becomes irregular in shape. It then becomes differentiated into a number of deeply stained granular chromosomes, which form the nuclear plate, and a less deeply stained substance in contact with them. In the daughter-nuclei the chromosomes can be distinguished still surrounded by this less deeply stained substance, with which they eventually become incorporated to form the daughter-nucleoli.

A somewhat different account is given by Van Wisselingh, who believes that two only of the chromosomes are derived from the nucleolus, the others being derived from the nuclear thread. In the reconstitution

[1] The Embryo-sac of *Myosurus minimus*, L. A Cell Study, Trans. and Proc. Bot. Soc. Edinburgh, 1892. See also Brit. Ass. Reports, 1892, p. 753.

[2] Ueber die Theilung der Kerne in *Spirogyra*-Zellen. Sitz. d. k. Akad. d. Wiss. in Wien, lxxxv, 1882, p. 268.

[3] Le nucléole des *Spirogyra*. La Cellule, 1888.

[4] Observations on Karyokinesis in *Spirogyra*. Verhand. der k. Akad. van Wetenschappen te Amsterdam, 1893.

[5] Bull. Soc. Bot. France, xlii, 1895, p. 319.

[6] Leçons sur la Cellule, 1896.

[7] Ueber die Kerntheilung bei *Spirogyra*. Flora, lxxxv, 1898, p. 81.

[8] Ueber den Nucleolus von *Spirogyra*: ein Beitrag zur Kenntniss der Karyokinese. Bot. Zeit., lv, 1898, p. 195, and Flora, lxxxvii, 1900.

of the daughter-nuclei the halves of these two chromosomes again give rise to the new nucleoli.

Whatever may be the exact method of division which takes place, it seems clear, as Strasburger says [1], that we have here a special behaviour of the nucleolus in giving rise to the chromosomes which, although opposed by Zacharias on micro-chemical grounds, appears to be morphologically decisive.

According to Golenkin [2] we have a somewhat similar phenomenon in *Sphaeroplea*. The nucleolus breaks up into a number of fragments, which arrange themselves in a nuclear disk, and then appear to split up and move to the two poles where they fuse into daughter-nucleoli. All the chromosomes appear to originate from the nucleolus. He says that similar nuclei occur in other green Algae, including all Volvocineae, and in Musci.

Rosen [3] states that in some plant-cells two kinds of nucleoli may be present, 'Eunucleoli' and 'Pseudonucleoli.' The Pseudonucleoli form part of the chromatin-network, and are used up in the formation of the chromosomes. The Eunucleoli are like the ordinary nucleoli, and do not disappear until a later stage in nuclear division. In the root-apex of *Phaseolus* there is only one kind of nucleolus present, which becomes lobular in the prophases of division, and does not entirely disappear in some cases until the separation of the daughter-groups of chromosomes is completed.

Macallum [4] has shown that some nucleoli give an intense reaction for iron. This indicates that they contain chromatin. He finds in *Erythronium* at least three kinds of nucleoli:—(1) nucleoli which give a weak reaction for iron, and which therefore contain little or no chromatin; (2) nucleoli rich in iron, and which give a reaction for iron in every respect like the nuclear thread; (3) nucleoli found in the embryo-sac. The nucleoli of the embryo-sac appear in the filaments during the retrogressive stage as spherical elements containing little iron. As the chromatin in the filament becomes reduced they give a stronger reaction for iron, and eventually are found to consist chiefly of chromatin, 'and in stained preparations appear to contain nearly all the chromatin of the nucleus.' 'When mitosis again commences the filament forms at their expense, the increase in size of the filament keeping pace, apparently, with the decrease in the quantity of chromatin which the nucleoli contain.'

V. Häcker considers [5] that nucleoli are not to be regarded as reserve

[1] Loc. cit., 1900.

[2] Bull. Soc. Imp. Nat. Moscou, 1899 (1900), p. 343. See J. R. M. S., 1901, p. 65.

[3] Beiträge zur Kenntniss der Pflanzenzellen, Cohn's Beitr. z. Biol. d. Pflanzen, v, 1892, and vii, 1895.

[4] On the Distribution of Assimilated Iron Compounds, other than Haemoglobin and Haematins, in Animal and Vegetable Cells, Q. J. M. S., N. S., xxxviii, 1895.

[5] Praxis und Theorie der Zellen- und Befruchtungslehre. Jena, 1899. Die Vorstadien der Eireifung: zusammenfassende Untersuchungen über die Bildung der Vierergruppen und das Verhalten der Keimbläschen-Nucleolen. Arch. f. mikr. Anat., xlv, 1895, p. 200.

substance, but as products of excretion resulting from the metabolic change taking place between the cytoplasm and nucleus, and especially a secretion of the chromatin, which is thrown out of the nucleus during division.

Farmer[1] states that in the Liverworts he examined 'the nucleolus was associated with the chromosomes in an unmistakable and remarkable manner.' Threads of delicate texture run from the nucleolus to the linin. These are especially well seen in *Fegatella*. In a considerable number of cases the decrease of nucleolar substance is contemporaneous with the growth of the chromosomes. In *Fossombronia*, for example, 'the nucleolus becomes angular, and, in extreme cases, almost star-shaped, whilst at the same time the linin begins to exhibit a very striking increase in the amount of chromatin which it contains.' In a later stage the very much distorted nucleolus is often connected with several of the chromosomes, and soon after disappears. In the daughter-nuclei the nucleoli appear early, first as two or three small bodies which finally fuse to one large one, and the linin concomitantly loses its chromatin constituent. This does not necessarily imply that the chromatin passes, as such, into the nucleolus. But some constituents of the chromatin may find their way into this body, since both chromatin and the nucleolus readily yield albumen on appropriate treatment.

Miss Ethel Sargant's observations on the formation of the sexual nuclei in *Lilium Martagon*[2] support the view that the nucleoli are concerned in chromosome-formation. The linin-thread during its growth appears to be fed from the partially dissolved nucleolus. Drops of nucleolar matter are found attached to the linin-thread in certain stages of its development. During the later stages of chromosome-formation the segments of the thread become shorter and thicker, and ultimately 'the colouring (staining) of the chromosome segments becomes uniform. Each is apparently homogeneous. There is no contrast between cyanophilous granules and erythrophilous ribbon, but the whole chromosome stains uniformly like chromatin.'

Miss Sargant has very kindly allowed me to examine her preparations of the embryo-sac nuclei, and I find that, as she and Professor Farmer[3] point out, there is a definite relation between the linin and the nucleoli. Both in the resting stage and in the synapsis the nucleolus is connected to the linin-network by delicate threads, and in the later stages the chromosomes in process of formation are also often connected to it by threads.

Carnoy and Lebrun[4] describe a series of complicated changes in the nucleus of the egg of the Batrachia from which it appears that the

[1] On Spore-Formation and Nuclear Division in the Hepaticae, Ann. Bot., ix, 1895, p. 469.

[2] The Formation of the Sexual Nuclei in *Lilium Martagon*, Ann. Bot., x, 1896 and xi, 1897.

[3] Loc. cit. (Hepaticae), p. 491.

[4] La cytodiérèse de l'œuf. La vésicule germinative et les globules polaires chez les Batraciens. La Cellule, xii, 1897, and xiv, 1898.

chromosomes are definitely derived from the nucleoli. The young nucleus contains a much convoluted, apparently continuous thread. A portion of this becomes transformed into nucleoli (primary nucleoli). The remainder becomes resolved into granules, some of which dissolve, and the others are converted into secondary nucleoli. The nucleus now contains only nucleoplasm and primary and secondary nucleoli. Both primary and secondary nucleoli become resolved into filaments which present very complicated figures. They are ephemeral, however, and again break up into granules, some of which contribute to the formation of new secondary nucleoli. These again produce new filamentous figures which are also ephemeral and from which comes a third generation of secondary nucleoli, and so on for a number of generations. All the filamentous figures found in the nucleus up to the time of the first polar kinesis have thus a nucleolar origin, and finally a portion of the products of this nucleolar resolution is dedicated to the formation of the nuclein elements (chromosomes) of the first maturation-spindle.

Montgomery[1] comes to the following conclusions concerning the structure and function of the nucleolus.

The ground-substance of the nucleolus is more or less dense, homogeneous, or granular, and either fluid or viscid in consistency. In *Spirogyra* it has a true membrane. Vacuoles are normal structures. The alveolar structure of nucleoli described by various observers (e.g. Cavara) is probably referable to the regular distribution of equal-sized vacuoles in the nucleolus. Nucleolini, granules within the nucleolus, have frequently been observed, but no particular morphological significance can be attached to them. They appear to be only detached portions of the nucleolar substance, and may in some cases be only small vacuoles.

Two kinds of nucleolus may be seen in some animal egg-cells, the nucleolus proper and the paranucleolus or Nebennucleolus. The paranucleolus usually stains less deeply with nucleolar stains. It is probably not present in plant-cells. Some observers consider the paranucleoli to be derivatives of the nucleolus. Hacker regards them as secretions of the chromatin. Montgomery considers that they may represent those portions of the nucleolar substance which are deposited last, after the nucleus has undergone important physiological and chemical changes, the first portion produced being the nucleolus proper. In some cases a double nucleolus is found, the component parts of which may each represent a true nucleolus; or such a double nucleolus may consist of a true nucleolus in apposition to a chromatin-nucleolus. The chromatin-nucleolus may be a metamorphosed chromosome (in *Pentatoma*), or, as in the larva of *Carpocapsa*, it may originate from one of the granules of the nuclear reticulum[2].

[1] Comparative cytological Studies, with especial regard to the Morphology of the Nucleolus, Jour. of Morph., xv, 1899, p. 265.

[2] Loc. cit., p. 519.

This seems to show that the chromatin-nucleolus always stands in genetic relation to the chromatin, whilst the nucleolus proper does not.

The mode of transportation of the nucleolar substance to the daughter-nucleoli is probably different in different objects. A discharge of nucleolar substance into the cytoplasm appears to take place in some cells and may disappear, hence 'the substance of the parent-nucleolus may be in many cases not transferred to the daughter-nuclei, but the latter (perhaps as a rule) may produce their own nucleoli *de novo*.' There is no substantial basis for Zimmermann's conclusion 'omnis nucleolus e nucleolo,' and there is no evidence that the nucleoli are genetically related to the chromatin.

The nucleolus 'is derived in the first place from the cytoplasm,' and consists 'of a substance, or different substances, taken into the nucleus from the cell-body.' 'It seems probable that these substances stand in some relation to the nutritive processes of the nucleus, and in a relation to the growth of the latter.' Thus growing nuclei are 'characterized by an especially large amount of nucleolar substance.' From this one might conclude that the nucleolar 'substance stands in some connexion with the processes of nutrition' and is either: (1) 'nutritive in function,' or (2) 'represents that portion of substances assimilated by the nucleus from which all nourishment has been extracted, and in this case it would be a waste product,' or (3) 'may represent accumulations of nutritive substance retained in the nucleus as a reserve supply; but this does not seem to be very probable, for by this assumption it would be difficult to explain the uniformity in the size of the nucleoli in a given species of cell.'

Cavara[1] has made an important contribution to this subject by his researches on the nucleoli of various plant-cells. I abstract briefly his chief conclusions. Nucleoli in a state of rest in cells still capable of division or growth are composed of two substances, one, internal, which forms the major part, a homogeneous and specially refractive substance, only slightly stainable, which corresponds to the plastin of Zacharias or pyrenin of Schwarz; the other, peripheral, of variable density, much more stainable than the plastin, with characteristics that connect it with chromatin or a modification of chromatin. These two substances are associated in various ways: sometimes the chromatin-like substance is uniformly distributed around the plastin, or more frequently is not so uniform, but presents breaks in its continuity which give to the nucleoli an alveolate structure or sometimes even *a real reticulate structure*. During the prophases of division the nucleoli decrease somewhat in volume and break up. At the same time the linin-thread contracts and breaks

[1] Intorno ad alcune strutture nucleari. Estratto dagli Atti del R. Istituto Botanico dell' Università di Pavia, nuova serie, v, 1898. Breve contribuzione alla conoscenza del nucleole. Bollettino della Società Botanica Italiana, 1902, p. 108. Bot. Cent., xxxix, 1902.

up into chromosomes. The nucleolar remnants which may remain in many cells, after the formation of the chromosomes, have no longer the characteristics of the nucleolus as presented before karyokinesis. They have become reduced in volume and in capacity for stains. This means that the chromatin-like substance has been subtracted from the nucleolus to build up the chromosomes, leaving the plastin which is employed in another manner, as in the formation of spindle-fibres and the new cell-wall. As the nucleoli are reconstructed during the anaphase, they become centres of attraction, not only for plastin, which is taken up from the nuclear sap, but also in part for chromatin, which is taken up from the nuclear thread. The differences which have been observed in the capacity of nucleoli for stains can be explained by the fact that they contain varying quantities of the two substances (see also Hertwig, loc. cit.). Without impugning the constancy of the characteristics of the nucleoli and chromosomes, 'non vi ha dubbio che gli uni e gli altri siano organi di una certa mutualità e transitorietà, e che come avviene dissoluzione di nucleoli o *pirenolisi* in seno al nucleo, altrettanto si possa dire di dissoluzione della cromatina, o di *cromatolisi*.' Chromatolysis ought not then to be regarded merely as an abnormal or pathological phenomenon, but it represents a normal condition, *sine qua non*, of the evolution of the cell.

Cavara's conclusions are combated by Longo[1], who says that from his observations the nucleolus is not formed of two distinct substances, and that the so-called plastin portion of the nucleolus represents only a vacuolization.

Buscalioni[2] also concludes that there is no connexion between the formation of the chromosomes and the disappearance of the nucleoli.

Bradley M. Davis[3] shows that in the tetraspore mother-cells of *Corallina officinale* the chromatin is scattered in a finely divided state in the nucleus, and that a linin-network is wanting. Each nucleus contains a single nucleolus which stains differently from the chromatin-granules. After division the chromosomes fuse together into a large deeply stained body on the surface of the prominent centrospheres. A nuclear membrane is formed and then the nucleolus reappears, at first smaller than the chromatin body, but afterwards becoming larger. The chromatin body then begins to fragment into a number of minute granules surrounding the single conspicuous nucleolus.

[1] Existe cromatolisi nei nuclei normali vegetali? Rendiconti della R. Acc. dei Lincei, 7ª ser., v, 1898. Contribuzione alla cromatolisi (picnosi) nei nuclei vegetali. Estratto dal vol. 9° degl' Ann. del R. Istit. Bot. di Roma.

[2] Osservazioni e ricerche sulla cellula vegetale. Ann. del R. Istit. Bot. di Roma, vii, 1898. See Bot. Cent., lxxix, 1899.

[3] Kerntheilung in der Tetrasporenmutterzelle bei *Corallina officinalis*, L., var. *mediterranea*. Ber. d. deut. Bot. Gesell., xvi, 1898.

Macallum [1] shows that the reaction for phosphorus obtained in nuclei by means of his nitric-molybdate reagent indicates the presence of chromatin in the nucleoli. 'The eosinophilous nucleoli in animal and vegetable nuclei give a strong reaction for phosphorus, but less marked than in the case of chromatin. On the other hand, the nucleolar elements in the nucleus of the ovary of *Erythronium* which are rich in "masked" iron, give a deep reaction for phosphorus. A similar result was obtained in the nucleoli of the nuclei of the embryo-sac of the same form, in the peripheral nucleoli of the maturing ovarian ova of *Menobranchus*, in the nucleoli of *Corallorhiza multiflora* and of *Spirogyra*, all rich also in "masked" iron.'

A very interesting case, on the animal side, in which the nucleolus takes part in the chromosome-formation is that of *Actinosphaerium*. Here, according to Hertwig [2], the nucleolus, at one stage in the life-history of the organism, consists of plastin only (plastin-nucleolus); at another stage both plastin and chromatin are collected into a single homogeneous body, the plastin-chromatin-nucleolus. There is, however, no sharp distinction between them, the one passing gradually into the other so that the more chromatin the nucleolus contains the more it reacts like the chromatin-nucleolus, and vice versa. In mitosis the plastin-chromatin-nucleoli produce fine ramifying threads, which exhibit a granular appearance. The granules react like chromatin, whilst the ground-substance reacts as plastin. The equatorial plate is formed out of these threads. In the division of the primary cysts, which result in the formation of secondary cysts (gametes), the daughter-nuclei are reconstituted in such a manner that the chromatin remains distributed on the nuclear network, whilst the nucleolus is composed of plastin. In mitosis the chromosomes are formed out of the nuclear thread. As they become grouped to form the equatorial plate, the plastin-nucleoli become drawn out into fine granular filaments, which surround the chromosomes and become more or less incorporated with them. The spindle is formed out of the nuclear network and cytoplasm, but the connecting fibres between the two groups of daughter-chromosomes appear to be formed out of the plastin-substance of the nucleolus. The rudiments of the plastin-nucleoli appear as blisters in the chromatin masses, from which Hertwig suggests the possibility that both plastin and chromatin are modifications of, or represent the same element in, the nucleus.

According to Němec [3], the nucleoli consist of a substance like plastin. The development of the spindle coincides with the disappearance of the

[1] On the Detection and Localization of Phosphorus in Animal and Vegetable Tissues, Proc. R. Soc., lxiii, 1898.

[2] Ueber Kerntheilung, Richtungskörperbildung und Befruchtung von *Actinosphaerium Eichhorni*. Abh. bayer. Akad. d. Wiss., 1898. Ueber die Bedeutung der Nucleolen, Sitzungsber. Bot. Ges. München, xiv, 1898. See L'Année Biologique, iv, 1898.

[3] Cytologicka pozorovani na vegetacnich vrcholech rostlin: Věstnik král. Ceské společnosti náuk Prag, xxiii, 1897 (see L'Année Biologique, iv, 1898); Zur Physiologie der Kern- und Zelltheilung, Separatabdruck aus Bot. Cent., lxxvii, 1899.

nucleolus. The mantle-fibres of the spindle condense during the anaphase into a granular mass, which becomes the nucleolus and passes into the daughter-nucleus. The extranuclear nucleoli which are sometimes seen at the anaphase along the new cell-wall also originate from the connecting fibres.

Němec, however, in a later paper[1], points out that, although his observations indicate the connexion between nucleoli and spindle-fibres, other explanations of the phenomena are possible, and remarks that these observations only prove certainly that there is some definite relation between the nucleolus and the division process, and that there is a definite connexion between the size of the nucleolus and the power of the nucleus to divide; in this he agrees with Schwarz[2]. The solution of the nucleolus and the formation of the spindle-figure might be two quite different processes, having this only in common that they go on side by side.

Chamberlain[3] finds a distinct connexion between nucleoli and chromatin in the oosphere-nucleus of *Pinus Laricio*. The chromatin takes the form of nucleoli, which collect from all parts of the nucleus to a definite area near the centre, and there develop into a typical spireme.

Duggar, in his studies on the pollen-grain and embryo-sac in *Symplocarpus*, *Peltandra*, and *Bignonia*[4], has some very interesting observations on the relation of the nucleoli and the chromatin-elements. The nucleolus takes the chromatin-stain mainly, the nuclear reticulum very little. During the formation of the chromosomes they remain attached to the nucleolus by minute linin-threads, and the nucleolus is often drawn out by these into a fusiform shape. In *Bignonia*, 'in the dispirem of the daughter-nuclei the chromosomes become very irregular in outline, and gradually diminish in size, while there is gradually formed a large nucleolar-like body with irregular outlines. This body takes the chromatic dyes as did the nucleolus generally before.' Speaking of the second division, the author says: 'Everything indicates that the nucleolus of the microspore-nucleus has thus resulted from the direct or indirect fusion of chromatic material used in division.'

Mottier[5] concludes that in *Dictyota* 'the behaviour of the nucleolus, during both the development of the nuclear figure and the construction of the daughter-nuclei, indicates that this body represents a substance which is utilized by the chromatin and not by the spindle-fibres.'

[1] Neue cytologische Untersuchungen: Sonderabdruck aus Fünfstück's Beiträgen zur wissenschaftl. Bot., iv, 1900.

[2] Die morphologische und chemische Zusammensetzung des pflanzlichen Protoplasmas. Cohn's Beitr. z. Biol. d. Pflanzen, v.

[3] Oogenesis in *Pinus Laricio*, Bot. Gaz., xxvii, 1899.

[4] On the Development of the Pollen-grain and the Embryo-sac in *Bignonia venusta*, Bull Torrey Bot. Club, xxvi, 1899. Studies in the Development of the Pollen-grain in *Symplocarpu foetidus* and *Peltandra undulata*, Bot. Gaz., xxix, 1900.

[5] Nuclear and Cell Division in *Dictyota dichotoma*, Ann. Bot., xiv, 1900.

Wiegand[1] points out that the nuclei in *Potamogeton* are all very peculiar, differing from the ordinary type in having the chromatin mostly aggregated in a ball at the centre of the cavity, instead of being distributed on the linin-network.

Andrews[2] shows that in the nuclei of *Magnolia* and *Liriodendron* the nucleolus is large, stains deeply, and has a conspicuous vacuole. The linin-network is chiefly connected with the nucleolus. The threads of the network are at first smooth and uniform in diameter. At a later stage granular masses of chromatin appear on it, which gradually increase in size and become the chromosomes. The nucleolus at about the same time disappears entirely. 'It is probably utilized as food in the growth of the chromatin masses, for they stain much more readily at this time than at an earlier stage.' The chromosomes therefore arise from the resting nucleus as irregular masses without a previous formation of the usual spirem, and their identity from the first to the second mitosis is not maintained.

Blanche Gardner[3], on the root-cells of *Vicia Faba*, comes to the conclusion that the chromosomes derive 'at least a large part of their material from the nucleolus.' 'The nucleolus is related to the nuclear reticulum in such a way that the fibres penetrate its substance.' Previous to the nuclear division the nucleolus divides into two. Then the thin, long, almost continuous nuclear thread can be seen 'at one or at several points' to 'dip into the nucleolus.' 'The nucleolus now begins to transfer its contents into the nuclear thread.' The thread becomes thicker and stains just like the nucleolus, which gradually disappears. This spirem thread then divides transversely into the chromosomes. In the daughter-nuclei the chromosomes 'aggregate to form a small, dense, blue-black coil.' Out of this mass the nucleolus is formed. The chromosome-coil gives up its chromatin and gradually loses its dark colour. 'At first these chromosomes are full of the dark chromatin-granules; these become fewer and fewer as the nucleolus becomes larger and more distinct[4].'

Methods.

The root-apices of varieties of the common French Bean, *Phaseolus vulgaris*, L., were used. Sections were cut both by hand and by the microtome. Various fixing-fluids were used, but Perenyi's fixing-fluid was the most useful. Among the many staining methods tried, Heidenhain's iron

[1] Wiegand, Karl M., The Development of the Embryo-sac in some Monocotyledonous Plants, Bot. Gaz., xxx, 1900.

[2] Karyokinesis in *Magnolia* and *Liriodendron* with special reference to the Behaviour of the Chromosomes, Bot. Cent., xi, 1901, Beih., p. 134.

[3] Studies on Growth and Cell-division in the Root of *Vicia Faba*. Contributions from the Botanical Laboratory, University of Pennsylvania, ii, 1901.

[4] Chamberlain, in a recent paper (Bot. Gaz., xxxvi, 1903, p. 28) concludes that in *Pellia* the nucleolus contributes material to the chromosomes and spindle.

haematoxylin gave the best results. Other stains, however, were used for special purposes. Very careful staining and washing-out is necessary in order to exhibit the finer details of nuclear structure, and a good illumination with high powers is required for the microscopic examination of the preparations. The apochromatic object-glass of Zeiss (2 mm. N. A. 1·40) and an oil immersion condenser, or Powell and Lealand's dry apochromatic condenser (N. A. ·98) were almost always employed; but a Leitz $\frac{1}{12}$ oil immersion lens was also found valuable. Daylight was often used for general work, but the light from an incandescent mantle passed through a bull's-eye condenser was used to determine the finer details of structure.

I am much indebted to my friend Mr. Norman Walker, Assistant-Lecturer in the Yorkshire College, who was kind enough to make some of the preparations for me.

The Resting Nucleus.

The resting nucleus does not appear to differ materially in structure from the ordinary nuclei of plant-cells. It is limited towards the cytoplasm by a thin deeply stained layer—the nuclear membrane—which exhibits in thin sections a fine granular structure. Inside this is a finely meshed nuclear network, which forms a thin layer at the periphery of the nucleus in close contact with the nuclear membrane. On it are distributed a number of small granules, which stain deeply in nuclear stains.

Each nucleus contains one or more nucleoli, which are usually much more conspicuous than the nuclear reticulum, and stain more deeply. The number present varies according to the age of the cell. It is only in the young cells, however, that two or more occur; as the cells come to maturity the nucleoli fuse together so that in all the older cells one nucleolus only is present. Each nucleolus is lodged in a cavity in the nuclear network, and is surrounded by a clear space. As in many other vegetable nuclei, it is suspended in this cavity by a number of delicate threads, radiating from it on all sides to the nuclear network (Pl. V, Figs. 3–7). These threads are only visible in stained specimens. They appear to be continuous with the nuclear network, and to form a part of it.

The relative size of the nucleolus and amount of nuclear network varies in different cells. In the actively growing and dividing cells of the meristematic region the nucleolus is the most prominent feature in the nucleus, the nuclear network forming only a very thin layer in close contact with, and scarcely distinguishable from, the nuclear membrane. In the cells of the root-cap the nucleolus is much smaller, but the nuclear network is relatively more abundant, and in the outermost older cells of the root-cap layer is in its turn the most prominent feature in the nucleus, the nucleoli being so small in some cases as to be scarcely visible. The same differences are

observable in other parts of the young root. It thus appears that the amount of nucleolar substance present in a nucleus is to some extent a measure of the nuclear activities.

Structure of the Nucleolus.

The nucleolus is spherical in shape or nearly so, and is usually placed in or near the centre of the nucleus. It does not lie free in the nucleus, but, as already pointed out, is in close connexion with the nuclear network, in which it is suspended by fibres which not merely surround it, as Montgomery states (loc. cit., p. 506), but actually penetrate its substance, and in many cases appear as if drawn out of it. The nucleolus in fact appears to form a part of the nuclear network. I have observed similar connecting fibres in the embryo-sac nuclei of *Lilium* in Miss Sargant's preparations, both in nuclei in the resting stage and in synapsis and later stages, in the apical cells of the stem of *Elodea*, in the root-cells of the Oak, and in many other cases. They have been observed by Farmer in the nuclei of Liverworts and *Lilium*; by Duggar (loc. cit.) in various plants; by Rosenberg in the nuclei of the suspensor of *Zostera marina*, L., and by other observers in various nuclei—both of animal and plant cells. Zimmermann's statement [1] that the nucleolus in no case appears to be in direct connexion with the chromatin-network is therefore not correct.

In some cases the nucleolus appears to be homogeneous throughout, but in the majority of nuclei it possesses one or more vacuolar spaces filled with a substance which appears to differ from the rest of the nucleolus (Figs. 1–7, 37). This vacuolization always appears to take place as the nucleus comes to maturity. It is absent only in young nucleoli. I have not seen the alveolar structure described by Cavara (loc. cit.), but it is obvious that the presence of a large number of vacuoles in a nucleolus would produce some such appearance as he describes.

In some of the larger nucleoli I have observed darker coloured granules (nucleolini) as described by Macfarlane (loc. cit.) and others, but I agree with Montgomery (loc. cit.) that no particular morphological significance can be attached to them at present. In the cases observed by me they seemed to form only the central portion of the vacuolar substance.

The vacuolate structure of nucleoli appears to be very common, and was first of all noticed in vegetable-cells by Schleiden, who speaks of the nucleolus as 'a small, sharply defined body, which, judging from the shadow that it casts, appears to represent a thick ring or hollow globule [2].'

Nägeli also observes that nucleoli may be homogeneous or 'hollow in

[1] Die Morphologie und Physiologie des pflanzlichen Zellkernes. Jena, 1896, p. 40.

[2] Contributions to Phytogenesis. Translated by Henry Smith in 1847 for the Sydenham Society, p. 234. See also Schleiden's Principles of Botany, Eng. Ed., translated by E. Lankester, 1849, p. 32.

the centre,' and that 'there may be one, two, three, or many cavities of various sizes and arrangement[1].'

Macfarlane[2] regarded the vacuoles as endonucleoli and ascribed to them an important part in the nuclear economy, and he still insists on their probable importance in living cells, and suggests that they play a 'special part in furnishing to the nucleolar substance some ferment or compound which may be utilized during the division period[3].'

Mann[4] regards the endonucleolus as 'the trophic centre for all the organs concerned in assimilation and dissimilation,' and says that it plays an important part in the conjugation of cells.

Cavara[5] contends that the vacuoles indicate the separation of the nucleolar substance into two distinct parts, chromatin and plastin, and Zacharias, who is opposed to the chromatin-plastin theory of the nucleolus, nevertheless concludes that it appears to consist of two distinct substances, a more refringent vesicular substance surrounded by a homogeneous, less refractive substance[6].

Chamberlain has described a case in which the central, less deeply stained portion appeared to be separable from the outer, more deeply stained peripheral part, for 'there seemed to be a crack in the nucleolus, and upon applying a gentle pressure the central portion came out of the shell[7].'

In haematoxylin-stained specimens of *Phaseolus* it is very clearly seen that the outer layer becomes more deeply stained than the vacuolar substance; in some cases there is a very marked distinction between them, and in some nucleoli the vacuolar substance is in contact with the exterior through an opening in the outer layer on one side of the nucleolus (Fig. 5).

In methyl green and fuchsin the nucleolus stains bluish-red, the nuclear network red, and in specimens from which the stain has been well washed out the outer layer of the nucleolus is coloured light blue, the vacuolar portion remaining colourless or nearly so. Similar results are obtained with methyl green and eosin. In gentian violet the outer layer is deeply stained violet and the vacuolar portion light blue. But in many of the larger nucleoli the gentian violet shows up a more complex structure. In such cases we find a deeply stained thin outer layer surrounding a less deeply stained inner layer, and in the centre one or more deeply stained masses which are irregular in shape, with coarse radiations into the lighter stained part, or even exhibit a structure akin to a coarse network, which recalls the description given by Carnoy of his *nucléoles-noyaux*.

The existence of a chromatin-like substance in the nucleolus is indicated by the fact that in all these stains the chromosomes stain like the nucleolus, except that there may be a difference in the intensity of the

[1] Memoir on the Nuclei, Formation, and Growth of Vegetable Cells, translated from Schleiden und Nägeli's Zeit. f. wiss. Bot., 1844, by W. Henfrey, p. 239. Ray Society, 1845.

[2] Loc. cit., 1882–5. [3] Loc. cit., 1901, p. 194. [4] Loc. cit., 1892, p. 396.

[5] Loc. cit. [6] Loc. cit., 1885. [7] Loc. cit., Bot. Gaz., 1899.

colouration. Thus in gentian violet and safranin the nucleolus stains red, the chromosomes deep red, whilst the cytoplasm and nuclear network are stained violet. In safranin and picro-nigrosin, well washed out, the larger part of the nucleolus stains deep red, but a thin peripheral layer stains blue, sometimes giving to the nucleolus the appearance of being surrounded by a membrane. The chromosomes stain light red or reddish-blue, the spindle deep blue, and the cytoplasm and nuclear network blue.

In gentian violet the chromosomes are usually more deeply stained than the nucleoli of the resting nucleus; but this appears to be due partly to the fact that the stain is more easily washed out of the nucleoli.

The chromatin-like substance thus appears to occur mainly in the outer more deeply stained portion of the nucleolus. This is further substantiated by the following observations.

In sections cut by hand from fresh root-apices and stained in dilute acetic acid solution of methyl green the protoplasm and nucleus both stain bluish-green, but the nucleolus is the most deeply stained, especially in the actively growing cells, and the outer layers of the vacuolate nucleoli are the most deeply stained. If fresh sections are placed in pepsin solution for from three to six hours, at a temperature of 36° C., a contraction and distinct diminution of the cell contents are shown. The nucleolus becomes much smaller and presents a bright glistening appearance. On staining in the methyl green solution, the protoplasmic remnant stains green, while the nucleolar remnant stains bright green, and in some cases a few granules, probably chromatin-granules in the nuclear network, take a similar bright green colour. The nuclear network stains like the cytoplasm.

Again, if sections, either fresh or in spirit, be treated according to the method of Macallum, we get a strong reaction for phosphorus in the nucleoli and chromosomes, but very little in the nuclear reticulum. In vacuolar nucleoli this reaction is confined mainly to the outer layers; we get very little in the vacuolar portion. And further, we get an intense reaction for phosphorus in the nucleolar remnant which is left after treatment with digestive fluid.

In gentian violet-stained specimens the nucleolar remnant left in contact with the chromosomes at the time of the formation of the nuclear plate (Fig. 21) is often much less deeply stained than the chromosomes, and in some cases probably represents that inner portion of the resting nucleolus which does not become stained deeply in nuclear stains. It is impossible to definitely decide this point, however, by microscopic examination.

The observations just described all point to the conclusion that there are at least two different substances in the nucleolus, one of which at any rate possesses the reaction of chromatin. It seems to me probable that, as Cavara suggests, the explanation of the different accounts which have been

[1] Loc. cit., 1898.

given of the structure and staining and chemical reactions, may be due to the fact that nucleoli at different stages of their development, or from different plants, may vary as to the relative amounts of plastin and chromatin substance which they contain. It seems almost certain that there are nucleoli which do not contain any chromatin or very little (the plasmasomes, true nucleoli, &c., of various observers), and others which contain a large quantity of chromatin (the so-called chromatin-nucleoli, *nucléoles-noyaux*, &c.), and between these two extremes we may have nucleoli with varying relative amounts of the two substances[1]. Miss Ferguson mentions, in her paper on *Pinus Strobus* (Ann. Bot., 1901, p. 433), that 'the occurrence of unstained nucleoli in the same nucleus in which others were deeply coloured was common, especially at about the time of synapsis.' Again, 'the attitude of this (egg) nucleolus towards dyes varies much at different periods of its history. It may or may not take the safranin of Flemming's triple combination; it may stain intensely with gentian violet or iron haematoxylin; it may show a weak reaction or may be absolutely unaffected by them.' It is not clear from the evidence available that the vacuolization of the nucleolus indicates a definite separation of the contents of the nucleolus into chromatin and plastin. It is more probable that the ground-substance of the nucleolus is plastin, and that the outer layer of it may become impregnated with chromatin or a modification of it.

Changes in the Nucleolus during the Prophase.

In the resting condition the nucleolus is suspended to the peripheral network by delicate threads, which are only visible in carefully stained specimens. As the nucleolus increases in size the suspending threads become more prominent, and it is then seen that they are intimately connected with the nucleolus, appearing as if drawn out of its substance, and on the other side are continuous with the peripheral network (Figs. 6 *a* and 7). In Fig. 6 *a* is seen a nucleolus at this stage which has become displaced from a nuclear cavity. The connexion of the threads with the nucleolus is clearly seen. In the root-apex of *Allium Cepa* Němec[2] points out that in younger cells the nucleoli are surrounded by a clear space, and are connected by achromatic fibres to the nuclear network. The hyaline appearance of this space may be simply due to the fact that chromatin-granules are absent from these threads. In somewhat older nuclei he points out that the radiating threads become more prominent, and chromatin-granules are now seen lying thickly on the surface of the nucleolus, and appearing as if fused with its outer layer. His figure illustrative of this stage corresponds almost entirely with my Fig. 6, for which I offer

[1] Cf. Montgomery, Woods Holl. Bio. Lectures, 1898.

[2] Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium Cepa*, Pringsh. Jahrb. f. wiss. Bot., xxxiii.

a different explanation, viz., that it indicates the beginning of the transference of nucleolar material to the nuclear thread [1].

The peripheral network of the resting nucleus as seen from the surface is shown in Fig. 2. It consists of a lightly stained network with numerous small chromatin granules. It stains less deeply in nuclear stains than the nucleolus, and is not very conspicuous in the resting stage. At the time that the suspending threads become more prominent, certain portions of the peripheral network become also more prominent and more deeply stained (Fig. 9), a number of thicker threads being visible, connected to one another by finer filaments of the original network. The impression conveyed to an observer on looking through a large number of such stages in the nuclear development is that the substance of the nucleolus is being conveyed into the surrounding threads, and this is borne out by observation of later stages, where the threads radiating from the nucleolus become larger and more definite, and stain exactly like the nucleolus, which at the same time is becoming smaller and more irregular or amoeboid in shape (Fig. 8). It is interesting to note that Zacharias, in his observations on the division of the nucleus in living rhizoids of *Chara*, observed that in the process the nucleolus becomes irregular in shape, and undergoes amoeboid changes of form, and then disappears just before the formation of the chromosomes. The stage of this amoeboid condition of the nucleolus in *Chara* corresponds exactly with what is observed in stained specimens of *Phaseolus*. In *Phaseolus* the amoeboid condition of the nucleolus coincides exactly with the thickening of the nuclear threads connected with it, and an inspection of Figs. 14 to 16 indicates pretty clearly that as the nucleolus decreases in size the nuclear thread becomes more and more prominent, while still in definite continuity with the nucleolus, and staining reactions show that it becomes stained in a similar way.

While these changes are taking place, kinoplasmic fibres appear at the poles of the nucleus, elongated in the direction of the future spindle. The nucleus contracts, and the nuclear thread tends to be drawn more closely around the nucleolus (Fig. 10). The nuclear wall breaks down gradually, and the fibres penetrate the cavity of the nucleus (Figs. 11 and 12). This may take place before the formation of the chromosomes, and the spindle-fibres may come into contact with the unsegmented nuclear thread (Figs. 15 and 16).

The thread then becomes bent sharply at different points, the nucleolus still being in connexion with it (Fig. 16), and then breaks up into short rod-like chromosomes. Fig. 17 indicates a portion of a nucleus at this stage, in which can be seen the remnant of the nucleolus and some of the chromo-

[1] The reference to the Figure in Němec's paper, p. 316, appears not to be correct, it seems to be Fig. 5, not Fig. 42, to which his description refers.

somes just on point of separation; Figs. 13, 18, and 19 show various appearances of the nuclei at this stage, with an irregularly shaped indefinite nucleolar mass surrounded by chromosomes. The chromosomes become shorter and thicker, and arrange themselves to form the nuclear plate (Fig. 20). The remains of the nucleolus can still be seen, and in Fig. 20 there is still visible an indication of its connexion with the chromosomes in the faintly stained band between the drawn-out portion of the nucleolus-remnant and the chromosomes (cf. Fig. 16). It appears that the numerous connecting threads around the nucleolus gradually disappear, until only one is left, the nucleolus being drawn out at this point into a kind of tail (Figs. 16 and 20).

It is extremely difficult, however, to be certain of the exact sequence of events, as the observations have to be made entirely on stained specimens. In many cases the nucleolus appears as if it was becoming directly transformed into chromosomes (see Figs. 12 and 13), but this I think is due to a contraction of the nuclear network around the nucleolus just at this time.

Finally all connexion of the nucleolus with the chromosomes ceases, It is now much smaller in bulk, stains less intensely than before, and often exhibits a somewhat spongy texture, and at the same time begins to divide into two generally unequal portions (Fig. 21) which separate to opposite poles of the spindle-figure (Fig. 23). Rosen has already observed this phenomenon of nucleolar division in *Phaseolus*, and similar phenomena have been observed in some other plants. These nucleolar remnants are also surrounded by a clear space as in the resting nuclei, and in Fig. 22 is seen a case in which the single nucleolar remnant left at one of the poles of the spindle is surrounded by a clear space across which suspending fibres are seen.

At a later stage these nucleolar remnants entirely disappear, and coincident with this the spindle becomes more prominent: the fibres increase in number and stain more deeply, so that one might easily conclude that there was some connexion between the two, and that a portion of the nucleolus is concerned in the formation of the spindle.

Reconstitution of the Daughter-nuclei.

We have now to consider the changes which take place in the chromosomes during the reconstitution of the daughter-nuclei. These have an important bearing upon the question of the relation of nucleoli to chromosomes, for it is clear I think, from the observations about to be described, that the nucleoli in the daughter-nuclei definitely originate by the fusion of the chromosomes, first of all into a number of small nucleolar masses, connected together by a deeply stained network, and then by a further fusion into the large nucleoli found in the mature cells.

The equatorial plate first of all splits into two groups of daughter-chromosomes which separate to opposite poles of the spindle. As this takes place the chromosomes are distinctly seen as very short rods or granules (Figs. 25, 26). Whether they are actually separated from one another or remain more or less connected together by fine anastomosing threads I could not definitely determine. On arrival at the poles of the spindle they become aggregated together into a more or less homogeneous mass, in which the chromosomes can with difficulty be recognized (Fig. 27). At this stage the cell-plate appears across the equatorial region of the connecting fibres which are now very numerous. A nuclear membrane then appears, and the nucleus begins to open out or expand, exhibiting the chromosomes connected together by a deeply stained network (Fig. 28). The nucleus appears at this stage as if lodged in a cavity in the cytoplasm, the limiting layer of the nucleus or nuclear membrane apparently consisting of the peripheral layer of the nuclear network in close contact with the cytoplasmic layer[1]. The cell-plate now gives place to the new cell-wall in the middle region of the figure, from which the connecting threads are fast disappearing, but at the periphery of the spindle the cell-plate formation is still going on, as indicated by the prominent and deeply stained connecting fibres. There is no indication whatever of any concentration of the spindle-threads to form nucleoli, as Němec states, although it is quite possible that a portion of them have been absorbed into the daughter-nuclei, and may enter into the constitution of the prominent network with which the chromosomes are connected.

The chromosomes now begin to fuse together into somewhat irregular masses, or in some cases into a thick, irregular band or thread, still connected by a well-defined and deeply stained network of threads (Fig. 29). The connecting fibres in the middle region of the cell have disappeared, but those at the periphery of the nucleus are still very prominent. Already there are indications that the nucleolar masses are the centres of radiation for suspending fibres. This becomes very clear at a later stage, as shown in Fig. 31, in which the nuclei contain two and four nucleolar masses respectively. Around each large mass and one of the smaller ones clear spaces are to be seen.

Fig. 30 shows a still further stage of chromosome fusion. In the upper nucleus there are now only two large nucleolar masses, and the way in which these have arisen by the fusion of smaller ones is shown in the lower of the two nuclei, in which four smaller nucleolar masses are to be seen fusing together in pairs. The nuclear network is still very prominent, and numerous granules of chromatin-like substance are visible on it. The

[1] Grégoire and Wygaerts, Beihefte, Bot. Cent., 1903, p. 13, and Lawson, Bot. Gaz., xxx, 1903, p. 305, also conclude, from their observations on various plants, that the nucleus is lodged in a cavity similar to a cell-vacuole.

cell-wall now extends nearly all across the cell. The formative fibres are completely separated from the nuclei, and appear free in the cytoplasm at the periphery of the cell, as shown in Figs. 30 and 31.

Fig. 31 shows a later stage of chromosome fusion. There are in each nucleus one large nucleolar mass, which has a form indicative of its production by the fusion of two smaller ones, and one or more smaller ones. In the upper nucleus one of the smaller nucleolar masses appears to be on the point of fusing with the larger. In both nuclei the large mass and one of the smaller ones is surrounded by a clear space. In all the cases of division figured, it is interesting to note that in each the two daughter-nuclei exhibit much the same conditions as regards fusion of the chromosomes, and are just in the same stage of development (Figs. 28–37).

Fig. 32 figures a case in which the transverse cell-wall is completely formed. In the daughter-nuclei are to be seen two large nucleolar masses in the same stage of fusion, and two smaller masses. The nuclear network is also clear, but with very few chromatin-granules.

In the next stage the fusion of the nucleolar masses is carried a step further (Fig. 33). In the lower nucleus there is one large nucleolus and near it are two smaller ones, of which one is just beginning to fuse with the large mass. The upper nucleus contains a single irregular nucleolus which has obviously just arisen by fusion with it of at least two smaller ones. I think we may consider, that the two prominent projections on it indicate the result of fusion of smaller masses with it.

It is not always the case that the result of the nucleolar fusion leaves the nucleus with one large and one or more smaller nucleolar masses. In Fig. 34 we have a case in which there are three about equal-sized masses of nucleolar substance which are just beginning to fuse together. In all these cases a delicate nuclear network is visible in contact with the fusing nucleolar masses, and in Fig. 35 is shown an interesting case of an irregular mass of nucleolar substance connected at one point in a very prominent manner with the linin-network (cf. Fig. 5).

When the nucleolar masses have become completely fused, the resulting nucleolus is at first a homogeneous irregular body of uneven outline (Figs. 33 and 35), but it gradually becomes more or less spherical (Fig. 36), and finally its homogeneity disappears, and it becomes vesicular (Fig. 37). As this proceeds the nuclear network becomes restricted to the peripheral part of the nucleus (Figs. 36 and 37), but the nucleolus remains connected to it, as already mentioned, by delicate connecting threads (Figs. 1–5).

During the time these changes are taking place, each nucleus gradually increases in size with the increase in size of the cell; the nucleolus also adds to its bulk, and goes on growing until, having reached a certain stage of development, the nucleus again begins to divide.

General Considerations.

It is not necessary here to enter into any detailed discussion as to the importance of the question of the function of the nucleolus. It will be sufficient to point out that the part ascribed to the nucleus, and especially the chromatin, as the bearer of the hereditary qualities in fertilization, renders necessary as precise a knowledge as possible of the chemical nature and function of each part of the nucleus before we can come to a definite conclusion that any one part or parts of it is more concerned in the process than another. The prominence of the chromosomes at certain stages during the division of the nucleus led to the enunciation of the doctrine that the hereditary qualities are transmitted by them; that they retain their individuality, more or less, in the resting nucleus; and that they must be regarded as individual units having an independent existence in the nucleus or cell.

The observations described in this paper show that we have in *Phaseolus* a phenomenon which, if it is found to be a widely spread one, must modify our conception of the significance of the chromosomes and nucleolus in heredity. The nucleolus is intimately bound up with the formation of the chromosomes, and Strasburger's contention that it is only concerned in spindle or kinoplasmic formation does not hold good, although it is not impossible that a portion of it—the plastin or pyrenin of Zacharias and Schwarz—may be used up in this way. There is no evidence either that the nucleolus originates from the spindle-fibres as stated by Němec; and Häcker's view that it is a product of excretion finds no support. A portion of it persists for a long time, even up to the stages of metaphase and anaphase, but it seems to disappear entirely within the region of the nuclear activity, that is in connexion with the chromosomes and spindle-fibres.

It seems clear also that the nucleolus does not originate *de novo* either from nuclear substance or from the cytoplasm. There is a definite continuity of nucleolar substance from mother-nucleus to daughter-nucleus through the chromosomes. How far this supports Zimmermann's conclusion, 'omnis nucleolus e nucleolo,' is perhaps difficult to determine. But if the nucleolus is simply a part of the nucleus in which nutritive substances are stored and perhaps partly elaborated, and not an independent nuclear organ, then, while there may be a definite nucleolar continuity, it seems to me that Zimmermann's conclusion simply becomes absorbed in the larger and more important—omnis nucleus e nucleo.

It is almost impossible to avoid coming to the conclusion that the nucleolus must be regarded simply as a part of the nuclear reticulum, in which chromatin-substance is stored for the use of the chromosomes during

division or other active condition of the nucleus. But it is not easy to arrive at any definite conclusions as to their chemical relations, or as to the exact rôle of the nucleolus in the metabolism of the nucleus. The increase in size of the nucleolus in the resting stage probably takes place at the expense of materials brought into the nucleus from the cytoplasm. In the transformation of these materials into chromatin there are, it seems to me, three alternatives as to the way in which they may be dealt with :—

(1) They may be taken up by the nucleolus directly from the cell-sap, and elaborated into chromatin, or (2) they may be first of all elaborated in the nuclear thread, and then passed on to be simply stored up in the nucleolus, or (3) they may be partly elaborated in the nuclear thread, and then passed on to be more completely elaborated in the nucleolus. Farmer's suggestion (loc. cit., p. 512), 'that the nucleolus, though not in itself containing chromatin, is able to furnish at least one, and that probably the albuminous constituent of this substance,' would not explain the presence of the phosphorus-containing substance, which both Macallum and I find in such abundance in many vegetable-nucleoli.

Miss Huie [1] has made some interesting observations which bear upon this point and which seem to support the second, or possibly the third, alternative. In the gland-cells of *Drosera* during food-assimilation the nucleolus becomes smaller, whilst the chromosomes (nuclear network) become larger. The cytoplasm becomes impoverished and scanty in amount. Some time after feeding, the nucleus again becomes normal and the cytoplasm returns to its former condition. This indicates that the nucleus is the seat of metabolic activity, and that it is in the nuclear network, and not in the nucleolus, that the changes take place. But the diminution in size of the nucleolus, which is coincident with the increase in size of the nuclear network, seems to show that nucleolar substance is required before this metabolism can take place, or, in other words, that the activity of the nuclear thread is set up by the passage of nucleolar substance—chromatin or nuclein—into it, and that when the nucleus resumes its normal condition the nucleolus becomes restored to its original size by taking up the chromatin-material again from the nuclear thread [2]. This is in harmony with Kossel's conclusion that the formation of new organic matter is dependent on the nucleus, and that nuclein (chromatin) plays a leading rôle in this process [3].

It appears to be always the case, that when cells are in an active metabolic condition, the nuclear thread becomes prominent, whilst the

[1] Changes in the Cell-organs of *Drosera rotundifolia*, produced by feeding with Egg-albumen, Q. J. Mic. Soc., New Ser., xxxix, 1897, p. 387. Further Study of Cytological Changes produced in *Drosera*, Part II, Q. J. Mic. Soc., xlii, 1899, p. 203.

[2] Cf. Farmer, Ann. Bot., ix, 1895, p. 495.

[3] See Wilson, The Cell, &c., p. 340.

nucleolus becomes reduced in size or disappears, as in the guard-cells of stomata, in the gland-cells of *Chironomus*, and in the male sexual cells of plants and animals. In all such cases the nucleolar substance probably migrates into the nuclear thread, and remains there so long as the nucleus is in an active state, to be stored up in the nucleolus again when the activity ceases. Farmer and Williams [1] and Strasburger [2] have shown, for example, that the sperm-nucleus of *Fucus* is very rich in chromatin, and at the time of fusion with the ovum-nucleus exhibits a network-structure but no nucleolus. After fusion, a second large nucleolus appears in the ovum-nucleus, apparently derived from the chromatin of the sperm-nucleus. Here we might suppose that the male nucleus, on its entry into the quiescent female nucleus, loses its intense activity, and that the chromatin-substance therefore becomes accumulated in the form of a nucleolus.

So also Ikeda [3] has shown, in his observations on the nutritive function of the antipodals in *Tricyrtis hirta*, that during their stage of metabolic activity the nucleolus decreases in size, whilst the original scanty chromatin-network shows an extraordinary increase of chromatin, which becomes variously aggregated within the nucleus.

It may be objected that chromatin-nucleoli, such as we have in *Phaseolus*, are only masses of chromatin-substance, and ought not to be confounded with those nucleoli which do not give a chromatin-reaction. There certainly appear to be two distinct types of nucleolus in some animal-cells, and it is possible that the same may exist in plant-cells also; but the evidence before us as to their chemical constitution, and staining, and other reactions, is not at present sufficient to differentiate them into two distinct categories [4]. We must rely mainly upon their morphological behaviour, and so far this has not produced much evidence of such a differentiation. As Montgomery suggests, it may be that all these bodies, which some observers are inclined to regard as fundamentally different structures, may be regarded as 'true nucleoli of a different chemical nature [5].'

In conclusion, it appears to me, from a careful consideration of the facts as presented to us by various observers, that the following statements are probably justified as a summary of our present knowledge of plant-nucleoli:—

(1) That the rounded bodies present in nearly all plant-nuclei, which

[1] Fertilization of *Fucus*, Phil. Trans., 1898.

[2] Kerntheilung und Befruchtung bei *Fucus*, Pringsh., Jahrb. f. wiss. Bot., xxx, 1897, p. 364.

[3] Studies in the Physiological Functions of Antipodals and Related Phenomena of Fertilization in Liliaceae: (1) *Tricyrtis hirta*, Reprint from Bulletin, Coll. of Agriculture, Tokyo Imperial Univ., v, 1902, p. 41.

[4] Cf. Fischer, Fixirung, Färbung und Bau des Protoplasmas, Jena, 1899, pp. 98–102, and Mann, Physiological Histology, Oxford, 1902.

[5] Wood's Holl. Bio. Lectures, 1898.

differ among themselves as regards various stains and reagents, are all to be regarded as ' nucleoli.'

(2) That these nucleoli may be composed of plastin (or plastin-like substance) only, or of plastin combined with chromatin in varying quantities, and that this variation in composition partly accounts for the varied accounts which are given of their staining reactions, &c., in various plant-cells (cf. Montgomery).

(3) That in those cases where the chromatin-network is prominent and gives a strong reaction for chromatin, the nucleolus may either be absent or, if present, may give only a slight reaction for chromatin or none at all, and that where the chromatin-thread is not prominent the nucleolus is large and gives a strong reaction for chromatin.

(4) That the nucleolus simply forms a part of the nuclear network, in which chromatin or chromatin-substance may be stored, and possibly to some extent elaborated, and that it is not therefore an independent organ of the nucleus.

(5) That the nucleolus is concerned in the formation of the chromosomes, and possibly also in the production of the spindle, and that a portion of it may in some cases be extruded into the cytoplasm, and there disappear.

(6) That in the reconstruction of the daughter-nuclei the chromosomes unite together in a more or less irregular mass or thick thread, out of which is evolved the nucleolus and nuclear network, the major part of the chromatin passing ultimately into the nucleolus, except in cases where division again immediately takes place.

(7) That the vacuolar structure of nucleoli is general, and may indicate, either the separation or partial separation of the nucleolar substance into plastin and chromatin, or a greater accumulation of chromatin in its peripheral layer.

(8) That Zimmermann's conclusion, ' omnis nucleolus e nucleolo,' is not justified by the evidence before us, and that the nucleolus cannot, in the majority of cases at any rate, be regarded as an independent organ of the nucleus.

(9) If these conclusions are correct, it is obvious that the conception of the part played by the chromosomes in heredity will have to be modified, and, as Dixon has already suggested [1], the nucleolus, as well as the chromosomes, will have to be taken into account in any new hypothesis that may be put forward.

[1] Ann. Bot. xiii, 1899.

EXPLANATION OF FIGURES IN PLATE V.

Illustrating Mr. Harold Wager's paper on *Phaseolus*.

Fig. 1. Resting nucleus, showing the large vacuolar nucleolus and delicate threads suspending it to the peripheral network.

Fig. 2. Peripheral network seen from the surface of a resting nucleus. The linin-network with minute chromatin-granules is seen.

Figs. 3 and 4. Resting nuclei, with nucleoli containing a single vacuole of irregular outline, less deeply stained than the peripheral portion.

Fig. 5. A nucleus, showing the nucleolus with an interruption in the peripheral deeply stained portion at one point, giving it the appearance of a flask-like structure enclosing a less deeply stained substance. The suspending threads are more prominent and more numerous around this opening.

Fig. 6. Nucleolus of irregular shape with prominent suspending threads. The substance of the nucleolus appears as if drawn out into these threads.

Fig. 6*a*. Nucleolus, which had fallen out of a nucleus, showing threads drawn out of the substance of the nucleolus.

Fig. 7. Irregularly shaped nucleolus, showing its substance drawn out into the suspending threads.

Fig. 8. Later stage than Fig. 7. The nuclear membrane not clearly visible. The nucleolar threads are prominent.

Fig. 9. Surface-view of a nucleus at about the stage between Figs. 6 and 8. A portion of the peripheral network has become thicker and more prominent, with a delicate linin-network in the meshes of it. Compare with Fig. 2.

Fig. 10. A nucleus, showing the kinoplasmic filaments at the poles. The nuclear membrane is still visible.

Fig. 11. Nucleus, showing the nuclear membrane disappearing on one side, and the penetration of the kinoplasmic filaments into the nucleus.

Fig. 12. Slightly later stage than Fig. 11.

Fig. 13. The nuclear membrane has disappeared, and the bipolar spindle is now visible around the irregular mass of chromosomes and nucleolus.

Figs. 14–20. Show the formation of the chromosomes.

Fig. 14. A slightly later stage than Fig. 8. The connexion of the nucleolus with the nuclear network is well seen.

Fig. 15. Later stage. The filaments of the spindle are now in close contact with the nuclear network, which has not yet begun to break up into chromosomes.

Fig. 16. About the same stage as Fig. 15, but from a different point of view.

Fig. 17. A section taken through a nucleus at a later stage than Fig. 16, showing the segmentation of the nuclear thread into rod-like chromosomes.

Fig. 18. Shows the irregularly lobed nucleolus surrounded by chromosomes. About the same or slightly later stage than Fig. 19, but a larger nucleus. The connexion of the nucleolus with the chromosomes not visible.

Fig. 19. Section through a nucleus at about the same stage as Fig. 18, showing nucleolus and a few of the chromosomes.

Fig. 20. The grouping of the chromosomes in the equatorial plane. Equatorial plate. The spongy remnant of the nucleolus in the midst of them, less deeply stained, can be seen, with apparently some connexion still to the chromosomes.

Fig. 21. The less deeply stained remnant of the nucleolus shown dividing into two unequal parts, one of which will pass to each pole of the spindle. Compare Fig. 23.

Fig. 22. A nucleolar remnant at one pole only of the spindle. This is surrounded as in a resting nucleus by a clearer space across which suspending fibres are seen.

Fig. 23. Two unequal spherical nucleolar remnants approaching opposite poles of the spindle. Each is surrounded by a clear space, and stains much less deeply than the chromosomes.

Fig. 24. The nucleolar remnants have entirely disappeared; as they disappear it may be noted that a deeper staining and increase in number of the spindle-fibres takes place. An indication of this is shown in the Figures. Compare Figs. 21 and 24.

Fig. 25. Separation of the chromosomes. Anaphase.

Fig. 26. Later stage of anaphase.

Fig. 27. Fusion of chromosomes at ends of spindle to form the daughter-nuclei. Formation of cell-plate.

Fig. 28. Daughter-nuclei with chromosomes connected together by linin-network. The new cell-wall is beginning to form in the centre of the connecting fibres, which are now more numerous at the periphery where the formation of the cell-wall is still in progress, and will continue until it reaches the lateral walls of the cell.

Fig. 29. The chromosomes fuse together into larger masses, still connected by a linin-network. The central connecting fibre here entirely disappeared, leaving peripheral fibres only.

Fig. 30. Later stage of the chromosome-fusion. One daughter-nucleus contains two large masses only (nucleoli), the other four nucleolar masses, which are on the point of fusing together to form two. The cell-wall now extends nearly across the cell.

Fig. 31. Shows each daughter-nucleus at a later stage, with only one large nucleolar mass and one or more smaller ones. Each larger mass is surrounded by a clear space across which suspending fibres are visible as in a resting nucleus. In the nucleus in the upper part of the figure, two of the smaller nucleolar masses are in this clearer space, and one of them is on the point of fusing with the larger mass.

Fig. 32. Shows cell-division completed. The two daughter-nuclei contain fusing chromatin-masses and a linin-network.

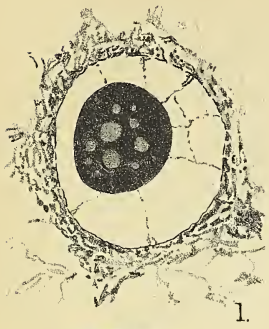
Fig. 33. Later stage than Fig. 32. The fusion of chromatin-masses is nearly complete. In the upper daughter-nucleus there is a single irregular lobed mass. In the lower one a large spherical mass and two smaller ones, one of the latter is on the point of fusing with the large mass.

Fig. 34. Nucleus, showing the last stage in the fusion of chromatin-masses to form the nucleolus. The three masses have just begun to fuse together.

Fig. 35. The fusion is now complete, but the nucleolus has not yet rounded itself off.

Fig. 36. A still later stage, showing the nucleoli as more or less spherical homogeneous bodies.

Fig. 37. A still later stage, showing the vacuolization of the nucleoli. The daughter-nuclei are now in the resting stage.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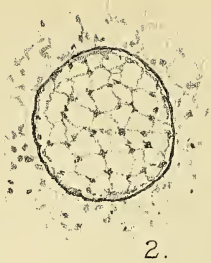
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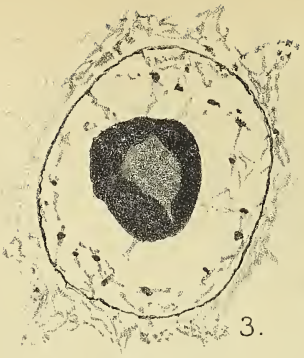
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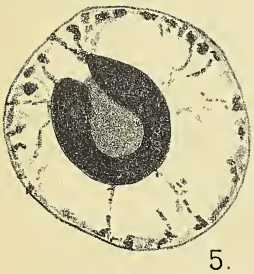
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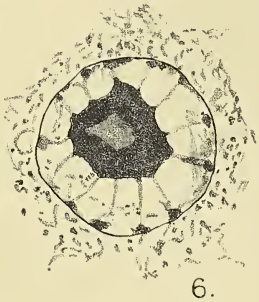
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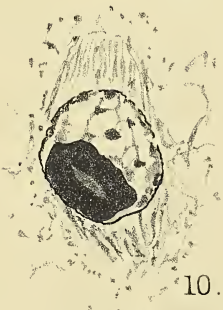
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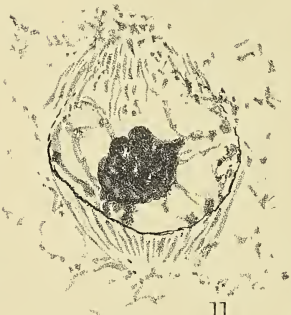
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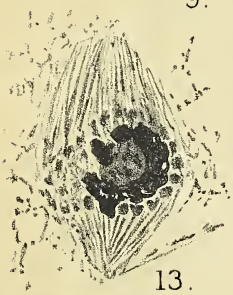
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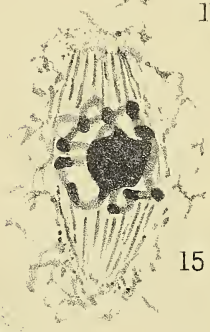
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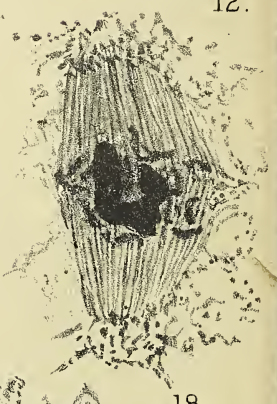
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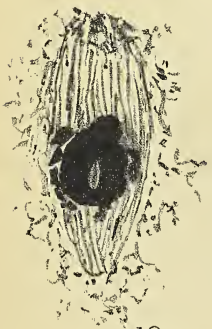
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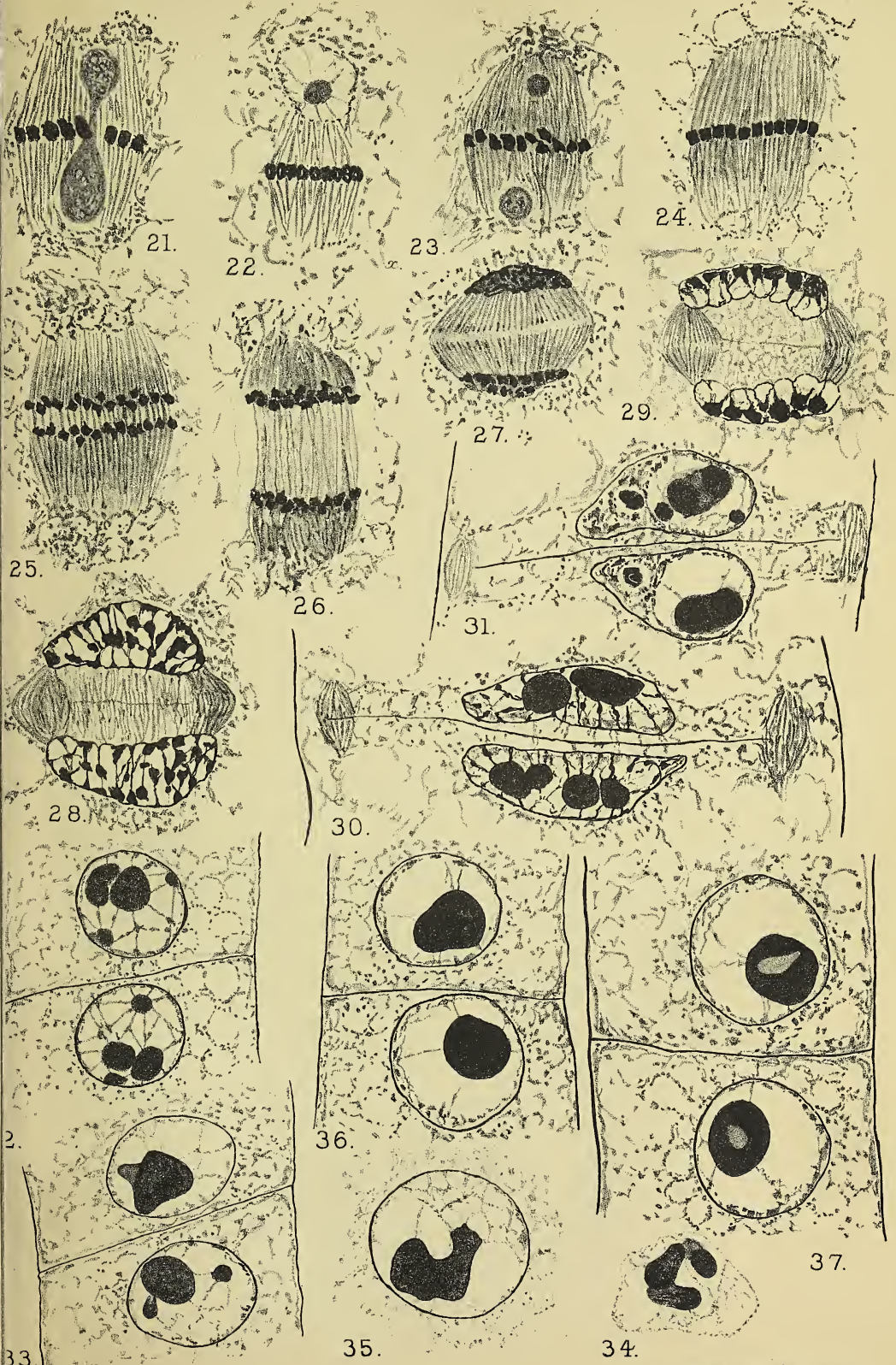
WAGER.— ROOT-APEX OF PHASEOLUS.



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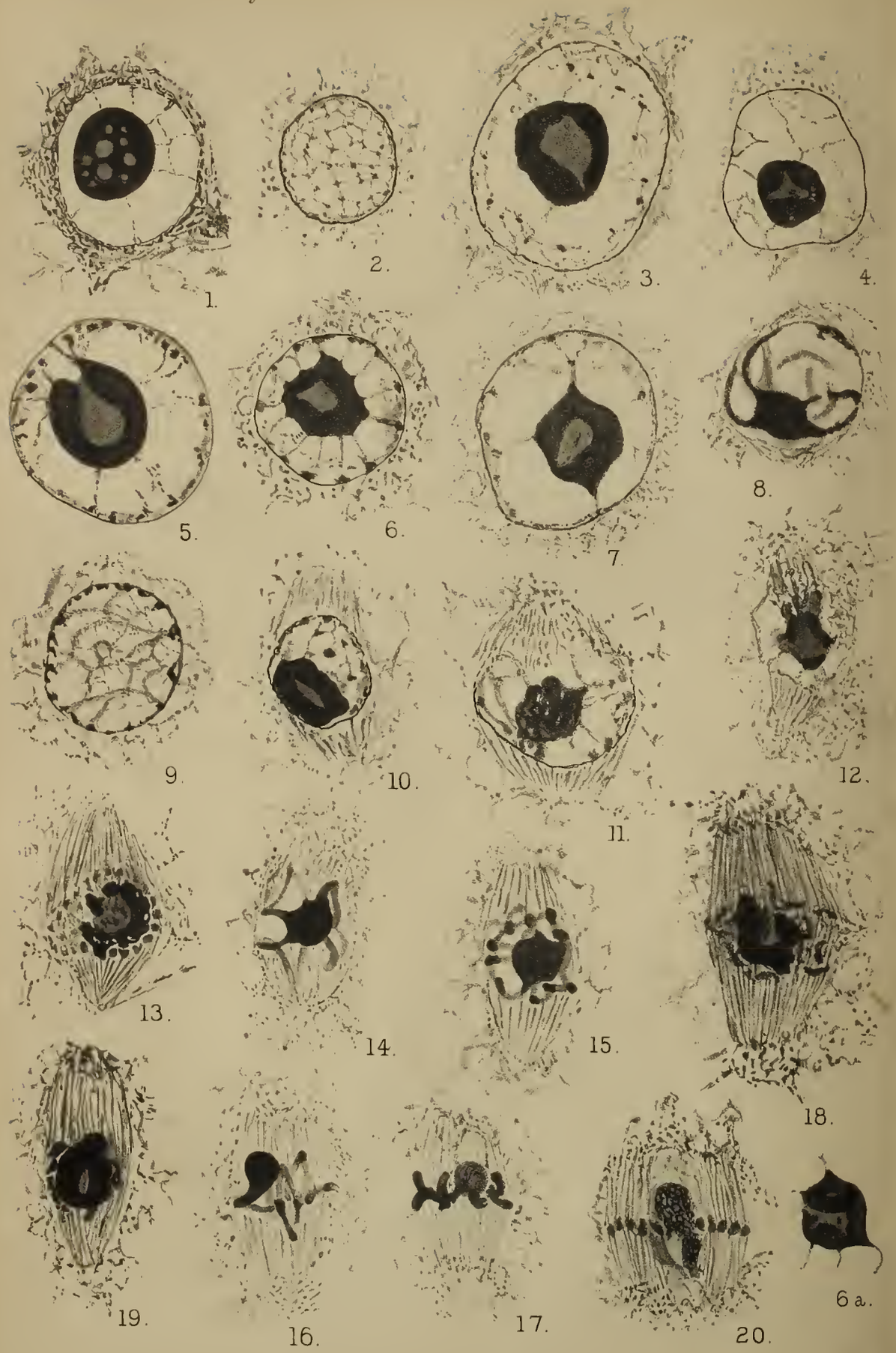

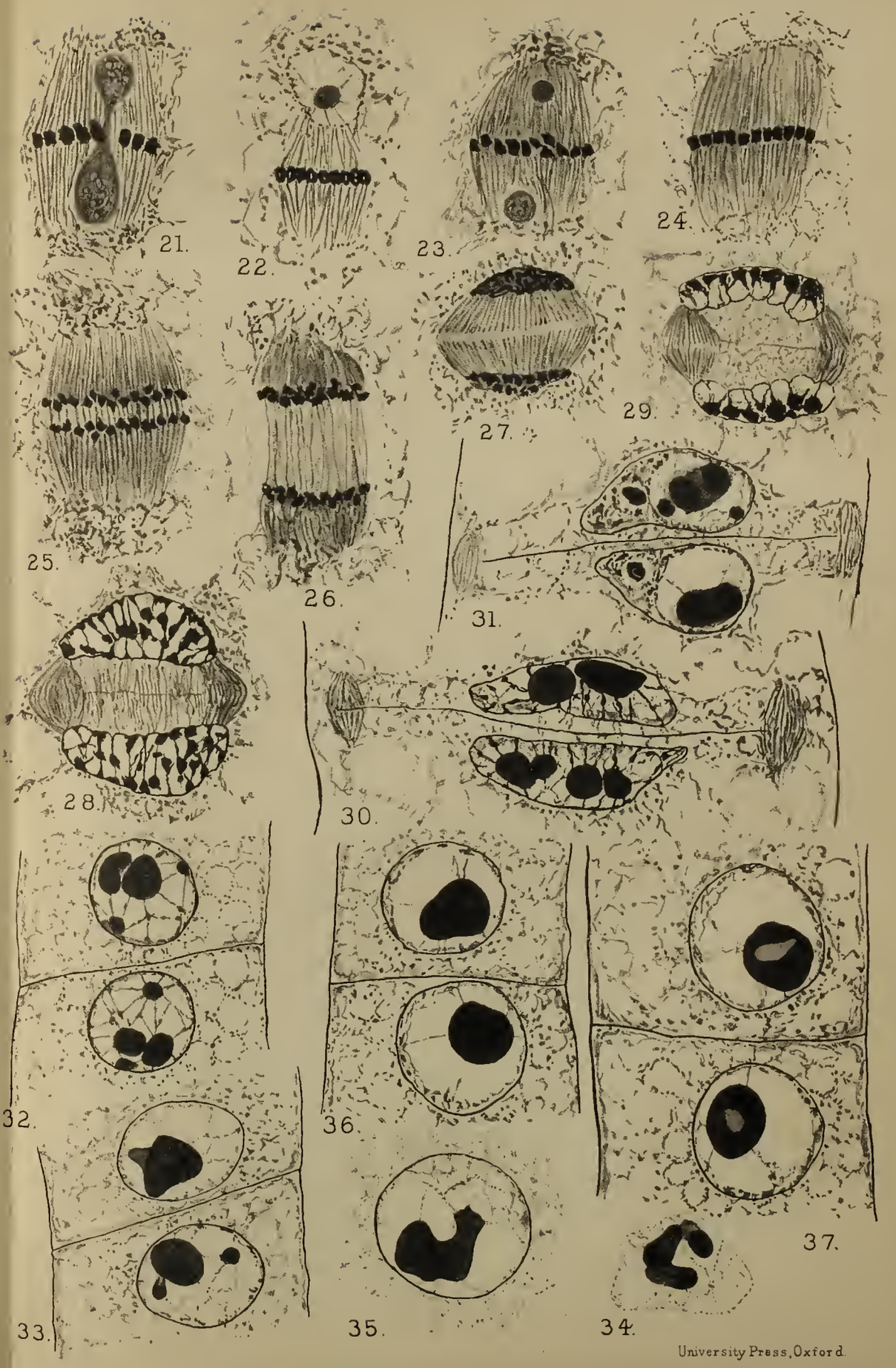

WAGER.— ROOT-APEX OF PHASEOLUS.

University Press, Oxford.

The Structure and Morphology of the 'Ovule.'

An Historical Sketch.

BY

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With twenty-seven Figures in the Text.

INTRODUCTION.

THE great aspect of botanical science known to us as Morphology comprises that department of study which is concerned with the form and the differential characters of the various structures or organs composing the individual plant. As this study advanced in the past it became clear that all the various parts or organs of the higher plants are capable of being grouped or classified into a few main categories, the *raison d'être* for the existence of these latter being that the organs constituting each, although exhibiting an extensive range of variation in accordance with the equally varied environmental conditions to which they inevitably become subjected, yet possess certain well-defined, exclusive characters of form, structure, and position which have rendered them during the course of ages of progressive differentiation so stereotyped and fixed as to preclude the possibility of the existence of any intermediate or transitional forms between any two of these categories. It is true that in some cases it is excessively difficult, perhaps even impossible, to determine the morphological category to which a given organ or structure pertains, and this owing to the extreme modification of the latter, resulting from certain special adaptive requirements, e.g. the submerged bladder-bearing portion of the *Utricularia*-plant and the seminiferous scale of the *Abietineae*. But this, it seems to the writer, is due solely to our ignorance and incapability of tracing all the stages of adaptive modification which, during its long phylogenetic history, the organ in question has undergone, the original and all the intermediate forms having long since become extinct; the ontogeny, or individual development, may yield us no clue; for in the majority of these cases the highly modified organ arises congenitally as such. If we are unable to discover, say, in the submerged organ of *Utricularia* a prevalence either of the distinctive characters of the phyllome

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or of those of the caulome, we surely dare not conclude that this organ exhibits within itself a fusion of those two categories! for if, in this particular case, such a fusion actually exists, we ought occasionally to find here and there in other plants, normally or abnormally, true transitional forms between, say, stem and leaf, or leaf and root, &c. If the existence of these could be demonstrated it would, in the writer's opinion, prove our morphological categories to be mere figments of the imagination; but he has no hesitation in saying that he believes such transitions never will be demonstrated.

Until within comparatively recent years it was usual to regard the categories as four in number, and as consisting of the caulome or stem, the phyllome or leaf, the trichome or hair, and the root. A few writers, however, have advocated the introduction of a fifth category, viz., that of the sporangium; a discussion of the validity of this view will be afforded at the proper time and place in this thesis.

The *ovule*, which forms the subject of the present paper, does so on the ground of its being one of those highly complex (as may be assumed from the evolutionary point of view), much modified structures whose morphological nature has on that account remained for so long a doubtful quantity and the cause of deep debate and argument on the part of many able investigators. It will be the writer's present object to endeavour to afford a presentation of the various views on the subject held by many of the leading botanists of the century, leaving his readers to judge for themselves which of those views contains the fullest measure of the truth. The facts thus collected and presented in a concise and accessible form will also, he hopes, prove useful to the student and the teacher of morphology.

At the outset let us consider the few, simple *facts* connected with the structure and position of the ovule as they are familiar to us to-day. The ovule is well known to appear in very various positions on the plant; in the majority of cases it appears as an outgrowth from the *margin* of the carpel; in other cases as a development from the *inner surface* of that organ, as in *Butomus* and *Nelumbium*; in older types of plant, the writer would submit, as *terminal* to a carpel of radial symmetry of structure, as in the fossil *Bennettites*; in some instances, as in Caryophyllaceae and Primulaceae, it is apparently a product of an upgrowing axile placenta; in yet others there are indications of its actually terminating the floral axis, as in Compositae [1], Piperaceae, *Najas*, Polygonaceae, *Taxus*. The facts connected with the *position* of the ovule have had no small share in influencing the decision of botanists as to its morphological nature. There have, indeed, been not a few who have placed their whole reliance on this set of data, constituting it the central pivot of their deductions. The ovule, whatever its position on the plant, consists, as a general rule, of

[1] But the ovule here is really lateral.

three distinct parts: a central structure, the *nucellus*, which assumes a terminal position on the ovular rudiment, and *two integuments*, which ontogenetically arise as annular outgrowths from the basal portion of that structure; the order of development of these integuments is basipetal, the inner, as regards the great majority of cases, preceding the outer (Fig. 1). To these facts of the normal structure a few exceptions occur: the integument may be entirely absent, as in *Crinum*; there may be only a single integument present, as in Ranunculaceae, Piperaceae, and apparently also in the group of the Gamopetalae[1]; on the other hand, the number of integuments may be increased, for C. Schimper is said to have found three in *Reseda lutea*, the third integument arising in the acropetal direction, i.e. within the two normal ones; such a case as this latter, however, requires confirmation.

In the present thesis the writer is purposely omitting any reference to the nature of the ovule in parasitic plants, as he hopes to treat this subject separately on some future occasion.

The facts of the normally constructed ovule being given, viz., a central papilla, the nucellus, enveloped by two basipetally-developed integuments, the problem which both past and present botanists have set themselves to solve is this: to which of the morphological categories does this important structure belong?

The views which have been held on this subject may be classified

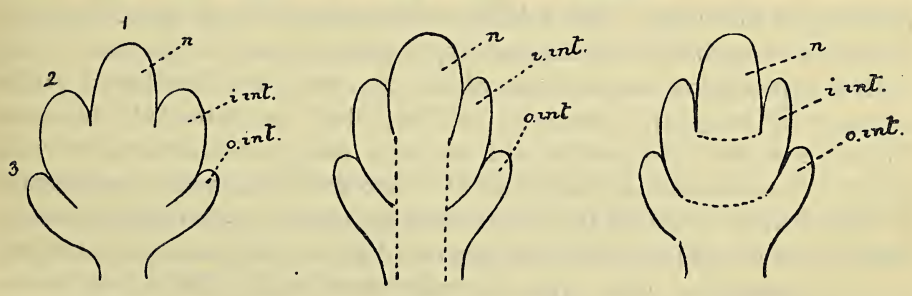


FIG. 1. FIG. 2. FIG. 3.

FIG. 1. Diagrammatic representation of the ovule to illustrate the *actual* structure apart from its morphological interpretation. FIG. 2. Diagrammatic representation of the ovule to illustrate its interpretation according to the axial-theory (after Čelakovský). FIG. 3. Diagrammatic representation of the ovule to illustrate its interpretation according to the foliolar theory (after Čelakovský). *n*, nucellus; *i. int.*, inner integument; *o. int.*, outer integument.

under three main headings, according as the ovule has been held to possess the morphological value of:—

1. a shoot;
2. a leaflet;
3. a new structure or sporangium.

[1] In this group of plants, at any rate, the 'single' integument is probably due to congenital fusion of the two integuments.

These views may be termed (1) the *axial*, (2) the *foliolar*, and (3) the *sui generis* theory respectively.

The most striking of the arguments which, during a period of some seventy years, have been advanced in support of each of the above theories will now be presented.

Historical Sketch.

As regards the first of these views, the shoot-, bud-, or axial-theory—whichever term most appeals to the reader—appears to have been the one prevalently held about the middle of the last century (Fig. 2). Its most weighty supporter is undoubtedly the great German botanist Alexander Braun (15), who, nearly twenty years after Schleiden, in a paper dealing more particularly with certain phenomena in the life-history of *Coelebogyne*, embodies ideas which, at first appearing to support the foliolar-theory of the ovule, are eventually seen to practically dispose of this in favour of the axial view. 'Leaves,' he says, 'suppose an axis; and as the idea that placentae are axile in nature as regards the majority of plants has been decisively refuted, the notion that ovules are *entire* leaves also falls to the ground.' So that 'the first explanation of ovules must be accepted, viz., that they are parts of a leaf, marginal structures, either peculiarly modified *teeth*, *lobes*, or *pinnae* of the carpel,' as Roeper and Brongniart stated, but with the recognition that the ovule must be regarded as something beyond a mere *continuation* of the carpel. But this idea of the ovule as a marginal lobe, &c., of the carpel cannot be of universal application, as seen in the case of free central placentation and in cases where the ovules are distributed, multiseriately or irregularly scattered on very thick or extended placentae. These cases show that ovules are not mere marginal structures, but outgrowths from the surface, comparable to the normal or abnormal emergences of many leaves. He says that numerous observations show that the flowers, leaves, or leaf-segments arising on expanded structures in cases of antholysis do not represent an entire ovule, but only part of such. There is to be distinguished a stalk (Träger), which must be regarded as a portion of the carpel, or in rare cases as an independent leaf, and sprouting from this a new structure—a *bud*. In metamorphosed ovules of *Adonis autumnalis* and *Nigella Damascena* he observed the outer integument expanded into a leaf-like structure. He remarks that in these cases there is no reason to regard it as part of the carpel, nor for aught else but an independent leaf belonging to the ovule (Eiknospe[1]). 'If this is correct, the bud-nature of the entire ovule is assured.' He is not decided as to which, the carpel or the ovule, the funicle belongs. The bud-nature of the ovule, the latter being

[1] The terms 'Eiknospe,' 'Samenknospe' ('Egg-bud,' 'Seed-bud'), clearly indicate the prevailing view as to the nature of the ovule held in Germany about the middle of the last century. The term 'Eichen' ('ovule'), as now generally adopted, is preferable.

a structure capable of further development, is proved by the following phenomena :—

(1) *Multiplication of integuments*, the latter arising in acropetal succession (he thinks that it is not strange that the abnormal integuments should arise in the opposite direction to that of the normal ones, seeing that the regions from which the annular protuberances arise are already formed).

(2) Unilateral expansion of one or both integuments.

(3) Proliferation of the ovule into an elongated shoot.

(4) Production of a leafy bud in place of the nucellus within the normal integuments. On one leaf of this bud he found an ovule, which he regards as a case of 'one ovule arising out of another.'

Having introduced Al. Braun first, as the most important of the supporters of this theory, let us now take up the views of the remaining authors in the historical order of their publication. We find that in 1840 Aug. de St. Hilaire (6), in his noteworthy 'Leçons de Botanique' (p. 543), in support of the axial-theory of the ovule remarks: 'We can only regard the ovule as a miniature branch composed of an axis and appendicular organs. The placenta, as we already know, is a continuation of, and represents the stem, whose branches are the ovules.' 'The primine and secundine are the appendicular organs of the young branch, and are comparable to the sheathing leaf-bases found in great numbers of Monocotyledons . . . ; it is therefore not surprising that the ovular axis, the least vigorous of all axial structures, should produce nothing but such sheaths.' On p. 490 he further says: 'We know that every part of a plant bearing ovules—or, rather, every placenta—springs from the axial system, and is nothing more than a prolongation of this latter ; so that in cases of axile or parietal placenta it is clear that the axis of the flower, after producing the carpellary leaves, has, in order to produce ovules, to divide by means of a partition, for supplying branches, double or equal in number to that of the carpels, but which, in the latter case, are susceptible of forming double placentas.'

This same view, as to the universally axial nature of the placenta, is put forward by the author in his earlier memoir on the Resedaceae. As affording a slight variant on the axial-theory, the coupled names of Endlicher and Unger (8) may be introduced, who held the ovule to be of the nature of a *disc* ('Nebenaxe') produced on the axial placenta.

Schleiden (14), who, in 1839, as also in his subsequent work the 'Grundzüge der wissenschaftlichen Botanik,' published in 1843, promulgated, like St. Hilaire, the view that the placenta is always an *axial* structure, and that the ovules borne on it are of the nature of buds. Doubtless, this view of the matter, owing to the great weight of the epoch-making work containing it, would have had much influence both among the author's contemporaries and among many of those who came after him.

Payer (23), in his great work on the organogeny of the flower, evidently—at least, in certain cases—regards the ovule, although he nowhere explicitly states it, as the morphological equivalent of a portion of the axis, this being the view definitely entertained by him with regard to all placental structures. In describing the development of the ovary in the Polygonaceae he says: 'At the bottom of this cup [the depression arising between the developing carpels] the apex of the axis is observed, which becomes successively clothed with two envelopes, thus constituting an erect and orthotropous ovule.' He applies the same interpretation to the nature of the ovule in Urticaceae, Chenopodiaceae, Paronychieae, and Amarantaceae.

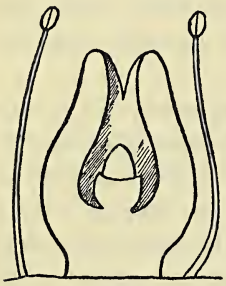


FIG. 4. Portion of flower of *Polygonum*, showing both ovary and ovule terminal to the axis (after Payer).

Magnus (44) offers a precisely similar explanation of the ovule, terminal to the axis, in *Najas*.

These two latter authors may be regarded as typical examples of those who, in their endeavours to determine the morphological value of any given organ are primarily influenced by the *position* in which it arises on the plant. Hence we may speak of such as *topical* morphologists.

The well-known observations made by Peyritsch on abnormal structures in the ovaries of Cruciferae and other plants will be referred to more fully hereafter.

Penzig (57) examined proliferated ovules of *Scrophularia vernalis L.*, which he found, in the extreme metamorphosed condition, were toothed leaflets bearing a nucellus at different levels on their upper surfaces or apex. He also found nucelli borne at the apex of elongated structures growing on the placenta which were often fused with the leaflet on the dorsal side; the appearance of the bundle in the nucellus showed the latter to be of ovular nature. He came to the conclusion that such phenomena are in greatest harmony with the bud-theory of the ovule.

Many other authors have put forward this theory of the ovule, reference to whose works will be found in the subjoined bibliography. A discussion of the validity of the axial-theory will be deferred until Čelakovský's views on the whole subject are brought forward.

We have next to notice that which we have termed the *sui generis* theory of the ovule; in this connexion there are the writings of four or five botanists to be taken into account.

Schmitz (43), in his study of floral development in the Piperaceae, and especially of *Peperomia repens*, makes this general remark: that 'as the ovule arises sometimes as an emergence on a leaf, and sometimes by metamorphosis of the vegetative apex, its relation to the shoot as a whole

cannot have the same morphological meaning. The ovule does not always possess the same morphological value.' The idea of an ovule, he thinks, only includes a tissue which encloses the embryo-sac and nothing more; and there need necessarily be no differentiation into integument and nucellus. 'The origin of the embryo-sac is not confined to the members of a definite morphological category.' He considers that the ovule in Piperaceae represents a continuation of the axis of the flower[1], the integuments being its leaves, and equivalent to the carpels and stamens. In order to form the ovule the apical portion of the axis undergoes a metamorphosis through the disappearance of all internal differentiation; the integuments arise *basipetally*; and on these grounds he regards the ovule as a *new structure*, for it cannot be made to fit into the morphological categories of caulome, phyllome, or trichome. From this author's views on the nature of the ovule generally, it appears justifiable to include his name under our present heading.

Sachs (51), in the second edition of his Textbook, enunciates a very similar view; on p. 573 (Eng. ed.) he says: 'I am induced to ascribe different morphological significations to the ovules, according to their mode of origin and their position.' On p. 575, after citing the various theories held up to that time on the ovule, he says: 'Of these views, the one which appears to be most true to nature is the one which allows the greatest latitude; but it is not always possible to refer an ovule to one of the categories, caulome and phyllome, for its position does not *necessarily* indicate its morphological significance. Thus a lateral ovule, as in Compositae and Primulaceae, might be either a leaf or a bud; its probable leaf-nature in these cases depends entirely on teratological evidence, which is of very doubtful value, for an organ in a monstrous condition does not necessarily assume its primitive archetypal form.' The following sentence affords us the key to his whole position and characteristic view of the matter: 'The difficulties met with in endeavouring to regard the ovule as a caulome or phyllome may be transcended by regarding it as an "emergence" borne sometimes on an axial, sometimes on a foliar member.' (This is practically equivalent to regarding it as an organ *sui generis*.)

Strasburger (47), in his time, has held two distinct theories on the subject; the earliest of these, embodied in his classical work, 'Die Coniferen und Gnetaceen,' supported the position of Braun and Payer. This view arose naturally from his researches on the Gymnosperms, the ovule of which he regarded as a metamorphosed bud whose stem was the nucellus and the integuments the leaves. But in course of time his views changed, until in 'Die Angiospermen und die Gymnospermen' we find him fully qualified for being classed among those who support the *sui generis* theory of the ovule, in regarding the latter as homologous with an independent

[1] In this order the single ovule terminates the floral axis.

structure like the sporangium of the Vascular Cryptogams, and with an *emergence*, which may arise promiscuously on either a foliar or an axial organ. It is to be noted that he founds his morphological views entirely on the phenomena of the *individual development* or ontogeny of the ovule. After stating that the funicle and sporangial-stalk, the nucellus and spore-capsule, are respectively parallel structures, he goes on to say that 'the integuments of Angiosperms cannot be directly identified with the indusia of Ferns, for they arise not from the structure which bears the ovule, but from the ovule, thus the sporangium itself, and from the upper margin of the funicle.' 'The nucellus is terminal to the funicle; the integuments, on the contrary, are of lateral origin.' After a careful investigation of certain ovular 'monstrosities' in *Rumex scutatus* and *Helenium Hoopesii*, A. Gray, he arrived at the following conclusions with regard to metamorphosed ovules generally: that metamorphoses (sports) are not retrogressive phenomena[1], 'but rather the expression of a competitive supplanting of one structure by another. In place of generative, vegetative rudiments arise, in accordance with the place of origin; so that, e.g., pinnae are formed as divisions of the carpels and buds as prolongations of the floral axis.' During the process of struggle between these two opposing tendencies, transitional forms occur, which will vary according as the one or the other tendency gains the upper hand. If the phenomena of oolysis were really retrogressive in character one would expect to see a cryptogamic sporangium appearing now and again, which, however, is never the case. In cases where an emergence appears on the surface of the leaflet, he regards the former as representing an entire ovule and not merely the nucellus; nor is the leaflet to be considered as the extreme form of the integuments, for he does not believe in the existence, as put forward by Čelakovský and others, of a series of transitional forms consisting at one extreme of almost normal integuments, and at the other of a vegetative leaflet. 'Each case must be considered in and for itself alone, and represents a compromise which has been arrived at between the struggle to form an ovule on the one hand and that to produce a leaflet on the other.' And again he says: 'If only a mere emergence is present on the leaflet, I regard this as the consequence of the tendency to leaflet-formation having early gained the upper hand, and not as the result of a reversion of already formed integuments into the leaflet. The hybrid-cases observed cannot be regarded as constituting a series of developmental stages which must be traversed in order to arrive at the extreme forms.'

He agrees with Čelakovský that where buds occur on fully formed parts of the carpel these are usually to be regarded as adventitious. But from his own standpoint, he must adhere to the view that the ovule may be directly supplanted by a vegetative bud.

[1] This has reference to Čelakovský's earlier view on the subject, which will be introduced in its proper place.

Finally, with reference to 'sports' generally, I may quote a sentence from his 'Lehrbuch der Botanik,' p. 132, where he says: 'Sports (Missbildungen) can only in rare cases be made use of in drawing morphological conclusions.'

Goebel (56) is a staunch upholder of the *sui generis* theory of the ovule and a severe critic of the method of settling morphological problems by the aid of 'sports.' In his 'Organographie' he recapitulates his views on the subject, put forward much earlier in Schenk's 'Handbuch der Botanik' and elsewhere. Speaking of the replacement of sporangia by vegetative organs, as in cases where stamens become foliaceous or petaloid, he says that 'a metamorphosis of the former into the latter does not occur, and the transitional stages between the normal and abnormal condition do not establish such a metamorphosis, but only show that there may be a varying degree of disturbance of the normal state of affairs.' The morphological character of stamens is determined by means of the development and a comparison with vascular Cryptogams. In treating of proliferated ovules, he considers it unjustifiable to regard these as reversions, and absurd to represent the simple leaf into which the ovule has become metamorphosed as the most primitive phylogenetic stage of development. 'The only conclusion it would be possible to draw from the proliferations would be that the integuments are formed from carpellary substance or represent outgrowths of the carpel which are the better adapted to vegetative development in proportion as the reproductive organs (the nucellus) are hindered in their growth.' We are to regard these proliferated ovules as crippled structures which have suffered a pathological modification of form; they may not be used for the determination of homologies. 'We are surprised to hear the assertion made that a leaflet bearing the abortive nucellus is homologous with the sorus- or sporangium-bearing leaflet of a fern. As if an abortive rudiment, showing no sign even of an embryo-sac, could in the remotest degree have anything to do with a sporangium.' He urges that 'it is a more profitable task to consider how such abnormalities have arisen and to discover the causes which condition the deviation from the normal development,' and this is the burden of his article on 'The Teratology of Plants' which appeared a few years ago in 'Science Progress.'

Here too we may place Eichler (49), who during his career held in turn all three views on the subject, but at length appears to have found repose for his ideas among those who support the theory we are now considering. In 1875, in the first volume of his 'Blüthendiagramme,' he upholds the axial-theory of the ovule, influenced largely by the facts resulting from Schmitz's recent work on the Piperaceae, and by those cases where the ovule is apparently replaced by a shoot; further, from the teachings of the theory of descent he felt bound to regard the ovule

as everywhere of similar morphological dignity; the placenta, on the other hand, as possessing a varying morphological value. But in 1878, in the second volume of the same work, he becomes an adherent of the foliolar theory, and says: 'I must side with Čelakovský and accept both his placental and ovular theory from beginning to end.' It is in the Botanisches Centralblatt for 1882, in his well-known thesis on the female flower of the Coniferae, that we find him finally throwing in his lot with the professors of the *sui generis* hypothesis; his observations on the coniferous ovule have evidently altogether influenced him in taking this step, for, after referring to the varying positions of this organ in the different genera of Coniferae, he says: 'We must, therefore, give up the idea that the ovule everywhere corresponds either to a leaf-segment or everywhere to a bud arising as a rule from the metamorphosis of those structures; it is rather the more or less modified macrosporangium of the higher plants [Vascular Cryptogams] which has been inherited by the Phanerogams, representing, like that organ, a structure *sui generis*. It may be compared to an emergence which it cannot be the exclusive privilege of leaves to produce, nor may it be regarded as invariably occurring on shoots; on the contrary, we must recognize that the ovule, like other emergences, may arise as well on the one as on the other organ, or on the margin of both, i. e. in the axil: this is not only clearly the case in the Coniferae, but undoubtedly also in the Angiosperms.'

Bayley Balfour's view (72) resembles that of Strasburger, as clearly shown by the following statement: 'I do not share a view which sees in integuments or other parts of the ovule anything of an axile or of a foliar nature. To me the funicle is a sporangiophore or a sporangial stalk, and the integumentary system is an outgrowth of the sporangial primordium of somewhat variable origin and development,' &c. As regards the schools of botanists who interpret the ovule from the standpoint of teratology and vascular anatomy respectively, he says: 'I do not accept the starting-point of either the one or the other.' Speaking of the sporangium his view is as follows: 'All recent investigations . . . tend to confirm the view that it is, and always has been, an organ *sui generis*. To that category the nucellus of the ovule is now pretty generally admitted. It is the body of a sporangium.' His general views on the nature of the ovule appear (as in the case of Strasburger) to be chiefly influenced by the facts of the individual development, or ontogeny of that organ; and he seems to hold the idea that the integuments, for instance, are structures arising *de novo* in the life-history of the nucellus or sporangium to subserve the special physiological function of water-carriers and food-reservoirs.

We have now to take up the consideration of the third, the '*foliolar*' theory of the nature of the ovule, that theory which explains this mysterious

organ as in fact representing the homologue of a *segment or leaflet of the carpel* which bears it (Fig. 3).

The notable founder of this view of the matter is Brongniart (9), who in 1844 made in the first place observations on abnormal carpels of *Delphinium elatum*, and noted the occurrence of transitions between the tridentate lobes of the foliaceous carpel and the ovules themselves. In his conclusions formulated from these facts he says that the veins of the placenta are really the lateral veins of the carpellary leaf; each ovule corresponds to a lobe or a large tooth of this leaf, while the funicle, as also the raphe, is formed by the median vein of this lateral lobe; the outer integument is the terminal portion of this leafy lobe folded on itself and forming a hood, the nucellus being a new production—a cellular protuberance arising on the upper surface of this lobe. It is thus impossible to regard the ovules together with the placenta as a production distinct from the carpellary leaf, and as part of the main or lateral axis. In *Brassica Napus* again, he observed transitions between the ovary and two foliage-leaves, and between the ovules and lobes of the leafy carpel. In abnormal ovaries of *Anagallis arvensis* the ovules are represented on the *axial* placenta by tiny *leaves*. In this case he evidently regards the ovules as the homologues of *entire* leaves, and also states his belief in a double origin for placentas: from the margins of carpels and from a prolongation of the floral axis. Finally, his estimation of the value of abnormalities, in the opening words of the paper, are worth quoting: 'There is hardly a botanist at present who does not recognize how much light the study of these aberrations from the normal structure, to which the name of monstrosities is given, sheds on the essential and fundamental structure of certain parts of the plants, or on that which is peculiar to certain groups of plants.'

Robert Brown (35), in his paper on *Rafflesia* (p. 211), says: 'The principal point in which the antherae and ovaries agree, consists in their essential parts, viz. the pollen and ovula, being produced on the margins of the modified leaf.' On p. 211 he says further: 'The marginal production of ovula not infrequently becomes apparent where its formation is in some degree imperfect, and is most evident in those deviations from the regular structure where stamina are changed more or less completely into pistilla,' as in *Sempervivum tectorum*, &c. On pages 379 and 556 of the first volume of his Miscellaneous Botanical Works he speaks of the margin of the carpels as the proper place for ovules, and he gives instances of exceptions to this rule, as in Nymphaeaceae, *Mesembryanthemum*. On p. 563 we find the remark that 'ovules belong to the transformed leaf or carpel, and are not derived from processes of the axis united with it, as several eminent botanists have lately supposed. That the placenta and ovula really belonged to the carpel alone is at least manifest in all cases where stamina are changed into pistilla.'

Caspary (25), in a paper, accompanied by beautiful illustrations, on abnormal ovules of *Trifolium repens*, states that the funicle in this plant is morphologically equivalent to the lower part of the leafy lobe or pinna of the carpel; at no stage is any limit between the funicle and the outer integument to be found. The inner integument, being so similar, must possess the same meaning and origin. It may disappear in the tissue of the leaflet, just as the outer integument has done in the funicle. 'The funicle, along with the integuments, is in *Trifolium repens* the morphological equivalent of a leaflet (pinna), whose stalk or midrib is in the lower part of the funicle and whose campanulate or conical outgrowths of the upper part are the integuments. The nucellus is the new shoot seated on this leaflet.' He thinks the nucellus also probably forms an integral part of the carpel.

Through that classical work of his, the 'Bildungsabweichungen,' Cramer (30) established his reputation as one of the most distinguished exponents of the morphological nature of the ovule. He observed metamorphosed ovules of Primulaceae; these were in the form of small foliar organs, developed on the free central placenta, which he regarded as entire leaves. He regards the nucellus as the product of a leaf. As for the integuments, if, he says, they were two distinct leaves and not part of one leaf they should both proliferate. That they are not such is shown by their becoming concave towards the upper side. The metamorphosed ovules are to be regarded as 'the outcome of the working of ideal successive combinations of formative forces fighting against each other, and not as developmental stages which have succeeded each other from time to time.' He is strongly in favour of the use of abnormalities for the solution of morphological problems. To him, the ovule of Compositae is, like that of Primulaceae, an entire leaf. He also observed proliferous flowers of *Delphinium elatum* in which the green carpels bore either sterile or vegetatively developed ovules in the form of lobes; the latter he held to be integumental in nature and the protuberances borne by them nucelli. In his paper 'On the Morphological Significance of the Ovule,' occurring in the same volume, he sets forth that the nucellus is a new structure and not of the nature of a shoot, as he does not believe that the latter, as asserted, could ever arise from an ovule. The nucellus is merely an *emergence*, like pollen-sacs and fern-sporangia. His researches in the development of the ovule in *Centaurea jacea*, *Lysimachia punctata*, and *Anthericum liliago* show that the primary papilla is *not* the nucellus but later gives rise to both this and the integuments. It is the funicle which first appears, and on this the nucellus is developed *laterally*. From his observations on *Trifolium repens* he concludes, with Caspary, that it is the *outer*, and not the inner integument which proliferates and which bears the nucellus in the middle of its upper surface.

Prantl (53) stands conspicuous as one of the bold, yet not necessarily

rash, thinkers who feel themselves at full liberty to compare certain organs of the Phanerogams with what they regard as related organs in the Vascular Cryptogams. It will suffice to quote a remark from page 11 of his work of 1875, where he says: 'Perhaps the integument (of *Cycas*) may be regarded as the equivalent of the indusium' (of Ferns). This view will be further elaborated when the tenets of the last author on our list are considered. Again, on page 13 he says: 'From my point of view I am unable to believe in actually axile ovules or anthers; these organs are derivatives of sori,' which are parts of the leaf. Further, he remarks: 'Ovules I must regard as under all circumstances originally parts of the carpel.'

At first (as seen from his paper of 1872, in which he says: 'Ovules are certainly most frequently metamorphosed axes') a defender of the axile nature of the ovule, Warming (46), in his most valuable thesis: 'De l'ovule,' published in 1878, and containing a series of researches into the development of the ovule-rudiment of the nucellus, unequivocally upholds the Brongniartian theory of the ovule. 'In every ovule we have considered two parts essentially different: the funicle and the integuments, which are of foliolar nature, and the nucellus, which is a new creation, a sporangium, a "sorus" composed of a single sporangium, as Prantl would say.' The origin and mode of development of the ovule-rudiment is similar to that of leaves, leaf-lobes, metablastema, emergences, and buds. Histogeny tells us nothing as to the morphological nature of the rudiment; it only informs us as to how the latter arises on the placenta as a new formation. 'There is but one method which can lead us to the goal: the gradual comparative study of allied forms, relying on all the means at the disposal of the morphologist.' On page 195 he writes: 'The comparative study of the carpels and placentation in the entire vegetable kingdom, the scrupulous examination of antholyses and the course of the vascular bundles, lead us, as Čelakovský and Van Tieghem have recently proved, to the conclusion that carpels and placentas are everywhere phyllomes, and that the ovule-rudiment is a leaf-lobe; I do not know if, in certain cases, they should not be regarded as metablastema, but the difference between the latter and a leaf-lobe is not essential and cannot be everywhere sustained.' On page 200 he proceeds: 'The so frequent foliar transformation of the ovule-rudiment which will later become the funicle, and its fusion with the carpel, is indomitable proof that this organ is really a leaf-lobe, a conclusion which had already been rendered very probable by the position and order of appearance of the ovules. Ovules are not buds; I know of no complete and well-studied teratological transformation which absolutely confirms the contrary, and I dare add that it will not be discovered. The ovular rudiment is therefore a leaf-lobe.' The developmental history and teratology show us:—

1. That the nucellus is a new formation on the ovule-rudiment, which is itself merely a lobe of the carpel.

2. That the pollen-sac and nucellus are identical as to their mode of development, which is here a proof of true homology, confirmed besides by a comparative study of these organs in the whole vegetable kingdom and by a series of teratological cases.

3. That the pollen-sac, like the sporangium (the common fundamental form of pollen-sac and nucellus), is everywhere attached to a leaf; conformably with this truth we are assured, in a totally different manner, that the ovule-rudiment is, in fact, of foliar nature.

This last view is supported by the study of Cycads. And he further says that we have seen that these metablastemata are everywhere attached to phyllomes and not to caulomes. As regards the true position of the nucellus, he says that in a general way it is to be regarded as *terminal* to the ovular rudiment. On the other hand, the teratological cases show it to be *lateral*, and appear to indicate the true, or at least the primitive, relations existing between the two organs.

At the end of Chapter III he says: 'It seems proved that the integument of Angiosperms is a special structure pertaining to the ovular leaflet and of foliar nature.' This author's treatise must be regarded, from its thoroughness of detailed investigation and breadth of treatment, as one of the most weighty contributions to this great and difficult subject, and of great interest in demonstrating how far developmental data serve in fixing the morphological dignity of the ovule.

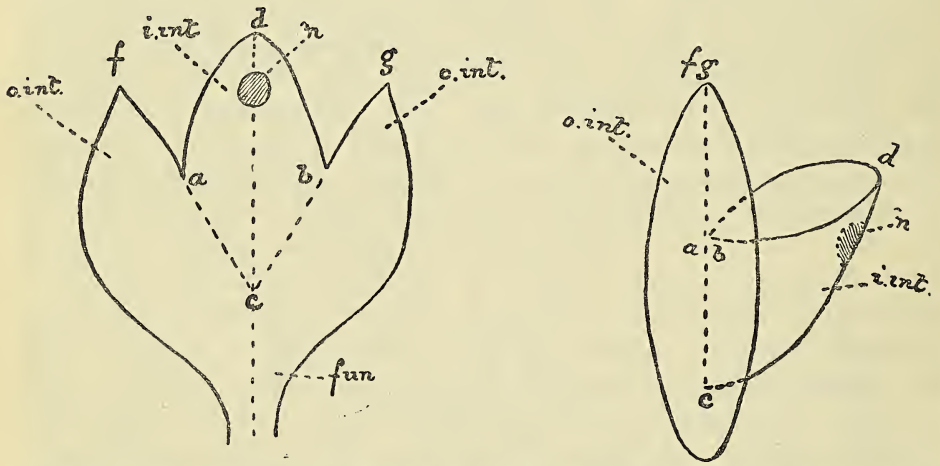
Čelakovský (38) stands out as a very brilliant exponent of the foliolar theory of the ovule, his contributions to the subject being, in the writer's opinion, remarkable for their deep insight into, and ingenious handling of, one of the most difficult problems in botanical science. As the writer is also inclined to believe that in the sum of the numerous treatises of this author on the subject is contained a probable solution of the great problem as to the real morphological nature of the ovule, more space will be allotted to the consideration of his views than has been the case with the other authors cited above.

Firstly, with regard to the various methods employed in determining the morphological nature of any doubtful structure, these may be said to be four in number, and may be termed: (*a*) the developmental, (*b*) the comparative, (*c*) the anatomical, (*d*) the teratological. Applying these methods in turn to the solution of the nature of the ovule, it will be seen, in the first place, that the study of the *development* or ontogeny of this organ tells us simply that it is a protuberance borne sometimes on an axis, sometimes on a foliar organ, producing in basipetal order two lateral, cup-shaped appendages. Relying entirely on the ontogenetic facts and assuming that the ovule is a bud would surely be dangerous; for we cannot be sure that it is not an organ of a totally different nature, arising from the earliest stage of development, as a *congenitally-modified* structure. Of this latter

the ovary of the Primulaceae is another example, affording us excellent proof of the unreliability of developmental evidence in such cases. The *comparative* method is a useful one, but taken alone is not finally reliable, for the standard of comparison is likely to be ever a shifting quantity varying with each botanist who employs it; indeed, it seems impossible to apply this method by itself to the *solution* of the nature of an obscure structure like the ovule. The Bohemian Professor further regards *anatomical* data as powerless when directed to the same purpose; Van Tieghem's method of research along these lines must be regarded as stilted and artificial; for vascular tissue is developed where it is needed and is quite independent of the morphological character of the organ which it supplies, and no *absolute* reliance is to be placed on the character of the course or structure of the bundles. Yet all three of these methods may be usefully employed as collateral aids and adjuncts in solving the morphology of a doubtful structure. For this latter purpose, however, the direct and principal method is that of the study of *teratological* phenomena; it is on the 'metamorphogenesis' that Čelakovský rests the whole weight of his argument; for he maintains that these abnormalities, or deviations from the normal structure, are not of the nature of 'sports' or 'monstrosities,' as is usually supposed, but, on the contrary, are phenomena *controlled by definite laws* of development, producing thereby structures which are *not new or monstrous* (except, indeed, as regards the limited life-cycle of the particular organisms or group of organisms concerned), but whose homologues will inevitably be found occurring as *normal* structures in organisms belonging to other sections of the vegetable kingdom. For all vegetable life is one, and the four types of organs: caulome, root, phyllome, and trichome, are the common heritage of all members of the vegetable world. But the question arises: how is the morphological value of any given doubtful organ to be determined by means of the abnormal structure which it assumes under the influence of these laws of 'metamorphogenesis'? It would not be justifiable, on the ground of a mere *supplanting* or *replacement* of the abnormal structure, to regard the two as homologous in nature. But if (and this the writer regards as Čelakovský's unassailable position) there can be traced in the metamorphogenesis *a series of gradual transitions* (whether occurring in a single individual or scattered over a number of individuals, matters not) between the normal form and the extreme stage of the metamorphosed structure replacing it, then this must prove the absolute homology of the two, i.e. that they possess an identical morphological value. It seems to the writer that a certain shallowness of thought and the influence of preconceived ideas on the subject are the cause of the rigid adherence by so many eminent botanists to the oft-reiterated statement that 'abnormalities can be made to prove anything'! It betrays an utter neglect of the possibility

that, as stated above, these same 'abnormalities' may be the outcome of the working of rigid laws of development whose activity is reproduced in other departments of the vegetable kingdom. It is a view which can be easily refuted along the lines laid down above.

Čelakovský regards the ovule as the homologue of a trilobed leaflet or segment of the carpel, of which the terminal lobe, involuted towards the upper surface to form a cup-shaped structure enclosing the nucellus (this latter being an organ of the nature of an emergence or sporangium borne on the *upper* surface of the lobe) is the *equivalent* of the inner integument; while the two lateral lobes, fused by their *inner* margins across the *upper* surface of the leaflet, *represent* the outer integument (Figs. 5, 6). It is to be noted that, in accordance with what the author terms the 'law of laminar inversion,' which ordains that two foliar laminae



FIGS. 5 and 6. Diagrams of a trilobed leaflet showing how, by fusion of the two lateral lobes across the upper surface of the terminal lobe through union of the lines *f a*, *a c* and *g b*, *b c*, a structure homologous with the virescent ovule is obtained. The nucellus is seen seated as an emergence on the terminal lobe of the leaflet (after Čelakovský). *fun*, funicle.

are invariably in contact with each other by means of the *similar* surfaces of each, the lower surface of the outer integument contacts the lower surface of the inner integument. The above-outlined general position of the author derives its entire support from the facts revealed by the so-called 'monstrosities' of ovules where gradual transitional forms between the normal ovule and the three-lobed or simple leaflet have been observed.

In the case of abnormal ovules of the Crucifer, *Alliaria*, it was the inner integument which exhibited the greatest amount of proliferation or virescence; and another remarkable feature consisted in the preponderating tendency to proliferation of the *funicle* rather than of the outer integument. So that the ovular leaflet (the virescent ovule) sometimes assumes the form

of a leafy structure (the funicle) bearing the inner integument, subtended by the rudimentary sheath of the outer integument, on its lower surface (Fig. 8); or, in some cases, the outer integument may be completely absorbed in the funicular lamina. (For all details, both in this and other cases of abnormal ovules described by our author, the reader is referred to the original papers, which amply illustrate the various stages of the metamorphogenesis.) In the case of *Trifolium repens* it was also the funicle which chiefly proliferated, assuming the form of a bilobed structure at the

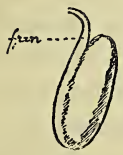


Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

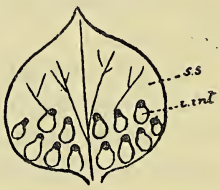


Fig. 14.

Fig. 7. *Alliaria officinalis*: normal ovule. Fig. 8. *Alliaria officinalis*: ovule in which funicle has proliferated, bearing at its base the outer integument ensheathing the inner integument. Fig. 9. *Alliaria officinalis*: ovule showing funicle subtending the much proliferated bifid inner integument, bearing the nucellus on its upper surface and an adventitious shoot (*ad*) at its base. Fig. 10. *Trifolium repens*: ovule in which funicle has proliferated, bearing inner integument in sinus between its two lobes (cf. Fig. 16). Fig. 11. *Trifolium repens*: virescent ovule proliferated as three-lobed leaflet. Fig. 12. *Hesperis matronalis*: virescent ovule with proliferated outer integument bearing inner integument on its lower surface. Fig. 13. *Hesperis matronalis*: proliferated outer integument bearing *several* inner integuments on its lower surface (Figs. 7–13 after Čelakovský). Fig. 14. *Cupressus*: seminiferous scale (= proliferated outer integument) bearing several ovules (= inner integuments and nucelli) on its lower surface (diagrammatic).

base of whose sinus sometimes occurred the small sheath of the outer integument (from the *upper* surface of which the funicular lamina is an outgrowth), enclosing the weakly-developed inner integument either in the form of a cup-shaped organ or as a simple leaflet bearing the nucellus on its upper surface (Figs. 10, 11). This is a very important stage, and will be referred to hereafter.

Hesperis matronalis differed from both of the last two plants, inasmuch as the leafy structure bearing the inner integument consisted solely of the *outer integument*, as is shown by its *sheathing base* and by the fact that the margins of the lamina passed gradually over into this sheath (Figs. 12 and 20). Hence our author terms it the 'basal lamina' (Grundspreite). The funicle takes therefore no part in its formation. In this plant the ovule frequently appeared as a simple leaflet bearing the nucellus on its *upper surface;* this leaflet cannot be the outer integument, because formation of the inner integument being always the primary process this latter organ could not arise as an emergence from the surface of the outer integument; it must also be situated invariably on the *lower* surface of the latter. The leaflet in question must therefore contain within itself *both* the inner and the outer integuments. This case resulted from the proliferating tendency setting in at the period when the ovule was nothing but a mere undifferentiated rudiment, this latter containing within itself the two integuments *in potentia*. The integuments, whether the outer or the inner, *once laid down as completely sheathing structures*, never proliferate as laminae. *Hesperis* is particularly interesting as having exhibited a case of a proliferated outer integument *bearing two or more inner integuments*, the extra ones occurring on the lateral lobes of the leaflet (Fig. 13); this case, as our author has elsewhere pointed out, is of considerable value for the interpretation of the female parts in *Cupressus* (Fig. 14).

With *Aquilegia* and its various stages of metamorphosed ovules our author winds up his investigations. The virescent ovules of this plant are probably the most difficult to understand and to unravel. The first stage, in which proliferation sets in rather late, shows the inner integument seated on the *upper* surface of the 'basal lamina'; the two lobes of the latter are bent back and fused together *behind* instead of, as in all other cases, in front of the inner integument. As this lamina is to constitute the outer integument, there here occurs an apparent contradiction to the usual law of 'laminar inversion'; but our author finds it to be *only apparent*, for differentiation into an upper and a lower surface has not yet taken place in the inner integument. In the second stage there is an anatropous cup-shaped structure which, from the mode of development and the various modifications occurring during the metamorphoses, is shown to be the *inner integument*, with which the outer integument is intimately fused along its whole length; the whole constituting a single undivided structure. This plant differs from *Trifolium* and *Alliaria* in the fact that the lower portion of the leaflet never grows out to form a separate individualized lamina. In the second stage just mentioned, where 'proliferation' sets in early, the outer integument remains stationary, while the inner integument alone proliferates as the apical portion of the entire leaflet. The usual relation-

ship between the outer integument and the ovular leaflet is described as follows: the former is an upgrowth out of the *lower* surface of the lower portion of the leaflet, after this latter has become *inverted and folded in towards the upper surface.* There exists, therefore, no essential difference between the case of *Aquilegia* and that of the other plants mentioned above.

As demonstration of the fact that these variable structures, known as the 'ovular leaflet' or virescent ovule, are all governed in their development by definite laws of growth and sequential differentiation, and are not the fortuitous and haphazard result of 'sportive' tendencies on the part of the plant producing them, it may be mentioned that *precisely identical phenomena* have been observed as curious modifications of the *entire* foliage-leaves of the common Lilac (*Syringa vulgaris*).

Again, exact homologues both of the metamorphosed ovule and of the unaltered ovule or 'ovular leaflet' may be found as *normal* structures in other departments of the vegetable kingdom. Čelakovský finds such in the apparatus of the female 'flower' of the Coniferae. The Taxaceae present the case of the normal ovule along with its two integuments; the remaining groups that of the semi-proliferated ovule, of which the seminiferous scale (or rather one-half thereof, seeing that each scale possesses two ovules) is the vegetatively developed outer integument bearing the involuted nucellus-producing inner integument on its lower (dorsal) surface. The case of *Cupressus*, in which a single seminiferous scale bears *several* such inner integuments on its lower surface (Fig. 14), finds its counterpart, as we have seen, in *Hesperis*; such a structure as this might conceivably arise out of a *compound ovular leaflet*, the terminal segment of each lobe becoming, as in the simple three-lobed leaflet, the inner integument borne on that lobe's lower surface. Descending lower in the scale, precisely the same set of structures (although naturally modified in accordance with the idiosyncrasies of the special group of plants in which they occur) are exhibited as *normal* stages of development in the sporophylls of the Ferns. In *Thyrsopteris* and Hymenophyllaceae (Figs. 15, 16) we see the case of the normal ovule of Angiosperms and of Taxaceae, in which the receptacle bearing its numerous sporangia (homologue of the nucellus), terminal in position, is ensheathed by the integuments, of which the indusium is the morphological equivalent of the inner, while the outer integument is represented by the laminar extension (when present) of the pinnule-segment *on either side* of the indusium. If now this structure be compared with the virescent ovule in *Trifolium repens*, its similarity to the bilobed funicular lamina enclosing the inner integument is obvious: the two structures are to be regarded as homologous although naturally presenting differing degrees of development of the respective parts. In *Dicksonia* the sorus is also terminal to the leaf-segment, but the indusium is here two-

lipped instead of cup-shaped. In *Davallia* and *Microlepia* the first stage in the projection of the sorus on to the lower surface is seen, this being caused by the elongation of the *upper* side of the indusium, which becomes green and is (in part) an extension of the pinnule-segment (the outer integument (Fig. 17).

The final stage is seen in *Cystopteris*, where the sorus, with the lower lip of the indusium, is projected completely on to the lower surface of the pinnule-segment (Fig. 18). In *Cibotium* and *Cyathea* there is a distinct cup-shaped indusium situated on the lower surface of the segment (Fig. 19); in the former this is at first terminal and marginal, becoming sub-

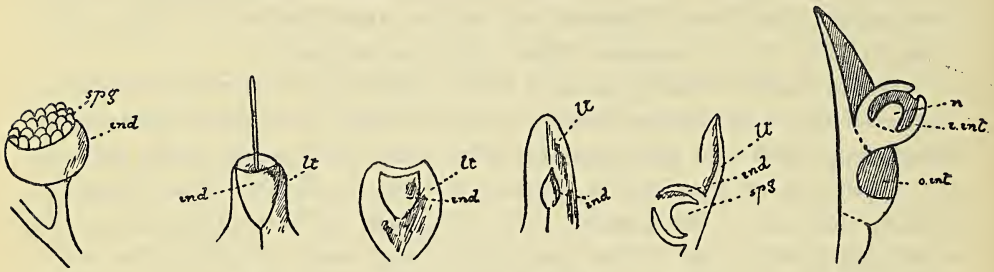


FIG. 15. FIG. 16. FIG. 17. FIG. 18. FIG. 19. FIG. 20.

FIG. 15. *Thyrsopteris*: sorus with indusium (*ind*) and terminal receptacle bearing sporangia (*spg*). FIG. 16. *Trichomanes*: receptacle and cup-shaped indusium (= inner integument) seated in sinus between two lobes of leaflet (= outer integument) (cf. Fig. 10). FIG. 17. *Davallia*: early phylogenetic stage in projection of indusium on to lower surface of leaflet; indusium one-sidedly developed. FIG. 18. *Cystopteris*: later stage in same process. FIG. 19. *Cyathea*: same stage, but indusium here completely formed; *spg*, receptacle bearing sporangia. FIG. 20. *Hesperis*: proliferated outer integument bearing inner integument on its lower surface; this is precisely the same structure as that in *Cyathea*. (All after Čelakovský.)

sequently displaced into an inferior position; in the latter it is inferior from the first. In these two latter cases we have the exact counterpart of an inner integument situated on the lower surface of a 'basal lamina' or proliferated outer integument or funicle, such as occurs in *Alliaria* or *Reseda* (Fig. 20).

As regards the Rhizocarps: in the Salviniaceae the fruit is equivalent to an ovule with one integument; the indusium represents the inner integument and the leaf-lobe bearing the sorus is probably homologous with the outer integument. There is a striking resemblance, admitted by most botanists, between the monangic sorus of *Azolla* and an ovule (Fig. 21). Prantl regarded the two as homologous structures, and Campbell suggests the same thing. In the Marsiliaceae the fruit has the value of a compound fruit of Salviniaceae. In *Pilularia* it is homologous with a pinnately quadri-foliolate leaflet of the entire leaf of *Marsilia*; in *Marsilia* with a pinnately multifoliolate leaflet; in these cases the leaflet of the sporangiferous leaf possesses the compound structure of the vegetative leaf,

while the leaflet of the latter is simple. The outer wall of the sporocarp is homologous with the *upper* surface of the outer integument of the ovule; here, as in *Hesperis* and *Cupressus*, it is polysorous, bearing a number of indusia or inner integuments (each with its enclosed sporangia) on the lower surface (Figs. 22–25).

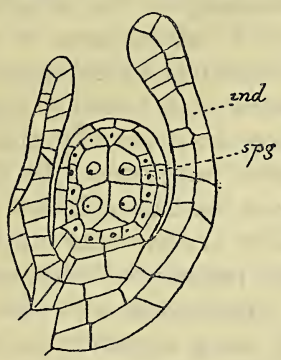


FIG. 21.

FIG. 22.

FIG. 23.

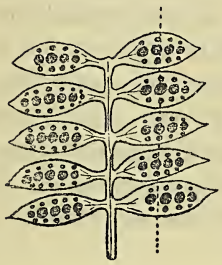


FIG. 24.

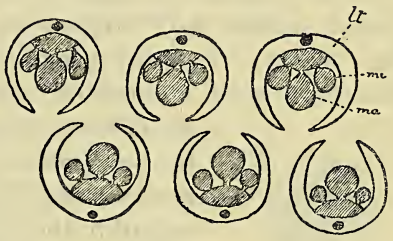


FIG. 25.

FIG. 21. *Azolla*: megasporangium enclosed by indusium (after Campbell). FIG. 22. *Pilularia*: diagram of morphological structure of sporocarp from two pairs of leaflets, each bearing a sorus on its lower surface. FIG. 23. *Pilularia*: transverse section (taken through dotted line in preceding figure) of leaflet, showing sorus and indusium; *ma* = mega-, *mi* = microsporangia. FIG. 24. *Marsilia*: diagram of morphological structure of sporocarp, from several pairs of leaflets, each bearing sorus on its lower surface. FIG. 25. *Marsilia*: transverse section (taken through dotted line in preceding figure) of leaflets here supposed to be folded together from opposite sides of midrib to form the sporocarp; sorus shown.

For a further elaboration of this subject of the homologies in the different groups the reader is referred to our author's exhaustive paper in 'Pringsheim's Jahrbücher.'

In the Lycopodiaceae are again found equivalents for our ovule and its various parts. The *ligule* of the Selaginelleae our author, in the above-mentioned paper, regards as the same leaf-lobe which later in the Schizaeaceae grows out over the sporangia, but here, owing to the ventral position of the sporangium, the ligule is also ventral, and as such is so weakly developed that it is partly fused with the carpel, so that it *de facto* springs from the carpel; the sporangium, as in the Schizaeaceae, springing

from the lower surface of this modified leaf-lobe. He regards the *velum* as 'unquestionably equivalent to the indusium of the Ferns.' 'The ligule along with the velum and sporangium is homologous with a leaf-segment of *Cyathea* with its integumented sorus, and in both cases the development of the leaf-segment precedes the formation of the integumented sorus.' 'As now a leaf-segment of a Fern with inferior indusium is homologous with a doubly-integumented ovule; thereby is also the homology of Isoëtes set forth: the velum corresponds to the inner integument, the ligule to the basal lamina of the half-proliferated ovule, the leafy equivalent of the outer integument.' The present writer regards the 'integument' of *Lepidocarpon* as homologous with the velum of *Isoëtes*; being more especially comparable with this organ in *I. echinospora*, where it completely envelopes the sporangium. In *Lepidocarpon* he regards the ligule as really (i.e. morphologically or ideally) situated *outside* the 'integument,' which is here open at its distal end (Figs. 26, 27). In other genera either the velum or both this and the ligule have quite aborted or never developed: the result, probably, of an efficient protection of the sporangia being afforded by the peltate ends of the sporophylls, as in *Lepidodendron* and *Spencerites*. The same may be said with regard to the Equisetaceae.

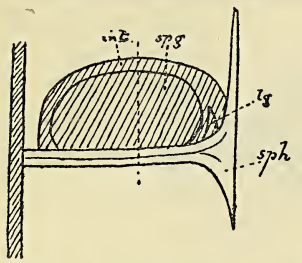


FIG. 26. *Lepidocarpon*: diagrammatic lateral view of sporophyll, showing 'integument' and its mode of attachment to sporophyll; position of ligule and sporangium are seen through the supposedly transparent 'integument.'

FIG. 27. *Lepidocarpon*: diagrammatic transverse section taken through dotted line in preceding figure. *int* = integument; *sph* = sporophyll.

Before proceeding further it is necessary to direct attention to one very important consideration. Čelakovský, until comparatively recently, regarded the virescent conditions of the Angiospermous ovule as *retrogressive* phenomena, as *reversions* to the primitive ancestral structures of the organ. After a deeper study, however, of the phylogenetic relationships of the various organs of plants, from which he gathered that the primitive position of the sporangium on the sporophyll was a *terminal* one, he was eventually led to regard the *normal* structure of the ovule as representing a more ancestral state of affairs, inasmuch as the nucellus or sporangium occupies a position *terminal* to the ovular leaflet. The more *vegetatively* developed is any structure, the more modified will it be in the direction of an *advance* away from the primitive reproductive condition. The *virescent* condition of the ovule merely reveals to us the *homologies* of the latter; it tells us nothing as to its phylogenetic origin; this can alone be determined by the *comparative* method of research.

We have thus been able to trace the homologue of the ovule in three great phyla of the vegetable kingdom; and this is only what one would *a priori* expect, for if there is a unity underlying vegetable life, and if all forms of plant-life own a common origin in the far past, the same organs, under a more or less modified or disguised form, will be produced by plants belonging to most of the great phyla of evolution.

Finally, some of the objections urged by Čelakovský against the other two theories of the ovule may be brought forward.

Against the bud-theory may be placed the fact that in almost all cases the integuments arise *basipetally* and not acropetally, a strange and exceptional state of affairs if they really represent foliar appendages of the nucellus. Eichler, Schmitz, and at one time Warming, held that the terminal organs belong to the caulome-category on account of their position; but this reliance on topical morphology is useless, as terminal organs other than axes are known. Hanstein showed that where the apex of the stem ceases to grow the apical periblem continues to do so and forms a terminal organ, which is a leaf, as the latter is always formed from periblem, whereas it is by growth of the plerome in length that an axis is continued in growth. The terminal cotyledon of Monocotyledons is another case in point. Thus, terminal ovules are not necessarily axial in nature. As regards the occurrence of adventitious buds in connexion with the ovules, our author determines, as far as his own investigations on *Alliaria* are concerned (Fig. 9), that the bud always represents a pathological new formation, and not a transformation of the ovule; he found never a trace of an axis on which integuments are borne as lateral leaves.

He enters into a lengthy criticism of Peyritsch's views and observations, which is in itself of considerable value towards the further elucidation of the nature of the ovule. The conclusions of this author, that the adventitious shoots are derived from and homologous with ovules, is shown to be groundless.

Čelakovský says: 'It may be stated with certainty that shoots replacing ovules are never met with in virescent structures, and much less is this the case with transitions between ovules and such shoots.'

Peyritsch's study of ovular metamorphosis is throughout influenced by the idea that the nature of the ovule can be determined from *developmental* data. He regards the ovular rudiment and the nucellus as identical (shown by Warming to be incorrect); that, therefore, the nucellus arises directly on the placenta and bears the integuments: but this never happens. He thinks, further, that the terminal ovule of *Rumex scutatus* is a shoot owing to its position; but it is no more so than is a terminal nucellus.

Other criticisms of Peyritsch's observations and theories could be

given, all going to show that that writer entirely misconstrued the nature of the various structures which came under his notice.

Referring to the case described by Penzig of adventitious shoots on ovular leaflets of *Scrophularia vernalis* which *bore nucelli at their apex*, our author holds that this phenomenon arises from a coincidence of the two constructive forces of the two structures respectively. The shoot arises *below* the nucellus after the latter has been formed and carries it upon its apex; but here no intermediate forms can exist between the two; 'in all truly intermediate forms an homology exists between all the parts, so that a transformation of the *same* basic structure obtains.' An analogy for such an *enclosed* apex is found in a case of *Helianthus annuus*, mentioned by Sachs, in which the normal apex was damaged.

From all this we gather that inasmuch as no true transitional structures between an ovule and a shoot have ever been seen, but that, on the contrary, all so-called ovular shoots are either axillary productions of the carpels or else of the nature of adventitious buds on the ovular integuments or the placenta, therefore the ovule can no longer be held to possess the morphological nature of a shoot.

Finally, as regards the *sui generis* theory of the ovule, Čelakovský's criticism of the views of Strasburger, the chief champion of this view, are very instructive and illuminating. This author's main theory, as also in the case of the abnormal female parts of Coniferae, with regard to the various virescent conditions of the ovule, is this, viz., that they represent the varying results of a simultaneous strife between two forces: the *generative*, tending to produce the ovule which is of the nature of a sporangium or emergence, and the *vegetative*, tending to form a leafy structure. Now, Čelakovský maintains that, according to this view of Strasburger's, the transitional forms which occur must be those between two *quite heterogeneous plant-organs*: a leaflet or segment of a carpel on the one hand, and a sporangial emergence on the other; this is, on the face of it, an absurdity, and represents the same fallacy as that underlying his position with regard to the female flower in Coniferae. For the transitional forms clearly betray their derivation from the self-same organ, their origin from the same morphological substratum.

Moreover, the virescent phenomena clearly show that the generative and vegetative forces assume possession of the ovular leaflet *successively* rather than simultaneously.

Further, 'if the ovule is not a metamorphosis of the carpellary segment, but a macrosporangium, an emergence, and this purely *in place* of the leaf-segment, the ovule with its integuments should contract more and more and gradually vanish, the leaflet or segment, on the other hand, become more fully developed.' There can be no other compromise. And yet, as has been repeatedly shown, this is precisely what does not happen, for the

integuments increase in size and become fused with and an integral part of the carpel.

Čelakovský does not suppose, as was thought by Strasburger, that the various transitional forms represent the different stages of development which are to be run through in order to reach the extreme form; nor does he regard it as ever possible that the integuments could, once they are laid low, revert back into a carpellary segment. A series composed of metamorphosed structures is of greater morphological importance than a developmental series.

Strasburger asserts that each of these forms ought to be treated and studied separately, in and for itself alone. Our author, on the contrary, affirms that 'each single case taken by itself is of little value; it is only the comparatively arranged complete series which can guarantee us real insight into the essential being, metamorphosis, and origin of the ovule.'

Our author further urges that a comparison of an orthotropous ovule with the macrosporangium and sporocarp of *Azolla* tends to destroy Strasburger's views founded on the ontogeny; for the integument of *Azolla* arises not from the sporangium, but from the leaf-segment beneath it. And, moreover, as the present writer would add, Warming has clearly shown that both nucellus and integuments develop *independently* from the ovular rudiment. The terminal position of the nucellus does not prevent it from being altogether distinct from the ovular rudiment producing it.

As regards the view held, for instance, by Balfour, that the nucellus is an organ *sui generis*, Čelakovský points out that inasmuch as, in the virescent ovule, the nucellus, as an emergence from the surface of the ovular leaflet, may become completely absorbed into, and form an integral part of, the latter, it cannot possibly constitute one of the distinct morphological categories; for the respective organs belonging to these latter have never been known to merge or be absorbed into each other in such a manner. The same will be true of the eusporangium of Vascular Cryptogams, which is the homologue of the nucellus.

General Summary.

In conclusion is appended a brief *résumé* of the various theories on the morphology of the ovule.

Axial Theory.

St. Hilaire (1830, 1840), Schleiden (1839, 1843), Payer (1859), Braun (1860), Peyritsch[1] (1872–76): the nucellus is of the nature of a bud bearing the two integuments as lateral foliar appendages.

[1] It must be borne in mind that this author nowhere makes any definite statement as to the morphology of the ovule, but his writings betray the trend of his ideas on the subject.

Sui Generis Theory.

Schmitz (1870); Sachs (1874); Goebel (1882–1901); Strasburger (1879); Balfour (1901): the ovule does not (necessarily) belong to any of the morphological categories, but is an independent structure, borne either on stem or foliar organs.

Foliolar Theory.

Brongniart (1844); R. Brown (1845–66); Caspary (1861); Cramer (1864); Prantl (1875); Warming (1878); Čelakovský (1874–1900): the ovule belongs morphologically to the category of the phyllome; it is the homologue of a (usually) three-lobed leaflet or segment of the carpel (or female sporophyll), the outer integument and funicles representing the lower portion of the leaflet whose lateral lobes, fused across its upper or ventral surface, constitute a lamina having the *lower* surface directed towards that of the involute cup-shaped terminal lobe; the representative of the inner integument. The nucellus is, like the eusporangium of Ferns, of the nature of an *emergence*, borne on the *upper* surface of the leaflet's terminal lobe. The morphological value of the various parts of the ovule here set forth is ascertained and demonstrated by the occurrence, in teratological conditions of the ovule, of gradual transitions between the structure of the normal ovule and that of the extreme virescent organ in the form of a carpellary leaflet. This latter must not be regarded as a reversion to the primitive condition; on the contrary, the normal ovule possesses a structure essentially primitive and archetypal.

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On the Structure and Biology of Fegatella conica.

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With Plates VI and VII and five Figures in the Text.

THE genus *Fegatella* Raddi (*Conocephalum* Wiggers) is represented by two species, of which one, *F. conica*, is very widely distributed in the North Temperate Zone, whilst the other, *F. supradecomposita*, is confined to Japan and China.

F. conica, which is fairly common in Britain, is one of the largest of the thalloid Hepaticae, the broad dichotomously branched thallus sometimes reaching a length of six inches (15 cm.) or even more; the older parts gradually die down as the new branches creep onwards over the substratum, which often becomes covered by a continuous layer of these plants, extending over several square feet. The plants grow chiefly in moist situations, especially on stones beside shaded streams, and sometimes become entirely submerged in water. This species was named *Hepatica fontana* by Micheli in 1729; the generic name, which was given on account of a supposed resemblance between the branched thallus and the lobes of the liver, was after Micheli's time applied to the whole group (*Musci hepatici*, *Hepaticae*).

The upper surface of the thallus is marked by lines dividing it up into polygonal (mostly hexagonal) areas and forming an extremely regular network. Each of these areas corresponds with an underlying air-chamber, and has in its centre a light spot which stands out in sharp contrast with the dark green colour of the thallus and marks the position of a pore. The central portion of each area is raised so that the pore occupies the summit of a low cone. When water is placed on the upper surface of the thallus, it collects along the lines separating the air-chambers and is quickly drained off by means of this network of channels.

In neither *Marchantia* nor *Lunularia*, which have a general external resemblance to *Fegatella*, is the areolation of the thallus so definite as in the latter genus, which is therefore readily distinguished from the other commonly-occurring genera of the Marchantiaceae, even when no reproductive structures are present. Another characteristic feature of *Fegatella*

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is its peculiar fragrant odour, which resembles that of oil of bergamot, and becomes very pronounced when the thallus is crushed.

The thallus is differentiated into a thicker median cylindrical portion (midrib) and a much thinner portion (lamina) on either side of it. This is more conspicuous on the lower than on the upper surface, since the midrib projects strongly below. On its lower surface the midrib bears two rows of scales, one row on either side of the middle line, together with numerous tufts of rhizoids, which are also arranged in two rows immediately outside of the scales and pass downwards into the soil.

The ventral scales are relatively small, being confined to the sides of the midrib and hardly reaching the lower surface of the lamina. Each scale (Plate VI, Fig. 16) is made up of an oval or kidney-shaped free anterior portion, red or violet in colour, and a colourless posterior portion which has the form of a long narrow band, attached by its outer edge to the side of the midrib, whilst its inner edge reaches and is parallel with the middle line of the thallus and overlaps the hinder portion of the next scale in front. On tracing the scales forwards and removing them one by one, it is found that the coloured appendages are, at the anterior margin of the thallus, curved upwards and backwards so as to cover the apical growing-point, which lies in the notch observed at the end of each branch. As seen from above, this notch appears to be largely filled up by the overlapping appendages, which serve to protect the young tissues of the growing-point (Plate VI, Fig. 1).

When the scales have been removed the lower surface of the midrib shows a median furrow, containing a strand of rhizoids which do not, like those arising in tufts from the sides of the midrib, at once grow downwards into the soil, but pass backwards in a compact bundle.

Apical Growing-point.

The growing-point of each branch is occupied by a transverse row of actively-dividing cells, lying nearly in the plane of the ventral surface of the thallus. These initial-cells are wedge-shaped, appearing oblong and rectangular in surface view, triangular in a longitudinal vertical section through the growing-point (cf. Plate VI, Figs. 8, 9, 18). From each initial-cell segments are cut off by walls parallel with its upper, lower, and lateral walls. The growth of these segments is less rapid in the region immediately behind the apex than it is on either side, so that the apex itself comes to occupy a deep notch. Whilst a large portion of the tissue formed from the segments consists of cells that remain closely packed together, the superficial cells of both the dorsal and the ventral segments show a specialized form of growth, the former giving rise to the dorsal layer of air-chambers, and the latter to the ventral scales and the rhizoids.

Apical Branching.

Under normal conditions branching only takes place at the apex of the thallus. In autumn, usually during October, resting-branches are laid down at the apex of each lobe of the thallus. During the winter months vegetative growth is practically at a standstill, but towards the end of January or beginning of February the resting-branches resume their growth. When branching is about to occur the initial-cells grow in breadth and undergo repeated divisions by vertical walls, thus becoming increased in number, whilst the row becomes laterally extended. The central cells for some time grow more rapidly than those on either side, and form a projecting lobe, but their growth soon ceases, and the cells on each side of the lobe, continuing to grow and divide, form the apical growing-points of the two branches (Plate VI, Fig. 9). This process is repeated, so that four growing-points occupy the end of each of the club-shaped outgrowths formed in autumn. These outgrowths, one of which is nearly always to be found at the end of each of the thallus-lobes, remain dormant during the winter, the four growing-points being protected by the coloured scale-appendages (Plate VI, Fig. 2). In early spring growth is resumed, and each growing-point may then give rise to a broad expanded lobe, or one of them may have its growth in length arrested by the development of a male or female receptacle (Plate VI, Figs. 3–7). The thallus acquires a more or less jointed appearance, the earliest part of each season's growth giving rise to a narrow stalk-like portion which then spreads out to form the broad lobes of the thallus. Occasionally the branching deviates from the type just described, owing to suppression or repetition of some of the divisions that normally occur. When plants are cultivated indoors the branches remain narrow and often cylindrical, and grow upwards instead of horizontally. This upward growth is especially marked when the plants are cultivated in a deep vessel illuminated from above. Plants kept in darkness present a somewhat similar mode of growth, the etiolated branches being long, narrow, and cylindrical.

Air-chambers.

Immediately behind the growing-point, each dorsal segment of the initial-cells becomes divided into an inner cell, which contributes to the formation of the compact tissue of the thallus, and an outer cell which gives rise to part of the spongy tissue. The outer cells soon begin to grow out and to become separated from each other so as to leave narrow air-spaces between neighbouring cells. As the tissues behind the growing-point become extended in area in consequence of active growth these spaces become widened, whilst the superficial cells grow up and remain attached laterally so as to form a series of vertical plates. The uppermost

cells then grow out horizontally and form a roofing layer (epidermis), the cells of which divide only by vertical walls. The epidermis thus forms a single layer of cells, a pore being left above each chamber (Plate VI, Figs. 17, 18). Each pore is at first surrounded by from five to eight cells, each of which undergoes repeated division by walls tangential to the pore, giving rise to five or six concentric rings of cells (Plate VI, Fig. 10). The dorsal region of the thallus thus comes to be occupied by a series of wide air-chambers, forming a single layer and separated from each other by vertical partitions, which are for the most part one cell in thickness and are united with each other so as to form a network. It is of course to these vertical walls between the chambers that the areolation of the upper surface of the thallus is due.

The concentric rows of cells surrounding each pore do not all lie in the same plane, the inner rows being higher than the outer, so that the pore becomes situated on the summit of a dome-like elevation. Immediately around the pore there is a thin but fairly wide membrane of cellulose, which shows in surface view a coating of granules; these are readily dissolved in alcohol, and probably consist of resin or wax. A similar coating of resinous or waxy grains is found on the cells surrounding the barrel-shaped pores of *Marchantia*, and it was suggested by Kny (1890, p. 369), that these serve to prevent the entrance of water into the chambers.

Whilst the chambers have been growing in width the cells forming the floor of each chamber grow out and divide by transverse walls, so as to form short rows of cells containing abundant large chloroplasts; the latter are also found in the cells forming the floor and sides of the chambers, and in much smaller numbers in the epidermis itself. At the sides of the chamber the filaments are generally attached both above and below, but the central filaments, lying below the wide pore, have a peculiar form, the terminal cell of each filament being elongated and produced into a narrow tapering point. In each of these pointed cells the chloroplasts are confined to the swollen basal portion of the cell, the upper portion containing only clear sap with a thin lining layer of protoplasm (Plate VI, Fig. 11). The writer has repeated the experiments of Kamerling (1897, p. 50), which show that these pointed terminal cells have the function of giving off relatively large quantities of water-vapour. A plant having its rhizoid-bundles intact is kept for several hours in a weak ($\frac{1}{2}$ per cent.) solution of red prussiate of potash (potassium ferricyanide), and afterwards treated with alcohol so as to precipitate the salt. On next placing the plant in a solution of ferrous sulphate it is found that the blue precipitate is almost entirely confined to the pointed terminal cells of the chlorophyll-bearing filaments, the cells below showing hardly any precipitate. This clearly shows that the evaporation of water is

localized in the pointed cells, and that the solution absorbed by the rhizoids had passed through the ventral tissue of the thallus into the green filaments, and had become concentrated in the terminal cells owing to continued evaporation.

Ventral Tissue.

The compact tissue underlying the air-chambers is well developed in the midrib, but in the lamina on either side is much thinner, becoming reduced to two or three layers of cells near the margin of the thallus (Plate VI, Fig. 18). This tissue consists chiefly of large colourless cells, containing starch-grains and having their walls thickened by anastomosing fibres, the unthickened portions remaining as slit-like pits (Plate VI, Fig. 21). Towards the lower surface of the midrib the cells are much smaller (excepting the large cells which grow out to form rhizoids), and have thick dark-coloured walls forming a kind of ventral cortex.

The cells of this compact tissue sometimes contain coiled chains of *Nostoc*; colonies of this Alga are frequently observed in the groove enclosed by the ventral scales and amongst the rhizoids (Plate VI, Fig. 20). Endophytic (perhaps symbiotic) blue-green Algae have long been known to inhabit specialized organs in *Blasia* and the Anthoceroteae, but apparently their occurrence in the tissues of the Marchantiales has only been recorded hitherto by Reinsch (1877, p. 234), for a *Riccia*, and by Mattirolo (1888, p. 7), for *Grimaldia dichotoma*. *Nostoc*-colonies have been observed by the writer in the compact tissue of the thallus of *Reboulia hemispherica*, *Preissia commutata*, and *Targionia hypophylla*, and it is probable that they are of general occurrence in the thalloid Hepaticae.

We may here describe two tissue-elements which are especially well developed in *Fegatella*, and which occur chiefly in the compact ventral tissue, viz. (1) oil-bodies, (2) mucilage-sacs.

The oil-bodies are spherical or ovoid in form, dark brown in colour, and from 20 μ to 40 μ in diameter. They occur singly in special cells, each of which is nearly filled by the oil-body (Plate VI, Fig. 21, *O. c.*). Similar bodies occur in many other liverworts, and they were first carefully studied by Pfeffer (1874), who found that they contain water, a proteid substance, oil, and in some cases tannic acid, these constituents being mixed and forming an emulsion, held together by an envelope consisting of proteid matter. Previous to the appearance of Pfeffer's memoir the oil-bodies of the Marchantiaceae had been noticed and described by various observers. Mirbel, in his classical work on *Marchantia polymorpha* (1835), first described them, and believed that they consisted of starch. Gottsche (1842, p. 287), showed that these bodies do not give the reaction of starch; he found that on treatment with alcohol the contents were dissolved and a sac-like membrane remained behind, and concluded that

the bodies consisted of resin or wax. Küster (1894) has recently confirmed and extended the results of Pfeffer's work by his exhaustive study of the oil-bodies. He finds that each oil-body consists of a ground-mass in which the oil and other substances are embedded. It would appear that in the living cell there is no special envelope around the oil-body, and that the membrane which becomes visible on treatment with alcohol is simply an artifact due to the action of this reagent on the substance of the ground-mass.

The oil-bodies are distributed throughout the compact ventral tissue, both in the thallus and in the sexual receptacles; they sometimes occur in the walls of the air-chambers, and occasionally in the epidermis. The only parts from which they appear to be invariably absent are the rhizoids and the sporogonia; the spores contain numerous small oil-drops, but no oil-bodies. It is evidently to the presence of the oil-bodies that the thallus of *Fegatella* owes its characteristic odour, which can no longer be perceived when the plants are soaked in alcohol and then washed in water. When once the oil-bodies are formed they appear to remain unchanged until the death of the cells containing them; plants may be kept in darkness for weeks or even months, and the new parts formed are invariably found to contain oil-bodies, whilst those already present in the older parts remain unaltered. These bodies can therefore only be regarded as products of excretion, but they appear to play an important part in the economy of the plant. As shown by Stahl (1888, p. 49), they serve to protect the plant against the attacks of snails, which will shun fresh pieces of the thallus of *Fegatella* even when there is no other available food. If, however, pieces of the thallus are soaked in alcohol and washed in water, they will be readily eaten by snails which reject the fresh pieces.

Mucilage-sacs. In a transverse section through the thallus (Pl. VI, Fig. 17), the midrib is seen to contain a number (from one to six) of large rounded elements which stand out sharply from the smaller polygonal cells surrounding them. Comparison with longitudinal sections shows that these rounded cells are arranged in continuous rows which traverse the midrib longitudinally and which may be traced forwards to the growing-point. These cells contain a highly refractive granular substance, which shows a stratified appearance, the layers being concentric with the cell-walls. On treatment with water, these cells increase in volume, their contents absorbing water and becoming homogeneous and transparent; the transverse walls between the cells of each row at the same time lose their definite outlines and become disorganized, so that these organs have generally been described as continuous tubes. The cells which will give rise to a row of mucilage-sacs are recognizable at an early stage, becoming differentiated just behind the growing-point (Pl. VI, Fig. 18, *M. o.*). These cells are distinguished from their neighbours by their dense contents and the absence of chloroplasts

and starch-grains. On tracing the cells of a row backwards, the nucleus is found to be broken up and diffused in the protoplasm, whilst layers of deeply-staining mucilage are successively deposited on the inner surface of the cell-wall. The mucilage appears, therefore, to be formed from the protoplasm of the cell, the remains of which can for some time be seen occupying the centre of the cell-cavity (Pl. VI, Fig. 20).

Besides these long coherent rows of mucilage-sacs, the thallus of *Fegatella* also contains large isolated mucilage-cells, which are especially abundant in the lamina, in the compact tissue below the chambers; they also occur abundantly in the sexual receptacles. The development of these isolated sacs agrees exactly with that of the constituent cells of the rows in the midrib. The latter, which are peculiar to *Fegatella*, were first described by Nees von Esenbeck (1838, Vol. IV, p. 188), who overlooked the mucilaginous contents and described the organs themselves as continuous air-canals traversing the midrib. Goebel (1879, p. 531) was the first to show that these supposed air-passages were in reality mucilage-organs, but his account of their development does not agree with the later description of Prescher (1882), with which the writer's own observations are entirely in accord.

Leitgeb (1881, p. 16) believed that the mucilage-organs might serve to confer rigidity on the thallus, and Prescher was also inclined to attribute a purely mechanical function of this kind to these organs, but it seems more reasonable to accept the view, suggested by Goebel, that they act as water-reservoirs. The writer has found that in plants growing submerged in water the midrib shows no trace of mucilage-sacs.

Ventral Scales.

The development of the ventral scales can be readily followed by examining series of longitudinal sections through the growing-point. Immediately behind the latter, some of the ventral superficial cells grow outwards and forwards, dividing rapidly so as to give rise to plates which remain one cell thick. A superficial cell first grows out and becomes divided by a transverse wall; the outer cell then grows rapidly in length, forming a long club-shaped mucilage-hair, which curves upwards over the growing-point. This hair then ceases to grow, but the cell below it divides actively and forms a plate, the hair being thrust on to the upper surface of this new outgrowth, which becomes the discoid appendage of the scale (Pl. VI, Figs. 12–15). In the meantime a zone of cells at the base of the appendage begins to show active growth, keeping pace for a time with the growth in length of the thallus and thus maintaining the position of the coloured scale-appendage in the notch at the apex of the thallus. The fully developed scale may therefore be divided into three portions, each representing a distinct stage in its development, viz. (1) the original

mucilage-hair, at first terminal but now standing in the axil of (2) the appendage, which is inserted by a narrow neck on (3) the long and narrow basal portion of the scale.

Rhizoids.

Each of the rhizoids springing from the lower surface of the thallus is formed by the outgrowth of a single superficial cell, and remains throughout undivided, though often reaching a length of an inch or more. The rhizoids are of two kinds, some being smooth-walled, whilst in others the wall shows numerous peg-like thickenings which project inwards and are arranged in a fairly definite spiral line. The smooth-walled rhizoids spring chiefly from the sides of the midrib, immediately outside the ventral scales, and pass straight down into the substratum, where their ends often become branched (Pl. VI, Fig. 25). The tuberculate rhizoids arise in small bundles, each bundle being borne in the axil of a ventral scale, and these unite to form the compact bundle which occupies the median groove on the lower surface of the midrib. The tuberculate rhizoids generally end freely in the median bundle and do not become branched.

The superficial cell which will produce a rhizoid is recognizable at an early stage on account of its large size and densely granular contents. This cell projects from the surface and grows enormously in length, but no cell-divisions occur, the rhizoid being simply an elongated cell (Pl. VI, Fig. 26). The young rhizoid shows strictly apical growth in length, the protoplasm and nucleus being found near the growing tip, whilst further back the rhizoid contains only cell-sap. The plain rhizoids give the reactions of cellulose, but in the tuberculate ones the tubercles themselves become altered in composition, and on treating the rhizoid with sulphuric acid the tubercles alone remain unaltered after the rest of the cell-wall has been dissolved.

Leitgeb (1881, p. 20) suggested that the tuberculate rhizoids of the Marchantiaceae not only shared with the smooth ones the function of attaching the plant to the soil and of absorbing water, but might also have a third function, namely, that of strengthening the thallus. As pointed out by Kamerling (1897, p. 12), however, the turgor of the cells composing the ventral tissue of the thallus is quite sufficient to maintain the rigidity of the latter, which remains unaltered when the median bundle of tuberculate rhizoids is cut through at various points. Kny (1890, p. 371) believed that the tubercles would serve to prevent the walls of the rhizoids from sinking in through lateral pressure or lack of water; Haberlandt (1896, p. 196), that they increase the area for absorption, these ingrowths causing the protoplasmic lining of the cell-wall to become spread out. There is little to be said in favour of either of these conjectures, for (1) neither the plain nor the tuberculate rhizoids collapse to any great extent when plants

are kept dry, and (2) the fully developed rhizoids can be shown by plasmolysis to have entirely lost their protoplasmic contents, whilst in younger ones the protoplasm is only found at the extreme apex, where tubercles are absent and where the absorption of liquids is shown by experiment to be most active. Kamerling demonstrated the existence of negative pressure in the rhizoids, by placing on a razor a drop of water which contained carmine-powder in suspension, cutting through a bundle of rhizoids, keeping the cut ends in the liquid for a few seconds, and then spreading out the upper part of the bundle (i. e. that attached to the thallus) on a slide and examining the rhizoids under the microscope. In the case of the plain rhizoids, the negative pressure is very marked, the liquids passing in for a distance of several millimetres, but in the tuberculate rhizoids the carmine particles are arrested by the tubercles immediately beyond the cut end.

As already stated, the tuberculate rhizoids spring from the angles between the ventral scales and the surface of the midrib, the scales evidently serving to protect the rhizoids against evaporation and mechanical injury, besides forming narrow spaces in which water is retained by capillarity. The apical appendage of each scale, after it has fulfilled its function of protecting the growing-point of the thallus, becomes, in consequence of the active growth of the latter, thrust on to the ventral surface and then soon withers. The basal portion of the scale, however, persists and covers the ventral groove in which lie the bundles of tuberculate rhizoids (Pl. VI, Fig. 17).

In the submerged aquatic form of *Fegatella*, a few rhizoids are borne in the axils of the reduced ventral scales, but though these rhizoids correspond in position with the tuberculate ones of terrestrial plants, the tubercles are almost entirely wanting. This observation lends support to the view that the tuberculate rhizoids are adapted for the storage of water, whilst the plain rhizoids serve to conduct water and to attach the thallus to the substratum.

Mycorhiza.

The thallus of *Fegatella* is frequently infested by the hyphae of a Fungus, regarded by Beauverie (1902) as a *Fusarium*. These hyphae are usually confined to a zone of cells underlying the air-chambers in the midrib, but are sometimes found also in the compact tissue of the lamina on either side (Pl. VI, Fig. 22). They penetrate the cell-walls and often become branched and coiled up within the cells, bearing here and there swollen vesicles, which may be either terminal or intercalary in position, and in the latter case are often arranged in chains (Pl. VI, Figs. 23, 24, *Ves.*). The writer has found that these vesicles are of two kinds. Those formed during summer are thin-walled, usually aggregated in chains, and sometimes become ruptured, so that the cells of the thallus become filled with

the densely granular fungus-protoplasm. The vesicles formed in autumn are usually terminal and isolated; they have thick walls and evidently function as chlamydospores, from which new hyphae grow out in spring, when the growth of the thallus is resumed. The plants containing the Fungus are larger and show more vigorous growth than those free from hyphae, and there can be little doubt that we have here a definite symbiosis, the Fungus forming a mycorhiza by means of which the life of the *Fegatella*-plant becomes to a certain extent saprophytic at the expense of the humus on which it is growing.

Golenkin (1902) has recently studied the mycorhiza of various Hepaticae, including *Fegatella*, and states that the infected cells never contain starch or chloroplasts. This is not quite true in the case of *Fegatella*, according to the present writer's observations, for here the fungal hyphae may be seen traversing cells which contain starch-grains, and in a few cases the hyphae were seen to have also invaded the chlorophyll-bearing cells that form the floor of each air-chamber. Golenkin suggests that the function of the mycorhiza in the Marchantiaceae is that of storing water and enabling the plant to resist drought, but this explanation will hardly apply to a thoroughly hygrophilous form like *Fegatella*, which possesses in its mucilage-sacs a special tissue that may very reasonably be considered as fulfilling the requirements of water-storage in an exceptionally complete manner.

Since the observations of Beauverie and of Golenkin are in many respects lacking in detail, and do not form a sufficient basis for conclusions as to the biological importance of the mycorhiza in the Marchantiaceae, the writer has made a series of cultures, the results of which, though in some respects incomplete, may be here briefly given.

On sowing ripe spores in heat-sterilized soil it was found that a relatively small number of young plants was obtained, and that these were always poorly developed, the thallus being long and narrow; the ventral scales were small and remained green throughout, and only smooth-walled rhizoids were formed. In no case were any fungal hyphae to be found in the tissues.

For comparison, an approximately equal number of spores (the entire contents of a ripe capsule) was sown in ordinary garden soil, under similar conditions as to light and moisture. A much larger proportion of young plants was obtained, and in many cases these plants showed broad thallus-lobes with well-developed air-chambers, scales with violet-coloured appendages, and tuberculate rhizoids. In the smaller plants the thallus was free from fungal hyphae, but these were found abundantly in the larger and more vigorous plants.

A third lot of spores was sown in rich humus (peaty soil). The young plants were more numerous and showed more vigorous growth than in the

other two lots. The thallus was larger and of a deeper green colour, and in every case examined there was a well-marked mycorhizal zone.

Similar series of cultures were made with very young adventitious plants, removed from old plants that had been found to be free from fungus, and also with the bulbils to be described presently. The results obtained agreed exactly with those just described for the spores. In the case of the bulbils, however, it may be mentioned that these structures themselves were frequently found to contain fungal hyphae at the outset.

Asexual Reproduction.

By the dying away of the older parts of the thallus, the branches become separated from each other and then grow out into independent plants. Besides this simple means of propagation, the observations of Schostakowitsch (1894), which have been confirmed by the present writer, show that the thallus of *Fegatella* possesses in a marked manner the power of regeneration. A plant is cut up into small pieces, which are cultivated on damp soil. After four or five days, given sufficient warmth, several shoots are seen to grow out from the lower surface of each piece of thallus, along the cut edge which in the intact plant was nearest to the apical growing-point. Each of these adventitious shoots arises through the active growth and division of a single superficial cell, forming at first a cylindrical body which grows out and after a time begins to become differentiated in the usual manner. When the cultures were made in darkness, new shoots were formed quite as freely as in the light, but they were long and narrow and did not grow into normal plants with typical air-chambers and scales; on being brought into the light, they gave rise to normal plants, otherwise they remained abortive after attaining a length of 2 to 3 cm.

According to the present writer's observations, the property of regeneration is much more restricted in *Fegatella* than was found by Vöchting (1885) to be the case in *Marchantia* and *Lunularia*. In all cases, the young plants were found to arise from the compact tissue underlying the air-chambers, and attempts to induce the formation of new shoots from the sexual receptacles and from the sporogonia gave negative results.

As already stated, the older plants become covered up by the new shoots, so as to form a kind of turf consisting of several superposed layers of plants. Along the ventral surface of these old plants, which have become for the most part brown and withered, there are frequently found numbers of small spherical or ovoid outgrowths, the bulbils or tubers, which appear to have been first described by Karsten (1887). In these tuber-forming plants, the cells of the compact tissue immediately within the ventral superficial layer of the midrib grow and divide actively, giving rise to a mass of tissue which later breaks through the superficial layer

and forms a stalked outgrowth. The tuber ultimately becomes detached and gives rise to a new plant, but it does not appear to be specially adapted for resisting drought, for tubers that have been kept dry (between sheets of filter-paper) for even a week were found by the writer to be very rarely capable of germination. The tuber bears numerous rhizoids, and its cells contain starch-grains; the superficial cells, except those that grow into rhizoids, have their outer walls cuticularized. In several cases abundant fungal hyphae were found in the cells of the tuber; the fungus-infested tubers were found to germinate freely, and the hyphae evidently belong to the mycorhiza of the thallus.

Sexual Organs.

Fegatella is strictly dioecious, and the sexual organs are borne on specialized portions of the plant, the receptacles. Each receptacle represents a branch of the thallus, or a system of branches. When a resting winter-shoot grows out, the four growing-points may either all give rise to ordinary broad thallus-lobes, or one of them may produce a sexual receptacle. The receptacles are laid down in spring, but the sexual organs do not become mature until about the end of June. The earliest stages in the development of the sporogonium are observed about the middle of July, as a rule, but the spores are not set free until the spring of the following year.

Male Receptacle.

The antheridial receptacle is sessile, forming an oval cushion studded with the openings of numerous cavities, each of which is found, on examining sections of the receptacle, to lodge a single antheridium, or sometimes two antheridia. Each of these antheridial pores occupies the summit of a conical prominence, and between them there occur numerous less conspicuous pores, each opening into an air-chamber (Pl. VII, Figs. 28, 31). At the anterior end of the receptacle are seen the appendages of the ventral scales, which curve upwards and backwards in the same manner as in the growing-point of a sterile branch. The receptacle is at first bright green in colour, but later the walls of the antheridial cavities, together with the prominences just mentioned, assume a deep red or purple colouration. The examination of serial sections through receptacles of different ages shows that the apex of the male branch undergoes repeated forking, so that several (usually from six to eight) growing-points are formed. Each of the latter then gives rise to a zig-zag row of antheridia, the oldest being found at the centre of the receptacle and the youngest at the margin (Pl. VII, Figs. 29–31).

Antheridium (See Fig. 28).

The superficial cell which will give rise to an antheridium projects above the surface and becomes divided by a transverse wall. The lower undergoes little further development, whilst the upper undergoes division by a series of transverse walls, so that the young antheridium consists of a row of cells, four or five in number (Fig. 28, V). The lowest cell of this row forms the short stalk, the body of the antheridium being derived from the upper cells, in each of which there next occur two vertical divisions, the walls intersecting each other at right angles and thus dividing the cell into quadrants (II, VI). The next divisions are also vertical, but the walls lie parallel with the surface of the antheridium, so that each tier of cells becomes divided into four central cells (sperm-cells) and four peripheral cells (wall-cells). The sperm-cells (III, IV, VII) are distinguished from the wall-cells by their denser protoplasmic contents, they undergo repeated divisions and give rise to the antherozoids, whilst the wall-cells divide only by anticlinal septa (i. e. septa perpendicular to the outer surface of the antheridium) and form the single-layered antheridial wall (VIII, IX). The sperm-cells divide with great regularity, remaining nearly cubical in form, and the first-formed walls suffer very little displacement, being easily traced in the nearly mature antheridium (IX). The writer hopes shortly to publish a detailed account of the development of the antherozoid in *Fegatella*.

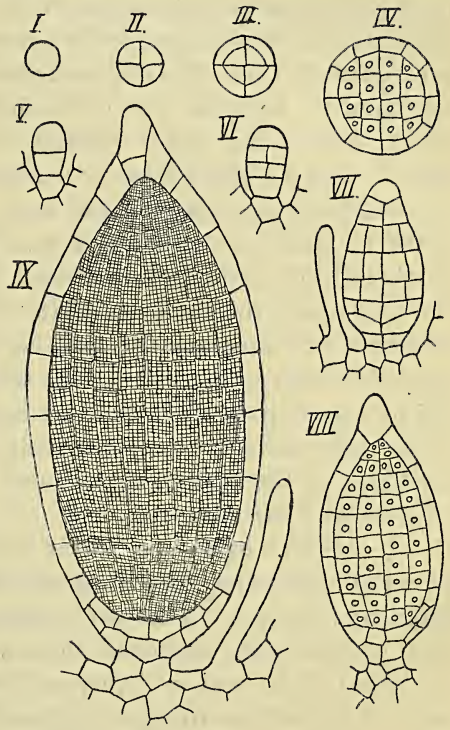

FIG. 28. I–IV. Transverse sections of developing antheridia. V–VIII. Longitudinal sections of corresponding stages. IX. Longitudinal section of well-grown antheridium, showing the regular arrangement of the sperm-cells. I–VIII, ×160; IX, ×80.

Each of the cells composing the wall of the antheridium contains a few large chlorophyll-grains, the colour of which usually changes from green to yellow or red during the ripening of the antheridium. The cells are flattened, except at the apex of the antheridium, where there is a pointed beak made up of elongated cells.

The development of the antheridia is accompanied by that of air-

chambers, which arise in essentially the same manner as those of the thallus. The dorsal portion of the receptacle thus acquires a spongy character, whilst the ventral portion consists of compact colourless tissue with mucilage-sacs and oil-cells. The air-chambers are at first long and narrow, each opening above by a pore which is surrounded by three or four superposed rings of cells (Pl. VII, Fig. 31, *p.*). Pores of this type, generally termed 'compound' or 'barrel-shaped,' are also found on the female receptacle, where they are more highly developed. Each antheridium becomes sunk in a deep cavity, formed in essentially the same manner as the air-chambers (Pl. VII, Figs. 29–31). As the antheridium grows in size, its cavity becomes flask-shaped, with a long narrow canal opening above on the surface of the receptacle by a very narrow pore (Pl. VII, Fig. 30). In consequence of the lateral pressure exerted by the growing antheridia, the air-chambers between the antheridial cavities become greatly compressed below, but in the upper portion of the receptacle they remain as wide spaces, each opening above by a barrel-shaped pore. The cells lining the air-chambers grow out here and there into filaments resembling those found in the air-chambers of the thallus. The male receptacle is therefore well provided with assimilating tissue. Water is taken up by means of the numerous smooth and tuberculate rhizoids which spring from the lower surface of the receptacle (Pl. VII, Fig. 30); part of this water is given off as vapour by the green cells lining the air-chambers, whilst large quantities can be stored up in the colourless cells of the ventral tissue. The margin of the receptacle is beset with scales derived from the growing-points, and the tissue of the thallus behind and on either side of the receptacle grows up to form a partial sheath.

In several cases the writer has found two antheridia occupying a common cavity, the sides along which they were in contact being strongly flattened (Fig. 29). The two antheridia were found, in those cases where fresh sections were examined, to be so closely joined that it was impossible to separate them without injury, and in a few cases the appearance of young pairs of antheridia strongly suggested the possibility of their having arisen from a common mother-cell. Similar examples of paired antheridia have been described by Leitgeb (1879, p. 67, Taf. 8, Fig. 17) for *Sphaerocarpus*, and by Campbell (1896, p. 500) for *Geothallus*, but they do not appear to have been hitherto recorded in the Marchantiaceae.

The cells forming the wall become mucilaginous, and on adding water to a ripe antheridium, these cells become swollen. The delicate walls of the antherozoid mother-cells also swell up in the same manner and ultimately become dissolved. Mucilage is also formed by a number of long club-shaped cells (paraphyses) which grow up from the bottom of each antheridial cavity, around the base of the antheridium. In consequence of the pressure set up, the antheridial cavities eventually come to be separated

from each other only by a few layers of flattened cells; the paraphyses are flattened between the antheridium and the walls of the cavity. The cells forming the beak of each antheridium are ultimately thrown off, and the semi-liquid contents of the antheridium are violently forced out of the opening at the top of the canal, arising from the surface of the receptacle in the form of jets of spray, as has already been described by the present writer (1903 A). A closely similar process had been described in the case of *Asterella californica* by Peirce (1902), whose observations were not known to the writer at the time of penning the note just referred to. In *Fegatella*, the jets of spray containing the antherozoids continue to be emitted from the same receptacle for several minutes, and were mostly observed on warm, sunny days. In most cases the jets reached a height of about 5 cm., but in a few cases the distance was greater, up to about 10 cm.[1]

Similar explosive discharges of antherozoids have been observed by the writer in *Reboulia hemispherica*, *Marchantia polymorpha*, and *Preissia commutata*, living plants of which were kept under cultivation in the laboratory, and it appears probable that this phenomenon will be found to be of general occurrence in the Marchantiales, where the antheridia are embedded in deep cavities sunk in the tissue of the receptacle, and communicating with the exterior by narrow canals. The biological importance of this mode of discharging the antherozoids is readily seen when it is observed in the dioecious forms, e.g. *Fegatella* and *Marchantia*. *Preissia* is generally also dioecious, but sometimes monoecious, and the same is the case with *Reboulia*. In *Fegatella* and *Marchantia*, especially, the female plants are often found to be removed several inches from the nearest male plants, and it is possible that the antherozoids on being ejected explosively from the male receptacles are carried by air-currents to the female plants. No doubt the same result is secured by rain-drops falling on the ripe male receptacles and then splashing over the female plants, as suggested by Goebel (1898, p. 310).

Female Receptacle (Carpocephalum).

The female receptacle arises just below the anterior margin of the thallus as a rounded outgrowth, which increases in size and becomes pushed forwards and upwards until it stands on the dorsal surface of the thallus (Pl. VII, Figs. 38–41). The archegonia are formed on the sides of this knob-like outgrowth, but are soon carried downwards on to its lower surface. A receptacle ready for fertilization has already attained the conical form to which the plant owes its specific name as well as the generic name (*Conocephalus*) used by some writers. It has a short stalk,

[1] See Postscript on p. 114.

though appearing to be sessile at the anterior margin of the thallus (Pl. VII, Fig. 32); it is protected in front by the overlapping ventral scales, behind and at the sides by a sheath formed by upgrowth of the surrounding tissue

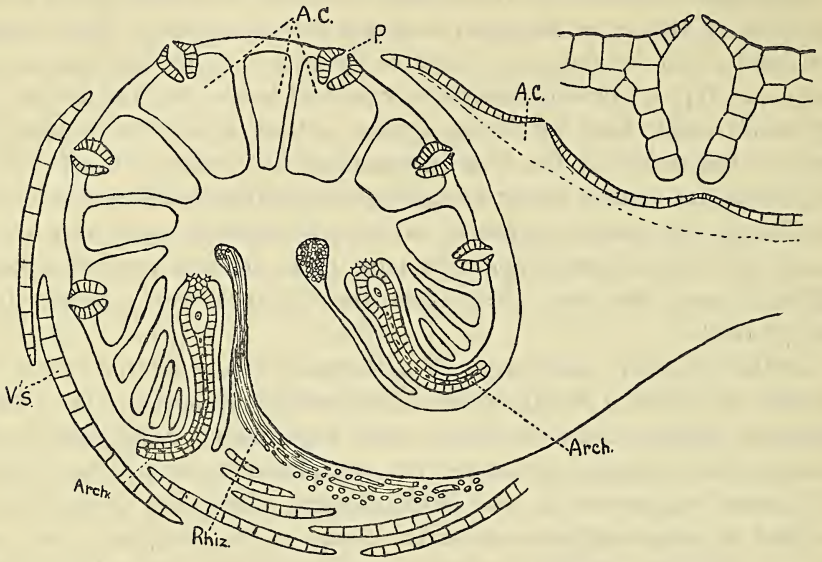

FIG. 29. Vertical longitudinal section of female plant, traversing a receptacle. *A. C.* air-chambers; *P.* pores; *Rhiz.* rhizoids springing from the lower surface of the receptacle and passing down the groove in the stalk; *V. S.* ventral scales; *Arch.* archegonium. × 15. One of the 'barrel-shaped' pores is shown above, in vertical section. × 120.

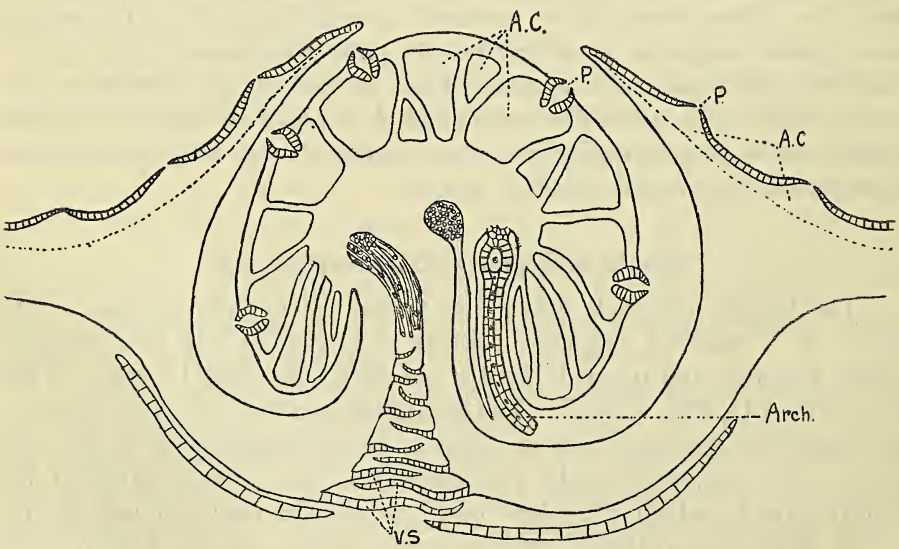

FIG. 30. Vertical transverse section of female plant, traversing a receptacle. Lettering as in Fig. 29. × 15.

of the thallus (Figs. 29, 30). After fertilization of one or more of the archegonia, the development of the sporogonia begins and is continued during the rest of the year. In the following spring, the stalk of the receptacle, which has up to this time remained very short, begins to grow in length and in a few days reaches a height of from 3 to 6 cm., carrying up the receptacle (Pl. VII, Fig. 33). Then the capsules open and the spores are set free.

The earliest stages in the development of the female receptacle are a little difficult to follow, but it would appear that here, as in the case of the male receptacle, the growing-point undergoes repeated branching. Leitgeb (1881, p. 94) leaves the question open, though bringing forward strong theoretical grounds for regarding the carpocephalum as a branch-system. The examination of numerous series of sections through young receptacles has convinced the present writer that branching does take place in the growing-point, repeated dichotomy giving rise typically to eight growing-points, which are separated by rounded sterile lobes, exactly as in the branching of the thallus. The carpocephalum of *Reboulia hemispherica* does not differ greatly in external form from that of *Fegatella*, but microtome-sections of the former show clearly the single growing-point lying below and in front of the receptacle. This is not the case in *Fegatella*, and there can be no doubt that here the apex of the fertile branch is used up in the formation of the whole receptacle, not merely of the stalk, and that the receptacle itself represents a modified branch-system, essentially homologous with the male receptacle.

The young receptacle first appears as a dome-like prominence, formed by active growth of the dorsal segments of the initial cells (Pl. VII, Fig. 38). These cells undergo repeated vertical divisions, so that the tissue of the young receptacle presents in horizontal sections a nearly circular outline, the cells being arranged in radiating rows (Pl. VII, Fig. 42). The next stage shows a slightly rounded lobe occupying the anterior end of the receptacle and recalling the 'middle lobe' seen in ordinary apical branching of the thallus. The cells composing this anterior lobe grow and divide very slowly, but those occupying the slight depression on either side of it divide actively, especially by vertical walls, and obviously constitute two distinct growing-points (Pl. VII, Fig. 43). Next, each of these growing-points becomes broadened, and in its centre there appears a secondary lobe (Pl. VII, Fig. 44). The receptacle now shows four growing-points, but each of these rapidly grows in breadth, and its central cells grow out to form a tertiary middle lobe (Pl. VII, Fig. 45). In most cases, the branching of the young receptacle does not proceed further than this, but sometimes one or both of the two anterior growing-points again becomes branched, so that the receptacle may show nine or ten growing-points instead of the eight which are normally found. On the other hand, one or more of the divisions

that normally occur may be suppressed, so that the receptacle may present only six or seven growing-points. The dorsal segments of each growing-point give rise at first to sterile tissue, in which air-chambers are formed in regular acropetal succession (Pl. VII, Fig. 46), whilst the ventral segments give rise to tuberculate rhizoids and ventral scales. Each of the latter consists simply of a club-shaped mucilage-hair terminating a short row of cells; sometimes the cells below the club-shaped hair undergo longitudinal divisions, giving rise to two or three rows of cells. Whilst the branching has been taking place the whole receptacle has become hemispherical in form. The tissue of the receptacle grows much more rapidly in all directions than does the tissue which lies below and behind it and which gives rise to the receptacle-stalk, so that around the insertion of the stalk there is formed a deep but narrow annular groove. This is continuous in front with the rhizoid-bearing groove on the anterior face of the stalk, which in its turn becomes confluent with the ventral furrow of the thallus. In addition to tuberculate rhizoids, this groove bears two longitudinal rows of scales, which are smaller and simpler in structure as they are traced upwards, every possible transition being found between the ordinary ventral scales of the thallus, with their coloured discoid appendages, and the greatly reduced scales already described as being formed behind each of the growing-points of the receptacle. Each growing-point gives rise to a single archegonium. The first archegonium invariably arises on the hinder margin of the receptacle, and it is closely followed by the appearance of the second and third at the sides of the receptacle, the latest-formed one being nearest to the front of the receptacle. Ultimately we find from six to nine archegonia in all, standing singly on the margin of the receptacle at approximately equal distances from each other. These early stages appear to be passed through very rapidly, and the archegonia are frequently found to be all in nearly the same stage of development, though the anterior ones are invariably less advanced than those occupying the hinder portion of the receptacle. Their development is obviously not simultaneous in any case, though it may sometimes be nearly so.

On comparing the carpocephalum of *Fegatella* with that of *Marchantia polymorpha*, the organization of which was long ago worked out correctly by Mirbel (1835), it will be seen that the only points of essential difference are (1) the absence in *Fegatella* of the long hollow sterile lobes which are so characteristic of *Marchantia*; (2) the development in *Fegatella* of a single archegonium from each growing-point, instead of the group of archegonia found in *Marchantia* between each pair of sterile lobes.

The higher Marchantiaceae, i. e. those having a stalked female receptacle, were divided by Leitgeb (1881, p. 49) into three sections, to which he gave the names Astroporae, Operculatae, and Compositae, and this

classification is adopted by Schiffner (1893) in Engler and Prantl's 'Pflanzenfamilien.' The female receptacle of the Compositae was shown by Leitgeb to consist of a branch-system, having a number of growing-points, from each of which archegonia are formed in acropetal succession; to this group Leitgeb referred *Marchantia*, *Preissia*, *Lunularia*, *Dumortiera*, and, doubtfully, *Fegatella*. In the Astroporae and Operculatae, however, the female receptacle was believed by Leitgeb to be merely a dorsal outgrowth of the thallus, formed behind the growing-point, which might in some cases (*Clevea*, *Plagiochasma*) continue to grow and to produce several receptacles in succession. Recent observations have shown, however, that the distinction drawn by Leitgeb between the receptacle of his 'Compositae' and 'Operculatae' cannot be maintained. Thus *Cryptomitrium* and *Fimbriaria* have generally been regarded as belonging to the lower type, but Abrams (1899) has shown that in the former genus the receptacle is formed directly from the apex of the shoot, which divides to form five or six growing-points, each producing about five archegonia in acropetal succession; and Campbell (1895, p. 57) found that *Fimbriaria californica* also shows a typical composite receptacle, the apex branching to form four growing-points, each of which gives rise to two or three archegonia. Moreover, Solms-Laubach (1897) has shown that although the genus *Exormotheca* is referred by Schiffner (1893, p. 29) to the Astroporae of Leitgeb, its bilobed receptacle presents two groups of archegonia, each group containing as many as five, though the number may be reduced to one; hence this form also would belong to the Compositae rather than to the lowest group of the Marchantioideae.

From the considerations here brought forward, it would appear that *Fegatella* represents the simplest type of the Compositae, the female receptacle being a branch-system, in which each growing-point usually gives rise to a single archegonium. In this respect *Fegatella* forms an interesting member of the series of forms that bridge over the gap between the two extremes—the simple dorsal outgrowth of *Clevea* and *Plagiochasma* and the elaborate branch-system of *Marchantia polymorpha*.

Archegonium (Fig. 31).

Since the number of archegonia developed in each receptacle is so small, and their differentiation is practically simultaneous, *Fegatella* is not a very suitable form in which to study in detail the cell-divisions that occur in the young archegonium. However, sufficient stages were observed to show that in this respect *Fegatella* agrees closely with the descriptions given for *Marchantia* by Strasburger (1870) and by Kny (1890), for *Preissia* by Janczewski (1872, p. 386), and for *Targionia* and *Fimbriaria* by Campbell (1895, p. 52). Each archegonium arises from a superficial cell which projects above the surface and becomes divided by a transverse wall; the

lower cell undergoes irregular divisions and gives rise to the short stalk, whilst the upper cell becomes divided by three vertical walls which intersect each other so as to separate a central cell from three outer cells (Fig. 31, I–IV). The central cell, which is triangular in cross-section, next divides by a transverse wall, cutting off a small upper cell ('cover-cell') from a large lower cell (V). The cover-cell then divides into four by vertical walls that intersect at right angles (VI, X), whilst each of the three outer cells divides into two by a radial longitudinal wall (XI). Transverse divisions then occur both in the large central cell and the six outer cells, so that the young archegonium now consists of two tiers of cells, the upper forming the neck and the lower the venter (VI). The axial cell of the neck undergoes repeated transverse divisions, giving rise to the row of neck-canal-cells, whilst the axial cell of the venter divides by a single transverse wall, cutting off a small upper cell (ventral-canal-cell) from the large egg-cell (VII, VIII). The four cover-cells either remain undivided or undergo longitudinal divisions, so as to increase in number to six or seven in the mature archegonium. The neck-cells divide repeatedly by transverse walls, giving rise to the long neck, which eventually consists of about twenty tiers of cells; the neck-canal-cells do not divide so rapidly, and there are never more than eight or nine altogether (Figs. 30, 31). During its development the archegonium becomes carried downwards on to the lower surface of the receptacle, whilst the tissue around it grows out to form a sheath, the tissue of which contains air-chambers; beyond the opening of this sheath hangs the long neck, which is curved upwards and outwards.

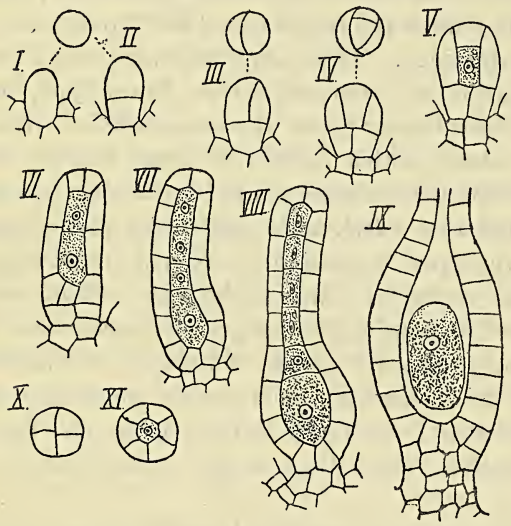

FIG. 31. I–VIII. Longitudinal sections of developing archegonia. The small figures above, I–IV, show the corresponding stages in transverse section. IX. Venter of a mature but unfertilized archegonium; the upper portion of the egg-cell is hyaline, forming a 'receptive spot.' X. Cover-cells in transverse section, corresponding to stage shown in VII or VIII. XI. Transverse of neck, showing six neck-cells and a neck-canal-cell. × 360.

In a paper by Gayet (1897), we find an account of the development of the archegonium in various Hepaticae which differs considerably from that here given for the archegonium of *Fegatella*, as well as from those

of all previous authors who have made a special study of this organ in the Hepaticae (Kny, Strasburger, Janczewski, Campbell). Gayet states that in all the Hepaticae examined by him, the cover-cell of the young archegonium acts as an apical cell, at any rate for some time, segments being cut off from it which contribute to the growth in length of the neck. Gayet's main conclusion is that both in Hepaticae and in Mosses the neck-cells are, at any rate to a large extent, derived from the segmentation of an apical cell (the cover-cell), and that in both groups the neck-canal-cells arise by division of the primary canal-cell—in short, that there is no essential difference in the development of the female organ in the two groups, such as has been generally held to exist. The Marchantiaceous forms studied by Gayet were *Targionia*, *Preissia*, and *Marchantia*. Owing to the fact that the archegonia of *Preissia* and *Marchantia* are developed in large numbers in each group, it is much easier to obtain a good series of stages than in the case of *Fegatella*, where each receptacle shows only seven or eight archegonia altogether. The result of the writer's observations on *Preissia commutata* and *Marchantia polymorpha*, using stained serial microtome-sections, has been to entirely confirm the accounts given by Strasburger, Janczewski, and Campbell for these and other Marchantiaceae. Gayet's figures are not convincing, and his methods of preparation are, as has been pointed out by Campbell (1898), quite inadequate for the exact determination of the points in question, for unless recourse be had to modern methods of fixation, paraffin-embedding, and serial sectioning by microtome, it is almost impossible to make out the precise sequence of nuclear and cell divisions in a developing organ which consists of a solid aggregate of cells.

The tissue of the receptacle, above and between the archegonia, contains numerous air-chambers, separated by thin partitions, consisting mostly of a single layer of cells (Figs. 30, 31); here and there we find large mucilage-containing cells, both in the compact tissue and in the walls of the chambers. The pores are of the compound or barrel-like type; the cells forming the upper tiers are narrow, whilst the lower tiers, projecting into the chamber are broader, the lowest cells being nearly spherical in cross-section. If sections of a fresh receptacle be examined in water, it is found that the cells of this lowest ring are so arranged as to leave a wide opening into the underlying air-chamber; but on irrigating the section with a five per cent. KNO_3 solution, these cells become flaccid and the opening becomes very much smaller, or the pore may become completely closed, the cells of this ring coming into contact with each other. On adding water, the cells resume their former state of turgescence and the pore again becomes open. It appears from this experiment that the lowest tiers of cells surrounding these barrel-shaped pores act in the same way as the guard-cells in the stomata of higher plants, and have the function of regulating the opening

and closing of the pores. This is not the case with the pores on the thallus, which remain permanently open when similar experiments are tried.

On the lower surface of the receptacle, immediately around the insertion of the stalk, there is a circular groove from the surface of which there spring numerous tuberculate rhizoids. Some of these rhizoids pass into a shallow groove on the anterior face of the stalk (Pl. VII, Fig. 47). This groove is continuous with the ventral groove of the thallus, and the rhizoids traversing it become merged in the median bundle of the midrib. Many of the rhizoids springing from the receptacle remain outside of this groove and hang down over the surface of the stalk.

The canal-cells of the mature archegonium become disorganized and converted into mucilage, and on the absorption of water the walls between these cells break down, the cover-cells being at the same time thrust aside and the mucilage being forced out of the open neck. The process of fertilization can be readily followed in *Fegatella*, owing to the comparatively large size of the antherozoids. A male plant with well-grown receptacles is kept dry for a few days, then a few drops of rain-water are placed on the upper surface of the receptacle; on drawing the water off with a pipette, it is usually found to contain large numbers of swarming antherozoids. Longitudinal sections are then made through a female receptacle, the razor being kept dry. In most cases, one or more of these sections will be found to include a mature archegonium, and on mounting such a section in water, it is often possible to see the opening of the neck and the discharge of the canal-cells. On adding to such a preparation some of the water containing the antherozoids, the latter are seen to swarm around the open neck of the archegonium in large numbers, being evidently attracted by the extruded mucilage. Since the cells forming the archegonium neck and venter are usually fairly transparent, one can follow the passage of the antherozoids down the neck-canal. In some cases large numbers of antherozoids were seen to enter the canal, and when they reached the egg-cell they caused it to exhibit a rocking movement. Ultimately one of the antherozoids penetrates the egg-cell, whilst the others perish.

Sporogonium (Fig. 32).

The fertilized egg-cell secretes a cellulose wall around itself and then begins to grow and divide. The first wall is transverse (I), and it is sometimes followed by a second transverse wall in the upper (epibasal) cell, but as a general rule the next walls are vertical and divide the embryo into regular octants (I, II). From the first, the embryo grows more vigorously in length than in breadth, and soon the lower (hypobasal) portion becomes separated from the upper by a constriction, the former giving rise to the foot, the latter to the stalk and capsule (VII). The foot becomes elongated and penetrates the compact tissue of the receptacle, the cells of which contain

abundant starch-grains. In the upper portion of the sporogonium there is evident at a relatively early stage a well-marked outer layer which gives rise to the wall of the capsule, the inner cells forming the archesporium, from which arise the spores and elaters.

Spores.

The archesporial cells are at first all alike, but later they become differentiated into two kinds, which show no definite relation to each other

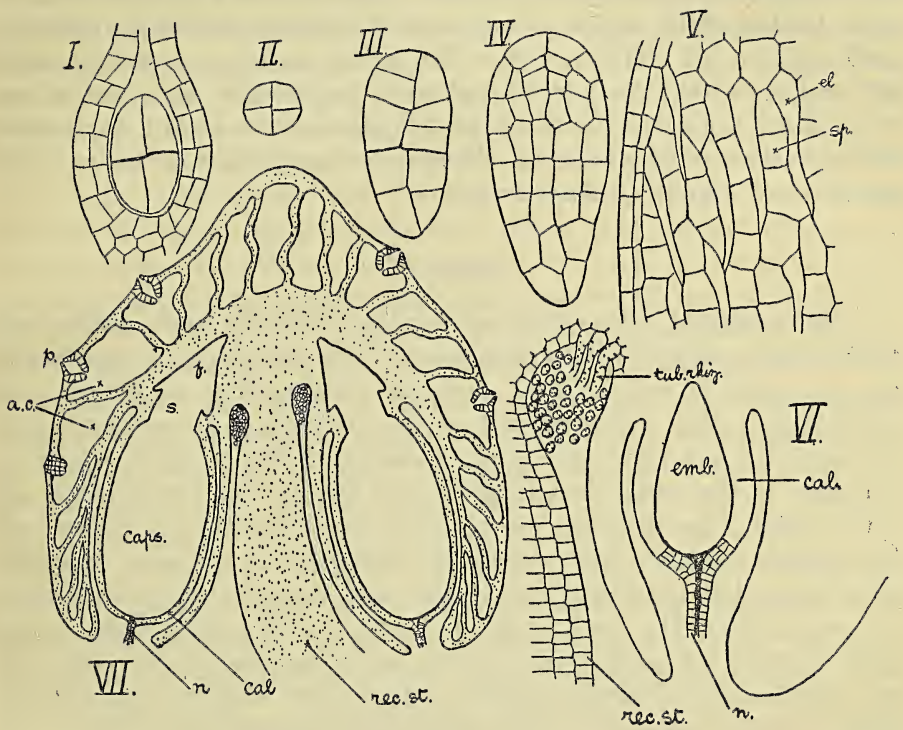

FIG. 32. I. Venter of fertilized archegonium, with eight-celled embryo (octant-stage), four cells being seen in the section. II. Transverse section of a similar embryo. III, IV. Older embryos in longitudinal section. V. Part of longitudinal section of developing capsule, showing differentiation of archesporial tissue into long narrow elater-forming cells (*el.*) and nearly isodiametric sporogenous cells (*sp.*). VI. Part of longitudinal section of receptacle, showing a young sporogonium in outline. VII. Similar section, showing two mature sporogonia in outline. *a. c.* air-chambers; *cal.* calyptra; *caps.* capsule; *f.* foot; *n.* withered neck of archegonium; *p.* pore; *rec. st.* stalk of receptacle; *rhiz.* rhizoids; *s.* seta of sporogonium. I–V, × 360; VI, × 76; VII, × 20.

beyond a tendency to become arranged in longitudinal rows (Fig. 32, V). Some of the cells grow in length and remain narrow, whilst the others grow almost equally in all directions, and are further distinguished from the long cells by containing more densely granular protoplasm. The larger cells eventually become rounded off and constitute the spore mother-cells. The nucleus divides into two, and then each daughter-nucleus divides

again, so that the mother-cell contains four free nuclei, which move apart and take up equidistant positions near the periphery of the mother-cell. As shown by Farmer (1895), a disc of cellulose next appears near the centre of the mother-cell, and this becomes joined by ingrowths from the periphery, so that the four spores become partitioned off. The spore is at first pyramidal in form, having three flat sides meeting at the truncated apex of the pyramid and marking the surfaces where the spore was in contact with the other three cells of the tetrad. The coat is thin, but is differentiated into two layers, the inner being thin and homogeneous, the outer thicker, light brown in colour, and bearing externally numerous small papillae (Pl. VII, Fig. 53). The spores begin to germinate whilst still enclosed within the capsule, each becoming divided up so as to form an ovoid mass of cells. In this respect *Fegatella* differs from the remaining Marchantiales, so far as known, though the same thing occurs in *Pellia* and in some species of *Dendroceros*.

Elaters.

The elongated cells which are mingled with the spore mother-cells remain sterile and give rise to the elaters. They continue for a considerable time to grow in length, their tapering ends passing in between the neighbouring cells, and then part of the protoplasm becomes arranged in a double spiral band on the inner surface of the cell-wall, whilst the remainder of the protoplasm, together with the nucleus, occupies the axis of the elater. Starch-grains are at first present, but these, together with the nucleus and the axial strand of protoplasm, eventually disappear, whilst the spiral band becomes thicker and at the same time assumes a brown colour. On tracing one of the turns of the spiral from one end of the elater it is usually seen to branch on its way towards the other end, and branches later, becoming united again; hence each end of the elater shows a small loop, whilst at the middle there may be from three to five parallel bands (Pl. VII, Fig. 52 *a*). The typical fully-grown elater is spindle-shaped, from 0.15 mm. to 0.25 mm. in length. As a rule, one end is blunt and rounded, the other pointed and tapering, and there is generally a slight bend near the middle and often also at each end. The elaters often assume very curious shapes owing to the occurrence of branching. Nearly every capsule examined by the writer showed a certain proportion of branched elaters. The branching takes place at a relatively late period, after the spiral bands have been deposited but before they have assumed their final deep brown colouration, and after the spore-tetrads have become separated. In the hundreds of young sporogonia examined by the writer not a single case of branching was observed prior to the rounding-off of the spore mother-cells and their

division into tetrads of spores. Moreover, the examination of longitudinal sections of the sporogonium shows that in nearly every case the branching really consists in a forking of that end of the elater that lies nearest the apex of the capsule, and that this is in the typical unbranched elater blunt and rounded, whilst the lower end tapers to a sharp point (Pl. VII, Fig. 52 *b*, *c*). From these facts it is obvious that the branching of the elaters is brought about by the loosening of the spores, in consequence of which the pressure on the upper portion of each elater becomes diminished, whilst its lower portion remains wedged in between the spores. In the lower part of the capsule the elaters either remain unbranched or slight branching takes place at their upper ends; towards the middle of the capsule branching takes place more freely, but still only at the upper end of the elater; but nearer the apex of the capsule branching may take place at both ends of the elater, the branches insinuating themselves between the spores. The writer has observed branching elaters in *Reboulia hemispherica* and *Targionia hypophylla*, where the relative arrangement of spores and elaters is much the same as in *Fegatella*, the elaters being relatively short and the spores being loosely packed, whereas in *Marchantia* and *Preissia*, where the spores and elaters are arranged in extremely regular longitudinal series, the elaters being very long and narrow for the most part, branching does not appear to occur. It is hardly necessary to describe and figure the various and often bizarre forms assumed by the branched elaters of *Fegatella*; this has already been done by a previous writer (Tilden, 1894). In most cases they are Y- or V-shaped, though in a few cases there were found towards the apex of the capsule a few X-shaped elaters in which both ends had become forked. The young elater contains numerous small starch-grains, but these become fewer as the differentiation of the bands proceeds, being evidently used up in the formation of these thickenings and disappearing by the time the bands have become brown. The bands at first, while colourless, give the reactions of cellulose, but later become lignified.

It is probable that the primary function of the elaters is to aid in the nutrition of the developing spores. They present a relatively large surface through which food-materials, passing up in solution through the stalk of the capsule, can readily be distributed to the spore-producing cells. This primary function of nutrition, the only one performed by the sterile cells in the lower Marchantiaceae (e. g. *Corsinia*), is in the higher forms superseded by a second, namely, that of assisting in the dispersal of the spores. This, however, only comes into operation after the dehiscence of the capsule, and it is probable that even after the elaters have ceased to be living cells and have become thickened, they still serve for the distribution of water to the developing spores.

Structure and Dehiscence of the Capsule-Wall.

The wall of the capsule remains one cell thick, except at the apex, where there is a small but well-marked cap projecting into the cavity of the capsule and consisting of several layers of cells (Pl. VII, Fig. 48). This cap appears to be derived from the uppermost portion of the archesporium. The cells of the capsule-wall are at first uniformly thin-walled, but thickenings appear later in the form of ring-fibres. At the summit of the capsule the cells are much shorter than in the lower portion, being cubical in form, whereas lower down the cells are elongated. The differentiation of the fibres begins in the upper part of the capsule, and we therefore find a gradual transition in passing upwards from the base, where the fibres are thinnest and about six occur in each cell, to the apex, where each cell contains a single broad ring, which is often so thick as to almost divide the cavity of the cell in two (Pl. VII, Figs. 49–51).

Besides the ordinary free elaters which are mingled with the spores we find a few which are attached at one end to the lowest layer of the apical cap, and others which arise from the bottom of the capsule (Pl. VII, Fig. 48). These fixed elaters are shorter and thicker than the ordinary free elaters, and are sometimes, especially at the apex of the capsule, intermediate in structure between the free elaters and the cells of the capsule-wall, bearing ring-fibres in addition to spirally coiled ones.

It is easy to isolate a single ripe capsule and to observe the manner in which it opens. Dehiscence takes place on drying, and may be hastened by gently warming the capsule. A line of cleavage runs round the upper portion of the capsule just outside the apical cap. The fissure thus formed is irregular and wavy, but it corresponds on the whole with the junction between the apical cap and the rest of the capsule-wall. Longitudinal splitting then takes place along several (usually from four to six) lines, which extend about half-way down the capsule, dividing the wall into the same number of valves. The apical cap remains intact, either becoming loosened all round and falling off, or remaining attached to one of the valves. As the longitudinal fissures extend towards the base of the capsule the tips of the valves become rolled backwards, thus exposing the mass of spores and elaters. The latter show hygroscopic movements, twisting about as they become dry, and thus helping to loosen the spores, which may then be either blown by the wind or washed away by rain.

After the archegonium has been fertilized its neck withers, but the venter grows actively and closely invests the sporogonium, forming the calyptra. Fertilization is immediately followed by the appearance of tangential walls in the cells of the venter (Fig. 32, I); the calyptra ultimately becomes five or six cells thick, and remains intact until the

sporogonium is mature. In early spring the stalk of the receptacle becomes suddenly elongated, the receptacle, which has hitherto been practically sessile, becoming in three or four days carried up to a height of from two to six centimetres. The writer has found that this sudden elongation is due to growth of cells already formed, and not to rapid cell-division. Should none of the archegonia of the receptacle be fertilized the stalk remains very short, and the whole structure soon becomes brown and withered, but fertilization of even a single archegonium is soon followed by active cell-division in the receptacle-stalk. This process continues during the autumn and winter, so that in early spring, before elongation has taken place, the stalk consists of longitudinal rows of very short but broad cells, densely filled with small starch-grains (Pl. VII, Fig. 35). During the elongation of the stalk the starch disappears, and when growth in length has ceased the stalk is found to consist of long cells which contain little but sap (Pl. VII, Figs. 36, 37). On comparing the length of the whole stalk and the average length of each of its cells, before and after elongation, it is found that the whole of this remarkable growth in length may be accounted for by the simple elongation of the cells, the starch being used up in the formation of the cellulose required to maintain the thickness of the cell-walls as they become stretched out.

The process just described is exactly similar to that observed in the elongation of the sporogonium-stalk in *Pellia* and other Jungermanniaceae, but does not appear to have been described previously in the case of the receptacles. In *Marchantia* and *Preissia* the conditions are entirely different from those here described for *Fegatella*. In these highest forms of the Marchantiaceae the growth of the receptacle-stalk is a gradual process, accompanied throughout by repeated cell-division.

In its final elongated condition the receptacle-stalk is a slender, pale-green filament, nearly as delicate in texture as the elongated sporogonium-stalk of *Pellia*, and very different from the rigid receptacle-stalk of *Marchantia* or *Preissia*, with its dorsal layer of air-chambers and its deep ventral furrows which contain rhizoids and are completely enclosed by the overlapping marginal sheaths. In *Fegatella* the receptacle-stalk is, after it has become elongated and has carried up the head of sporogonia, a purely temporary organ; it only lasts until the spores have been shed. Soon after this has taken place the whole carpocephalum withers, its remains usually becoming covered up by the new thallus-lobes. Hence the botanist who does not begin his outdoor observations before Easter is likely to receive the impression that *Fegatella* rarely fruits, even when the very patches examined by the belated observer had been a short time before covered with abundant carpocephala. The writer has found *Fegatella* to be one of the most freely fruiting of the Hepaticae growing in various localities which were visited at regular weekly or fortnightly intervals

throughout the entire year. The importance of systematic outdoor observations at all seasons of the year can hardly be over-estimated in the case of the Hepaticae and the Mosses; even in the middle of winter these plants yield material which is absolutely necessary to the investigator who wishes to follow the successive stages in their life-histories.

Before the dehiscence of the ripe capsule takes place its seta grows in length, owing to simple elongation of its constituent cells, the starch-grains originally present in these cells becoming used up in the process. The seta finally becomes as long as, or a little longer than, the capsule itself, so that the latter is thrust out through the ruptured calyptra and projects beyond the opening of the sheath (involucre). It frequently happens that the seta becomes broken off at the base, so that the entire capsule falls from the receptacle.

Germination of the Spore.

As already stated, the ripe spore begins its germination before the dehiscence of the capsule takes place, so that on being liberated it encloses an ovoid mass of cells, usually five or six in number. At either end of the longer axis there is a bluntly conical cell, distinguished from the middle cell by containing fewer chloroplasts. When the spore is set free and its germination is resumed, one or other of these terminal cells grows out to form a colourless rhizoid (Pl. VII, Fig. 54), the exospore not showing any definite rupture but simply becoming stretched out. It appears that here, as in other cases that have been investigated (cf. Zimmermann, 1879; Heald, 1898), light is the chief factor in determining the point of origin of the primary rhizoids, which invariably spring from the end which is furthest from the light, whilst the illuminated end grows out to form the apex of the young plant. The latter at first appears to grow by a single initial cell, but soon the typical transverse row is established, and the differentiation of the tissues proceeds as in the adult plant. At first only smooth-walled rhizoids are formed, whilst the ventral scales are simpler in structure than in the fully-grown thallus, but soon the typical structure of the latter is attained with the appearance of tuberculate rhizoids, coloured scale-appendages, mucilage-sacs, oil-cells, and well-developed air-chambers. The spores will usually germinate promptly on being sown directly after the dehiscence of the capsule, but they are not adapted to undergo a resting period, and if allowed to become dry they soon perish. In darkness they usually remain unaltered, though sometimes cultures in the dark show the development of rhizoids from one or both ends of the mass.

Postscript.

In a recent number of 'Torreya' (April, 1903), there appeared an interesting note by C. A. King on the explosive discharge of antherozoids in *Fegatella*, in which reference was made to the previously published accounts of a similar phenomenon in *Asterella californica* by Peirce, and in *Fegatella* by the present writer. At the time of writing the note which appeared in the 'Annals of Botany,' January, 1903, I was not aware of any previous accounts of such discharges, which are not mentioned in any of the numerous works on the structure and biology of the Hepaticae to which I have had access (see list of literature consulted). It appears, however, that the violent discharge of antherozoids in *Fegatella* was observed and described by the late M. Thuret nearly half a century ago. M. Ed. Bornet, who kindly wrote informing me of this observation of Thuret's, which appears to have remained unnoticed by practically all subsequent writers on this group of plants, says in his letter: 'L'émission à distance des anthérozoïdes du *Fegatella conica* a été observée en 1856 par G. Thuret. Il en a donné la description dans une note insérée dans le tome iv des Mémoires de la Société des Sciences naturelles de Cherbourg, p. 216. J'ai rappelé cette observation dans la Notice biographique sur G. Thuret qui se trouve dans les Annales des Sciences naturelles, Botanique, sér. v, tome ii, p. 336, 1875. En général les ouvrages consacrés à la systématique ne reproduisent pas les observations biologiques. Voyer Botanische Zeitung, 1858, p. 144, où la note de M. Thuret est analysée.' Thuret's observations are also briefly referred to by M. Le Jolis in his 'Remarques sur la nomenclature hépaticologique,' 1894, p. 130.

Summary.

1. Although the pores are of the simple type, as opposed to the complex or barrel-like type found in *Marchantia* and *Preissia*, the thallus of *Fegatella* presents a higher degree of internal differentiation than is found in any other Marchantiaceous form. The air-chambers are lined by long hyaline cells, from which localized evaporation of the water into the chambers takes place. The midrib contains highly developed mucilage-organs, arising as rows of large cells which are devoid of chlorophyll and starch, but contain dense protoplasm; the concentric layers of mucilage are derived from the protoplasm, not from the cell-walls.

2. The ventral tissue of the thallus, underlying the air-chambers, is frequently infested by a Fungus forming a mycorhizal zone. The relationship between the Fungus and the host-plant may be regarded as symbiotic in character, enabling the host to assume a partially saprophytic mode of nutrition at the expense of the humous substratum.

3. The sessile cushion-like antheridial receptacle presents from four to eight growing-points, each producing rows of antheridia in acropetal succession; it clearly represents a branch-system, as in *Marchantia* and *Preissia*. The receptacle contains air-chambers lined by inwardly-projecting hyaline cells, as in the thallus, and the pores are barrel-shaped.

4. The antheridia are usually sunk in separate cavities, but in some cases a single cavity contains two antheridia closely joined together and strongly flattened along the surface of contact.

5. The antherozoids are explosively ejected from the openings of the flask-shaped antheridial cavities; the essential factor in the process is the absorption of water by the mucilaginous antherozoid mother-cells and those forming the antheridium-wall, leading to considerable pressure, which is relieved by the discharge of the antheridial contents in the upward direction, that of least resistance. The antherozoids are larger than in other Marchantiaceae; each consists of at least two complete turns of a spiral, and the thicker posterior end often shows a vesicle, the remains of the mother-cell.

6. The archegonial receptacle, like the antheridial, represents a branch-system, but each of the (5–9) growing-points only produces a single archegonium. The stalk of the receptacle remains very short until after the sporogonia are ripe, and immediately before the dehiscence of the capsules it suddenly attains a length of 3–6 cm.; this elongation is due solely to growth in length of cells already present, the starch contained in the cells of the stalk being used up during the growth in length of the latter.

7. In the development of the archegonium, the cover-cell becomes immediately divided by intersecting vertical walls and takes no part in the growth in length of the archegonium, as asserted by Gayet to be the case in *Marchantia*, *Preissia*, and other Hepaticae.

8. The young sporogonium usually shows an octant-stage, and does not grow by means of an apical cell, as stated by Hofmeister.

9. The large, green, thin-walled spores begin to germinate within the capsule, each forming an ovoid cell-mass. Beyond the occasional formation of short rhizoids no growth takes place in darkness. The spores are not adapted to resist desiccation.

10. The relatively short elaters are frequently branched; the branching takes place at the time when the spore-tetrads become separated and loosened within the capsule.

11. The capsule opens by throwing off a thickened discoid apical cap, the rest of the wall then becoming split longitudinally into 4–8 valves.

12. *Fegatella* may be regarded as the lowest member of the Marchantioideae-Compositae, with which it agrees in the structure and development of the thallus, male receptacle, and sporogonium. In the organization of the female receptacle *Fegatella* approaches the Marchantioideae-Operculatae. It appears, therefore, to occupy an intermediate position between the two highest series of the Marchantiaceae.

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EXPLANATION OF FIGURES IN PLATES VI, VII.

Illustrating Mr. Cavers' Paper on Fegatella conica.

The drawings for Figs. 32 and 33 (Pl. VII) were kindly made for the author by Miss Lucy M. Phillips.

Fig. 1. Surface view of anterior portion of thallus, from above, showing the areolation of the surface; each area has in its centre a pore situated on the summit of a dome-like elevation; the growing-point lies in the notch at the margin of the thallus, and is covered by the overlapping appendages of the ventral scales. × 12.

Fig. 2. Similar view, showing a new shoot arising in the notch at the anterior end of the thallus. × 4.

Figs. 3–7. Successive stages in the growth of a winter-shoot. × 1.

Fig. 8. Horizontal section of thallus, showing the transverse row of initial-cells (*x*, *x*, *x*): *v.s.* ventral scales. × 360.

Fig. 9. Similar section, showing dichotomy of apex: *x*, *x*, the initial-cells of the two new growing-points; *v. s.* ventral scales. × 360.

Fig. 10. Surface view of a pore, showing the concentric rows of cells, and the granular rim immediately bounding the pore. × 360.

Fig. 11. Vertical longitudinal section of thallus, showing an air-chamber, lined by chlorophyll-bearing filaments, those below the wide pore being produced into long hyaline processes: *ep.* epidermis, forming roof of chamber; *st.* starch-grains in cells underlying chamber. × 360.

Figs. 12–15. Stages in development of ventral scale, in surface view: *p. p.* primary papilla; *app.* appendage; *b. sh.* basal portion of scale, in the axil of which spring tuberculate rhizoids (*rh.*), whilst its free margin bears mucilage-hairs (*m. h.*). × 200.

Fig. 16. Fully grown ventral scale; lettering as in Fig. 15. × 15.

Fig. 17. Part of transverse section of thallus: *a. c.* air-chambers; *lam.* part of lamina; *m. o.* mucilage-organ; *sm. rh.* smooth-walled rhizoids; *tub. rh.* tuberculate rhizoids, borne in bundles in the axils of the ventral scales (*v. s.*). × 75.

Fig. 18. Vertical longitudinal section through growing-point of thallus: *a. c.* developing air-chambers; *ep.* epidermis; *m. o.* mucilage-organ; *p.* pores; *v. s.* ventral scales; *x.* initial cell. × 360.

Fig. 19. Cells in ventral tissue of midrib, containing Nostoc-chains. × 360.

Fig. 20. Part of a longitudinal section through midrib, traversing a row of mucilage-cells. × 360.

Fig. 21. Cells in ventral tissue of lamina, showing pitted walls: *o. b.* oil-body. × 360.

Fig. 22. Part of a longitudinal section of midrib, showing cells of the ventral tissue traversed by branching fungal hyphae. × 360.

Fig. 23. Cell with fungal hyphae bearing thin-walled vesicles (*ves.*). × 540.

Fig. 24. Cell with fungal hyphae, one of which bears a large terminal thick-walled vesicle (*ves.*). × 540.

Fig. 25. Branched ends of two smooth-walled rhizoids. × 120.

Fig. 26. Two young rhizoids, with nuclei and protoplasm. × 200.

Fig. 27. Transverse sections of tuberculate rhizoids, showing the peg-like thickenings. × 360.

Fig. 28. Antheridial receptacle, seen from above; *v. s.* ventral scales. × 10.

Fig. 29. Part of horizontal section through antheridial receptacle: *v. s.* ventral scales. Two antheridia are shown occupying a single cavity, in lower part of figure. × 200.

Fig. 30. Vertical transverse section of male plant, traversing an antheridial receptacle: *a. c.* air-chambers of thallus; *an.* antheridia; *sm. rhiz.* smooth-walled rhizoids; *tub. rhiz.* tuberculate rhizoids; *v. s.* ventral scales. × 10.

Fig. 31. Median longitudinal section of young antheridial receptacle: *a. c.* air-chamber; *an.* 1, *an.* 2, developing antheridia; *p.* pore; *v. s.* ventral scales. × 360.

Fig. 32. Female plant, with two receptacles (carpocephala). × 2.

Fig. 33. The same, after elongation of the carpocephalum-stalk. × 2.

Fig. 34. Carpocephalum as seen from below, showing cut end of stalk with its ventral furrow and the slit-like opening of the sheath around each of the six sporogonia. × 12.

Figs. 35–37. Cells in tissue of carpocephalum-stalk, as seen in longitudinal section before, during, and after its sudden growth in length. × 360.

Figs. 38–41. Longitudinal sections of developing carpocephala: for description see text. × 6.

Fig. 42. Horizontal section of young carpocephalum, corresponding with the stage shown in Fig. 38. The cells occupying the anterior margin of the outgrowth show active divisions, constituting at this early stage a single growing-point, which may be compared with that of the thallus, shown in Fig. 8. Below the row of initial-cells there stand numerous mucilage-hairs, shown in cross-section; two of the ventral scales which curve upwards over the young carpocephalum are also shown in section. × 200.

Fig. 43. Similar section of a later stage, showing dichotomy of the apex, a 'middle lobe' having been formed between the two growing-points, as in ordinary dichotomy of the thallus (cf. Fig. 9). × 200.

Fig. 44. A still later stage: here each of the two growing-points has again undergone dichotomy, so that four growing-points have been established; between these are the projecting 'middle lobes.' × 200.

Fig. 45. Part of a horizontal section through a carpocephalum with eight growing-points, four of which are shown. × 200.

Fig. 46. Part of a longitudinal section through a carpocephalum of about the same age as that in Fig. 40, showing a young archegonium: *a. c.* air-chambers; *p.* pores; *rhiz.* tuberculate rhizoids springing from the lower surface of the receptacle, near the insertion of the stalk (*rec. st.*). × 200.

Fig. 47. Horizontal section of receptacle bearing six nearly mature sporogonia: *a. c.* air-chambers of receptacle, now reduced in size owing to the pressure exerted by the growing sporogonia; *cal.* calyptra; *caps. w.* capsule-wall; *rhiz.* rhizoids; *st.* stalk of receptacle. × 75.

Fig. 48. Longitudinal section of ripe sporogonium, showing the foot (*f.*), seta (*s.*), and capsule (*c.*); the spores and the free elaters are omitted, in order to show more clearly the apical thickening of the capsule-wall and the apical and basal tufts of fixed elaters. × 60.

Fig. 49. Apical portion of capsule-wall, showing the strongly thickened cells of the apical cap, in surface view. × 200.

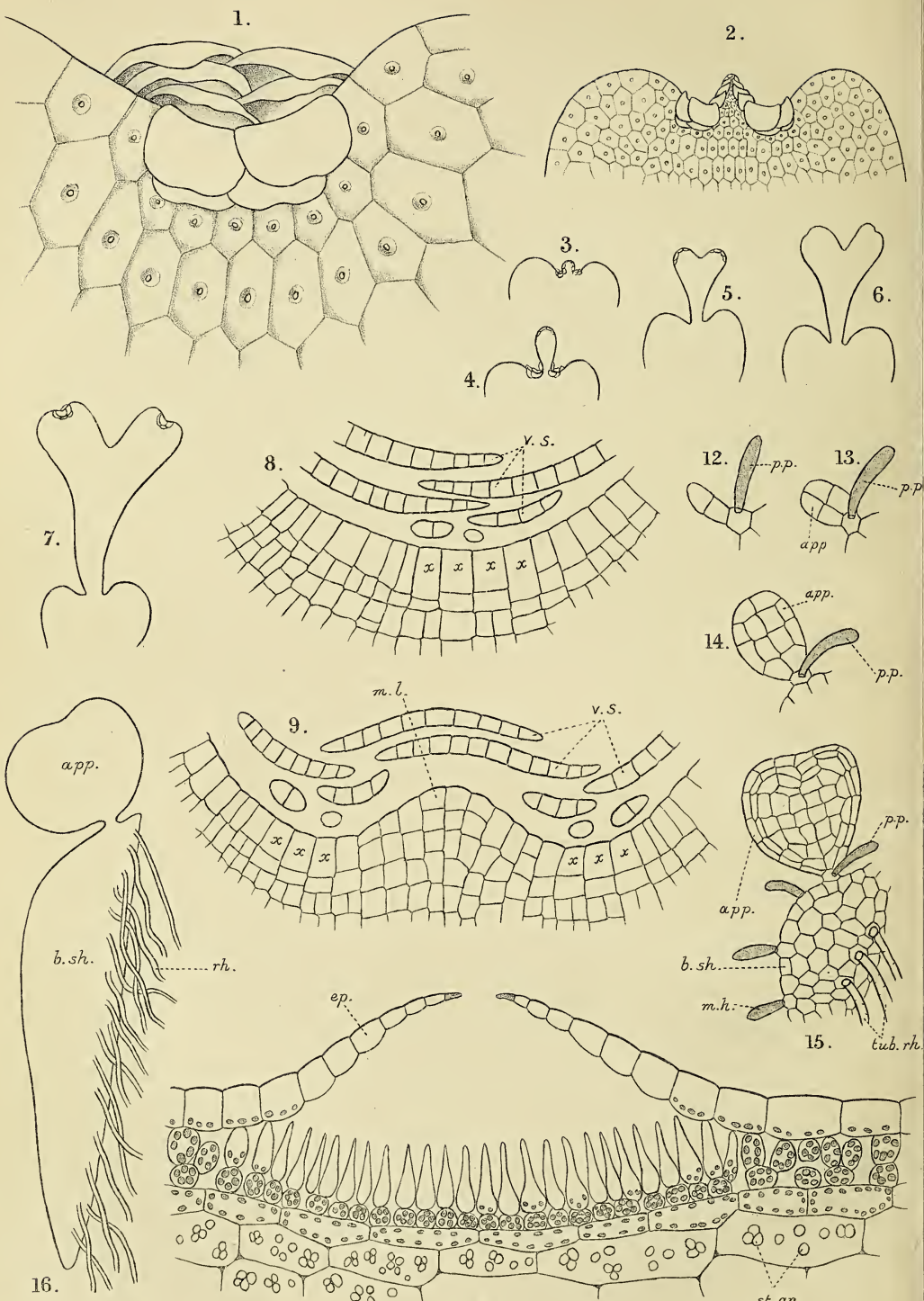
Fig. 50. Cells in upper half of capsule-wall, as seen from outer surface. × 200.

Fig. 51. Cells in lower half of capsule-wall, as seen from outer surface. × 200.

Fig. 52. Fully formed elaters: *a*, normal form; *b*, *c*, branched elaters. × 200.

Fig. 53. Median section of spore at time of dehiscence of capsule. × 200.

Fig. 54. Germination of spore (optical section): *rhiz.* rhizoid. × 200.



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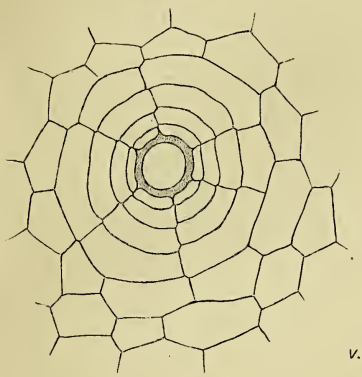

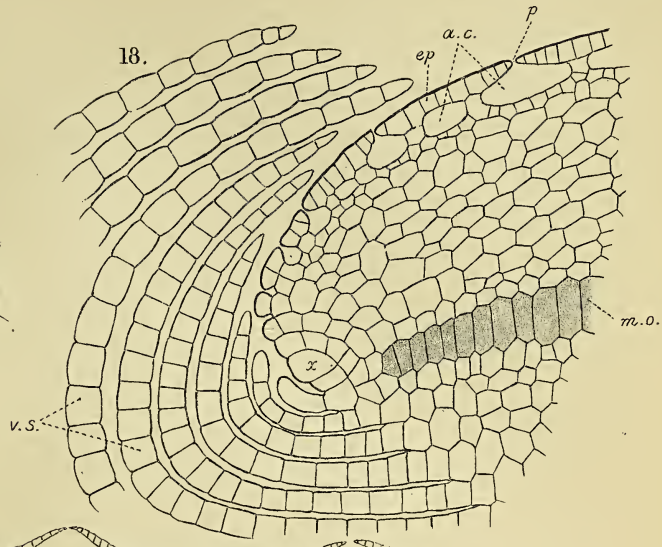

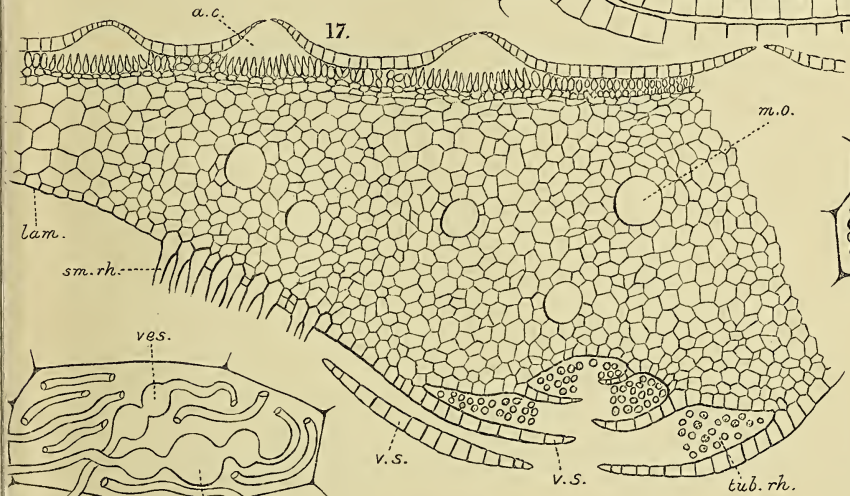

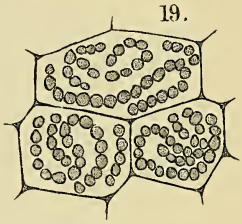

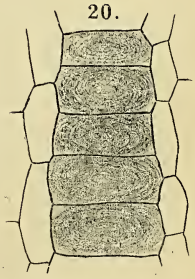

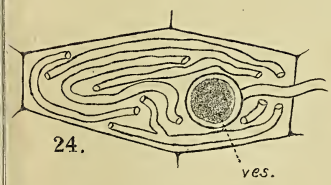

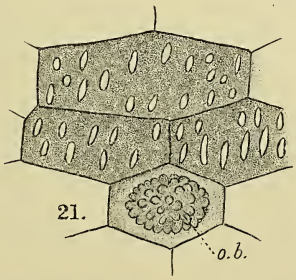

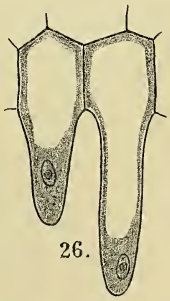

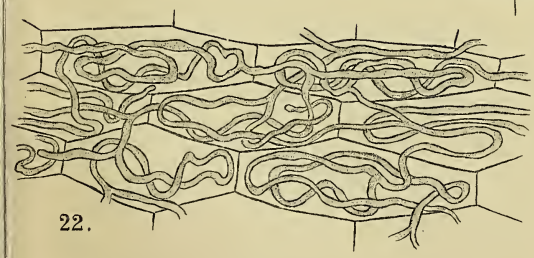

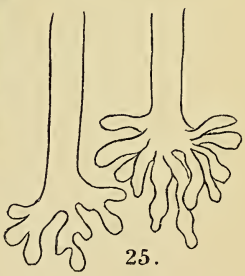

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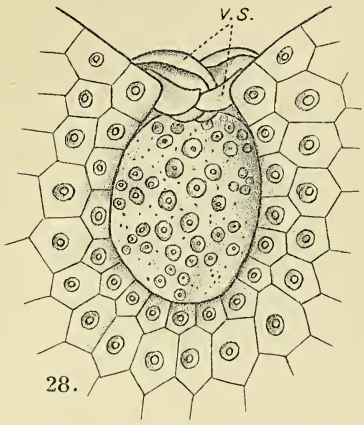
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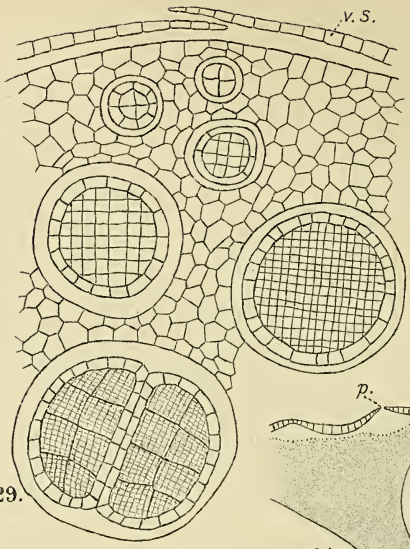
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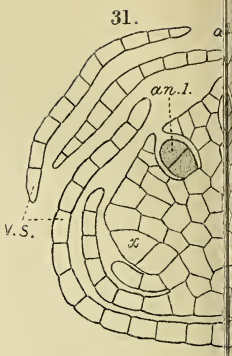
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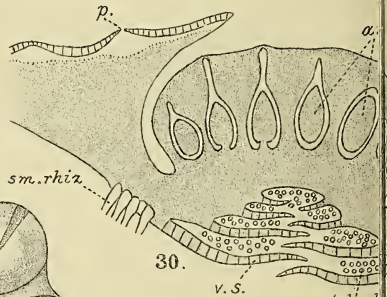
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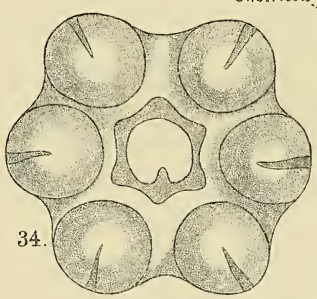
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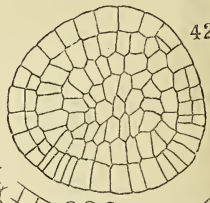
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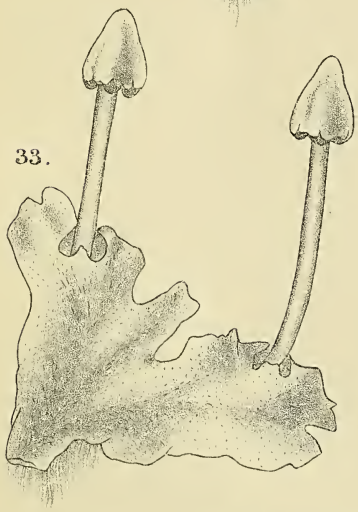
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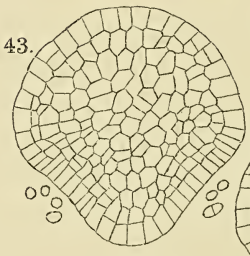
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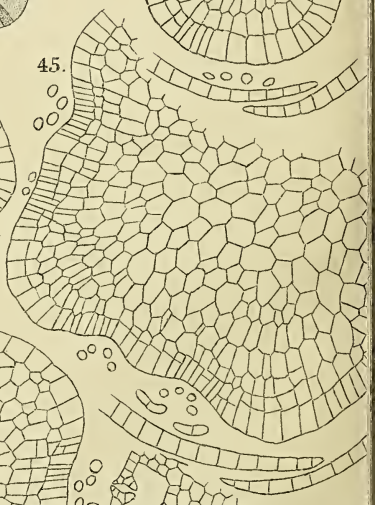
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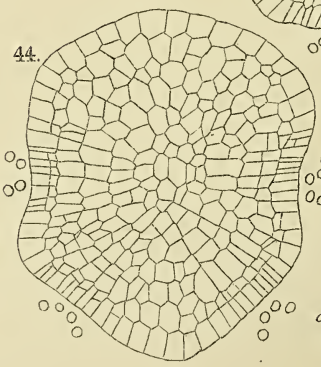
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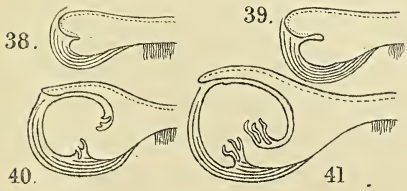
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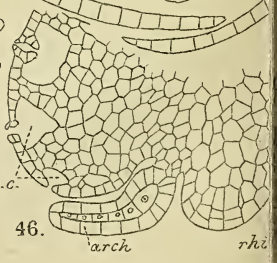
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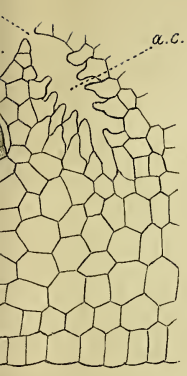

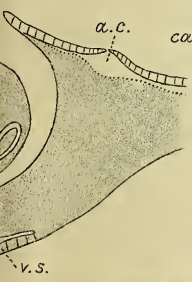

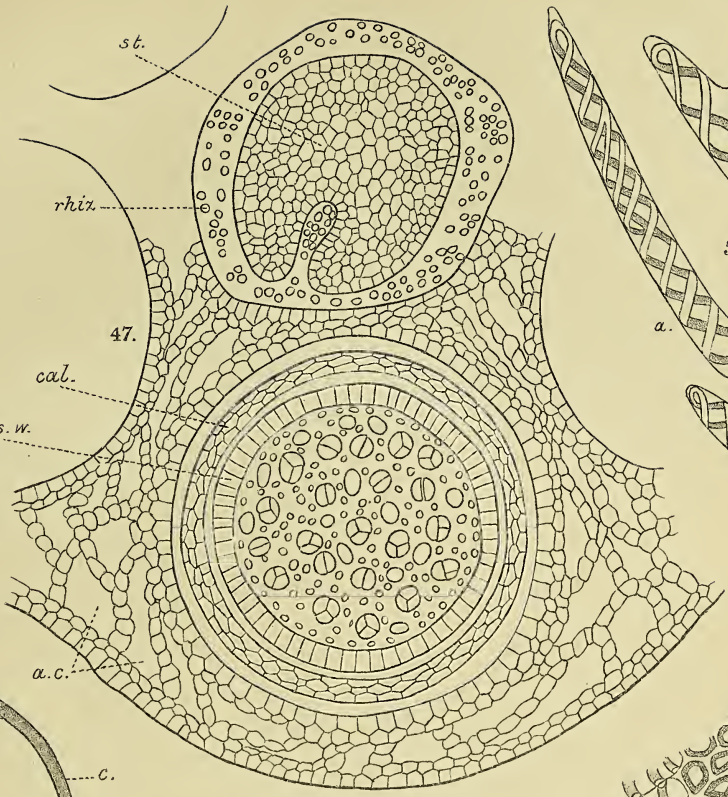

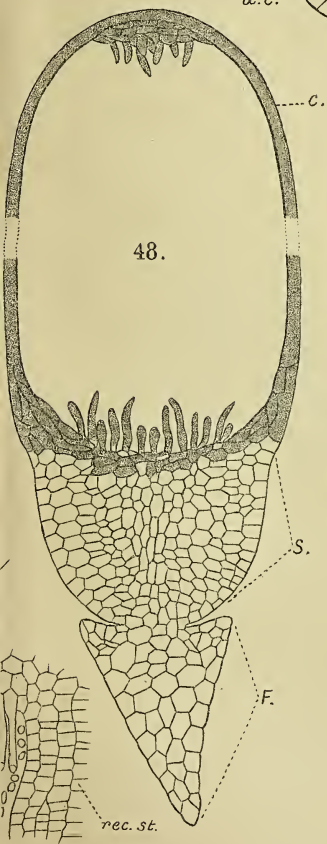

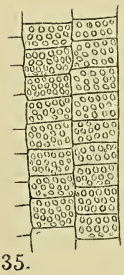

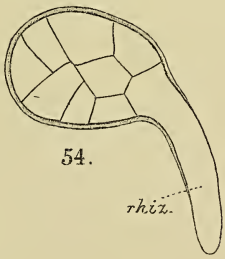

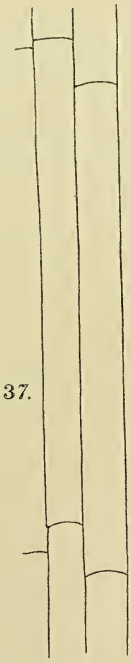

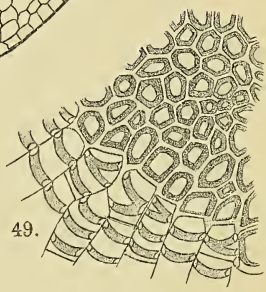

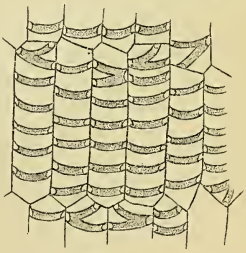

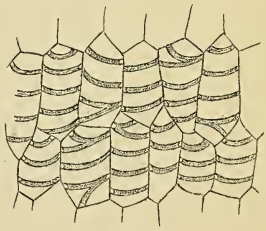

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F. C. del.

University Press, Oxford.

On the Occurrence of Cellulose in the Xylem of Woody Stems.

BY

M. C. POTTER, M.A.,

Professor of Botany in the University of Durham College of Science, Newcastle-upon-Tyne.

With Plate VIII.

THE appearance of cellulose in the xylem of various trees when attacked by certain wood-destroying Fungi has been noted, and has been attributed to the action of a delignifying enzyme secreted by the Fungi. Hartig ('78), in his classic work upon the destruction of the wood of Conifers and of the Oak by certain Fungi, has given an account of the appearance of cellulose in the wood when under the influence of such attack. In the Oak, he describes in detail the special action upon the wood of *Telephora perdix*, *Polyporus ignarius*, *P. dryadeus*, and *Stereum hirsutum*, and states in each case that in the process of decay the lignified walls become converted into cellulose, attributing this change to the delignification by the hyphae. In the wood of the Conifers the same appearance is noted and ascribed to the influence of *Trametes* and other Fungi.

Mayr ('84) attributes the presence of cellulose in the stem of *Betula* to the action of the parasitic Fungi *Polyporus betulinus* and *P. laevigatus*.

Marshall Ward ('97), studying the action of *Stereum hirsutum* upon the wood of *Aesculus*, has demonstrated the presence of cellulose in the xylem when infected by a pure culture of this Fungus. The swollen inner layers of the cell-wall become delignified and consist entirely or almost entirely of cellulose, as shown by the differentiating colour-tests of chlor-zinc-iodine, and phloroglucin. The hyphae attack the walls of the tracheides and other wood-elements, and, he considers, gradually delignify them from the lumen outwards. Marshall Ward assumes the presence of an enzyme which effects the delignification, but 'did not succeed in extracting the enzyme from his cultures.'

Biffen ('01) has published some investigations upon the biology of *Bulgaria polymorpha* and the effects of its action on wood. Sections from a block of Oak thoroughly permeated by hyphae showed a swelling of the

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thickening layers of the woody elements, which, on treatment with colour stains, gave indications of the presence of cellulose, suggesting that the action of the Fungus was one of delignification. Biffen also observed a sharply defined zone of cellulose surrounding many of the pits, especially in the vessels. He considers that the action of *Bulgaria polymorpha* upon Oak-wood causes the dissolution and probably decomposition of the lignin, and has assumed the existence of a delignifying enzyme to explain these results.

Czapek ('99) claims to have isolated a delignifying enzyme from *Merulius lacrymans* and *Pleurotus pulmonarius*. From the fungus-extract, by precipitation with alcohol, he obtained a white precipitate, soluble in water, which destroyed the lignin of the woody cell-walls. This fungus-extract, he states, lost its wood-destroying properties when once boiled.

Hermann von Schrenk ('00) has described a disease of the Red Cedar caused by *Polyporus Juniperinus*, which attacks the walls of the wood-fibres, and extracting the lignin substances leaves a basis of almost pure cellulose. The amount of wood-fibre reduced to cellulose is very considerable, and it is supposed that some very potent lignin-splitting enzyme is concerned in the changes which are brought about. Schrenk ('03) also described a delignification in the wood of *Fraxinus* due to *Polyporus fraxinophilus*, which is said to give off an enzyme which attacks the inner parts of the wood-cells, extracting the lignin, and leaving an impure cellulose.

Some evidence may be gathered with reference to cellulose occurring normally in the xylem.

Writing in 1860 Sanio ('60) states that the tertiary thickening gelatinous layer of lignified cells was first pointed out by T. Hartig and confirmed by von Mohl, and finally claimed by Schacht as an element present in all woody cells, which never becomes lignified but consists always of pure cellulose. Sanio regards this latter statement as too sweeping, and maintains that the share which this tertiary layer takes in the thickening of the woody cells varies greatly, sometimes being very strongly pronounced, at other times hardly perceptible. He mentions *Acer platanoides* as a stem in which this layer, which is coloured violet with chlor-zinc-iodine, is most distinctly to be observed; and adds a list of seventeen species in which this cellulose thickening is found in the wood-fibres.

In his general considerations on the structure of the Oak (p. 94), Hartig, speaking of the wood-fibres, says that in the walls of these fibres two, often three, layers can be distinguished, differing in their chemical behaviour. The most external, always a very thin layer, which is united with the corresponding layer of adjacent organs, encloses a very thick inner layer. This is lignified, and only in isolated cases is bounded internally by a third layer, which colours blue with chlor-zinc-iodine. Hartig himself makes only slight further allusion to this occurrence, as when discussing *Hydnum*

diversidens, he mentions the most internal, somewhat gelatinous layer of many healthy Oak wood-fibres, which colours blue with chlor-zinc-iodine. He evidently regarded the character as so limited as to be negligible, and the fact as noted by him appears to have escaped attention.

Strasburger ('91) in the 'Leitungsbahnen' mentions incidentally that the inner thickened layer of the wood-fibres of *Robinia pseudacacia* colours violet by potassium iodide; and also that in *Wistaria* the wood-fibres show an inner thickened layer, sometimes almost completely closing the lumen, which on treatment with potassium iodide and with chlor-zinc-iodine gave a beautiful wine-red colour.

In the case of *Pinus sylvestris* it has been shown by Schellenberg ('96) that the primary medullary rays remain unlignified until the third year[1]. He has also observed that among the wood-fibres generally the middle lamella is very often more lignified than the other layers, but that completely unlignified inner lamellae are only seldom met with, as in *Triosetum perfoliatum.*

With these exceptions I can find no reference[2] to the presence of cellulose in mature wood, except as the result of the destructive action of certain Fungi. Abromeit ('84) mentions that after the use of chlor-zinc-iodine he has been unable to detect the tertiary gelatinous layer in the wood-fibres of the Oak, and, so far as I have been able to trace, it has come to be very generally accepted that the elements of the wood become entirely lignified, using the term in its simplest acceptation.

After an extended examination of a very large series of specimens I have detected the presence of cellulose in the xylem of many trees as a normal condition in perfectly healthy and vigorously growing stems. It occurs chiefly in the wood-fibres, and proves to be by no means an exceptional occurrence.

My attention was first drawn to this question when studying the action of a Bacterium (*Pseudomonas destructans*) ('99) upon the cell-walls of storage tissues, such as in Turnip, Carrot, &c., and following up some investigation into the destructive action of this parasite upon the xylem. Small pieces of the xylem of *Quercus* and *Fagus* were sterilized by steaming in test-tubes in which they were half immersed in water, and afterwards were sown with a pure culture of this Bacterium. After an interval of fourteen days transverse sections showed a very marked presence of cellulose, as indicated by chlor-zinc-iodine and haematoxylin. As this seemed to suggest an action by the Bacterium corresponding to that of the Fungi above mentioned, it became necessary to determine whether this cellulose was present before inoculation.

[1] I have observed cells in the medullary rays remaining unlignified for a much longer period.

[2] De Bary (Comp. Anat., Engl. ed., p. 482), quoting Sanio, mentions the occurrence of this gelatinous layer, which remains unlignified.

On examination of pieces of wood from the same branch, before sterilization, I was surprised to find that in many sections a thick layer was present in several of the fibres which gave the violet colour characteristic of cellulose, when treated with haematoxylin; and similar sections showed a layer in the fibres which refused to react to phloroglucin. Treatment with iodine and sulphuric acid also gave the characteristic blue. This unlooked-for result in apparently sound wood led me to examine a number of shoots of Oak of various ages, carefully selected for their vigorous growth and entire freedom from disease.

For the purpose of this investigation it was specially important that fresh material only should be used. Pieces of felled wood left in the open, even for a short time, would be liable to the objection that any cellulose present in the xylem might be due to the process of decay. For this reason freshly cut branches were always used and the sections made generally, on the day following that on which the material was obtained, when it was still fresh and before any change could have taken place. Shoots of Oak were selected from trees growing near sheltered river banks and from those fully exposed in the open country. At first transverse sections were cut by a microtome through stems of varying ages and about 1.3 cm. in diameter. The sections thus obtained were stained with phloroglucin and anilin sulphate, and it was found that in certain areas the internal layer of the walls of the fibres refused to act to these stains and remained as a white layer inside the otherwise coloured layer, but when treated with Schultze's solution, with iodine and sulphuric acid, or with haematoxylin, the characteristic blue colour was at once given by this layer. These tests are all confirmatory one of another, and leave no doubt that the substance of the inner layer was of the nature of cellulose. As haematoxylin alone sufficiently indicated these results permanent preparations were made by using Delafield's haematoxylin and mounting in Canada balsam.

In transverse section this cellulose layer appeared as a broad violet lining, gradually shading off towards the middle lamella, sometimes being partially detached and lying kinked in the lumen (see Pl. VIII, Fig. 2). Longitudinal sections confirmed the above observations, the cellulose lining being sometimes torn out by the razor and resembling an isolated prosenchymatous element. It was noticeable in longitudinal sections that around the bordered pits a layer of violet was often present when stained with haematoxylin or Schultze's solution, showing that the bordered pits were also surrounded by a ring of cellulose.

The fibres containing the cellulose lining occurred singly, in isolated groups, or in broad bands concentric with the annual rings and interrupted by the medullary rays; but they seldom passed round the whole of the stem. It was necessary to cut across the entire stem, for, although the

wood fibres in certain small sections might give no cellulose reaction, on taking complete transverse sections I never failed to detect this reaction in some area or another.

When stems of a larger diameter were employed it became necessary to divide them into smaller areas, and for this purpose they were cut into blocks about 1.3 cm. square. These blocks were numbered, and by this means a transverse section of a large stem was obtained. In one instance the base of a young branch approximately sixteen years old, measuring 5.4 by 3.8 cm., was divided by a fine saw into twelve blocks; sections were cut from each of the blocks, stained with Delafield's haematoxylin and mounted in Canada balsam. Although not one of these block-sections could be found which did not contain some fibres with a broad violet inner lining, in some they were quite isolated, while in others (more especially on the lower side) they presented a very conspicuous feature, so much so that their presence could be distinctly discerned by the unaided eye.

In another instance I specially felled a young Oak, approximately forty years old, the stem measuring 14 cm. by 12 cm. This was divided by a fine saw into eighty-eight blocks, all of which were numbered in order, and transverse sections were cut from each, stained in haematoxylin and mounted in Canada balsam. In this way a complete transverse section of this trunk was obtained. As in the case of the smaller branch some fibres with a violet lining could be detected in almost every one of the eighty-eight sections, but in certain portions of the stem they occurred in very conspicuous groups. These fibres were well marked in the blocks situated in the centre of the stem, that is around the pith, and containing the first few years of growth, which showed that the oldest wood was not completely lignified and contained a large proportion of cellulose. Its presence in the wood of the first years of growth was also noticed in many smaller stems. The cellulose was somewhat irregularly distributed, though it was evidently developed more markedly on one side of the tree than the other, and it is probable that the orientation has some influence in this direction (Fig. 1).

The trees above-mentioned were growing in the neighbourhood of Newcastle-upon-Tyne. It became an interesting question to determine whether climatic conditions had any influence upon the composition of the xylem, and specimens of Oak were obtained from other parts of England, i. e. from Warwickshire, Yorkshire, and Surrey. Transverse sections from Oaks growing in all these localities presented the same appearance, when stained with haematoxylin, as has been already described. Fig. 2 represents a section of an Oak from Wimbledon, taken from a branch eight years old, diameter 1.3 cm., showing strikingly the inner cellulose layer of the fibre-walls. The cellulose extended about one-third round the stem. From Warwickshire I was fortunate in obtaining,

ten days after being felled, the base of a well-grown sound Oak, approximately sixty years old, and in this specimen too a considerable quantity of cellulose was found in the first years of its growth, and also distributed throughout the duramen and alburnum.

Branches of various other trees were next examined. In the wood of *Fagus* the occurrence of cellulose was equally as strongly pronounced (Fig. 3) as in *Quercus*, if not more so, in trees grown both in the north and central parts of England. It occurs chiefly in the broader annual rings which exhibit special development during a season of vigorous growth. As in the case of *Quercus*, the cellulose may be found at the very centre of the stem, and branches of twenty-five and thirty years both showed this peculiarity in the wood of the first and second years of their growth.

In *Aesculus* the cellulose, as indicated by the reaction to haematoxylin, was not so immediately apparent, and in specimens I first examined it was found only in the very young wood, and then quite locally. A further study, however, of numerous transverse sections of different trees showed it to be fairly prevalent in the growth of other years. The softer wood of *Aesculus* is very different in character to that of the trees considered above, and the inner lining of the wood-fibres is represented by a much thinner layer than is the case in many of the harder woods. In certain parts of the sections a thin lining can be observed inside the fibre-walls, which is stained violet with haematoxylin, and this is the most characteristic appearance; but in other regions, quite locally, the violet-blue layer may assume a thicker, more gelatinous appearance, and lie quite detached and often crumpled in the lumen. This thicker layer sometimes occupies a large part of the fibre-lumen, and shades into a paler colour towards the middle lamella, being edged inside with a darker line. In one instance I found this disposition of the cellulose along a radius extending throughout the third to the ninth years, though most noticeably present in the third and sixth years, which showed a more vigorous development, while in other parts of the annual rings it was entirely absent. In another section it was well marked in the spring wood of the current year. The cellulose is more generally to be found in the spring than in the autumn wood.

In *Salix* the presence of the cellulose lining to the wood-fibres is often very pronounced. In one branch, with a diameter of 1.6 cm. and containing ten annual rings, cut from a willow growing at the edge of a large pond, hardly a single fibre could be detected which did not show a broad violet inner layer when treated with chlor-zinc-iodine. Sometimes this layer completely closed the lumen of the fibre. In another instance, in a branch with a diameter of 3.7 cm., with twelve annual rings, from a tree growing upon a hillside with northern aspect, in many of the fibres no violet colour due to chlor-zinc-iodine could be observed, and also where the edge of the lumen was coloured deep violet this colour gradually

shaded away toward the middle lamella. The difference in the manner in which the fibre-walls reacted to chlor-zinc-iodine was most marked in the two cases, and this may perhaps be attributed to the different situations.

In longitudinal sections these appearances are well-marked, the cellulose lining of the fibres being easily recognized. The cellulose ring round the pits on the walls of the vessels was exceedingly striking when stained in chlor-zinc-iodine. Fig. 4 is taken from the branch of the tree growing at the water-side. The iodine and sulphuric acid (76 per cent.) reaction agreed with the results obtained from chlor-zinc-iodine. In longitudinal sections, especially, it was most beautiful to see the edges of the pits turn blue in turn as the acid gradually swept over them. Further confirmation was obtained by treatment with Congo-red and also with haematoxylin.

In the wood of *Ulmus* each annual ring commences with large wood-vessels succeeded by wood-parenchyma and fibres. When stained with haematoxylin and mounted in Canada balsam the wood-fibres are especially conspicuous by their thick yellow walls, inside which, in many instances, a distinct blue-violet lining of cellulose may be detected.

An *Alnus* stem obtained from the banks of a stream in Warwickshire, about ten years old, and having a diameter of 2 cm., showed the presence of cellulose very distinctly round one half of the stem, while in the other half it was entirely absent. The development occurred chiefly in the third, fourth, fifth, and sixth years, being especially prevalent in the very wide fourth year, as indicated by the inner thickened layers staining clearly blue-violet with haematoxylin.

A branch of *Betula* six years old, with a diameter of 1.4 cm., was also examined. This, upon treatment with haematoxylin, indicated a special prevalence of the swollen cellulose layers in the fourth and fifth years.

In *Fraxinus*, unlike the other woods examined, no well-marked patches of wood-fibres, in which a broad lining of cellulose is present, could be seen. The specimen from which my sections were taken was a vigorous and healthy sapling, with a diameter of 2.7 cm. and twelve annual rings. The stem had therefore grown quickly. It was obtained in July, and the next day sections were cut, stained with Delafield's haematoxylin, and mounted in Canada balsam. In none of the sections, however, could any characteristic development of the thickening gelatinous layer be found; at most on the internal surface of some of the fibres there might be detected a very thin line of blue, and this was found to be true of all the trees afterwards examined.

It had now become perfectly plain that a thickening layer of cellulose occurred quite commonly as a natural feature in the woody fibres of

a variety of trees in all situations, probably representing a stage of arrested development. As this fact has not hitherto been recognized the observation is important, and has some bearing upon the study of fungoid action in timber.

It is well known that boiling water has a destroying and dissolving action upon the cell-wall. As long ago as 1882 Singer ('82) has shown that four substances can be extracted from xylem by boiling water, namely, vanilin; a substance which shows the reactions of coniferin; a gum, which is soluble in water; and a substance soluble in water not identical with the other three; these all entering into the composition of what is known as lignin, though in what relationship is not determined.

Recently, Van Wisselingh ('98) has shown that by heating sections of the root of *Beta vulgaris* for six hours in distilled water, at a temperature of about 125° C., a pure cellulose wall remains behind, and the pectin substances are destroyed and removed. It seems probable that the operation of sterilizing by discontinuous boiling would have some such effect upon the lignified walls, and that some substances might be destroyed or removed from the cellulose matrix during the process; in other words it would have a delignifying action.

The wood of *Fraxinus* affords very suitable material for studying the action of boiling water upon the xylem; since, as far as I have been able to determine from the examination of numerous sections taken from various trees, no broad inner lining of cellulose is present in the fibres. The xylem appears to undergo complete lignification. Transverse sections were taken from a stem ten years old and about 2·4 cm. in diameter, and some of these were examined at once with chlor-zinc-iodine. Fig. 5 is a good illustration of the appearance presented, the walls are thick and hard and show no trace of violet colour, the only effect of the chlor-zinc-iodine being to turn the walls yellow. The other sections were placed in a boiling tube half filled with water and steamed for about two hours on consecutive days, remaining immersed in water the whole time. Some of these sections were removed at intervals and subjected to treatment with chlor-zinc-iodine. After two days some delignification was slightly indicated; after three days it was well pronounced. Fig. 6 represents the general appearance after four days: the middle lamella is a bright yellow, while the inner layers of the fibre-walls are swollen and stained violet, more deeply on the inner surface and shading away towards the middle lamella, showing almost complete delignification. A comparison of the sections before and after boiling represented in Fig. 5 and Fig. 6 shows in a most marked manner the delignifying action of the boiling water. It should be noted that in the sections steamed four times a delignification had already commenced in some of the wood-vessels.

The xylem of *Fraxinus* is somewhat resistent to the action of boiling water, the same effect being produced in other woods after a less severe treatment; but it has been selected for illustration because of the certainty of obtaining sections not exhibiting incomplete lignification in any part before boiling.

In the case of *Aesculus* the delignifying action of boiling water is well marked. A portion of a healthy stem of *Aesculus* was procured, with a diameter of 2·2 cm. and ten annual rings. Sections containing the whole surface were cut by means of a microtome and placed in a boiling tube half-filled with distilled water and steamed for two hours on three consecutive days. The sections were then treated with chlor-zinc-iodine and phloroglucin, and permanent preparations made by staining with Delafield's haematoxylin and Congo-red (Fig. 7) and mounting in Canada balsam. (Similar preparations to serve as controls were made before steaming.) When stained with chlor-zinc-iodine the appearance was most striking and in strong contrast to the unboiled sections. Instead of only in certain areas, the fibres and wood-cells in nearly every part contained a swollen blue-violet-coloured layer, which was frequently broken away and lying in contorted shapes in the lumen (see Fig. 7), while the middle lamella remained yellow; the walls of the vessels were yellow, swollen, and striated, but lined with a faint blue tinge. The action of phloroglucin fully confirmed that of chlor-zinc-iodine. The middle lamella of the fibres showed the characteristic red, while the inner layers were quite white and colourless; the walls of the vessels also stained red, but they were swollen, and a white unstained lining might in some cases be detected. The annual rings stood out distinctly red with phloroglucin, but when stained with chlor-zinc-iodine the elements composing these rings showed the inner layers swollen, striated, and yellow, enclosing a blue lining. Prolonged steaming further emphasized these results.

Sections subjected to discontinuous steaming for six days when stained with chlor-zinc-iodine showed the inner layer of the fibres a deep blue-violet, much swollen and in many cases obliterating the lumen; the walls of the vessels and tracheides were much swollen and showed distinctly violet; the elements forming the boundary of the annual rings too had a well-marked violet lining. With phloroglucin the walls of the fibres remained white, and in some cases even the middle lamella refused to stain; in the vessels too there was a distinct inner white layer, the medullary rays staining red.

Among the many stains for lignin, Hegler ('00) has shown that thallin sulphate stains the vanilin yellow and phenol the coniferin a blue-green. The action of these stains on steamed wood is of importance, and gives confirmation of the results already obtained with phloroglucin. Steamed transverse sections from the same piece of *Aesculus* treated with thallin

sulphate showed the middle lamella of the wood-fibres a distinct yellow, but enclosing an inner layer remaining white and unstained. The walls of the wood-vessels and tracheides stained yellow. In the longitudinal sections the same peculiarities of staining were noted, but in the wood-vessels the borders of the pits remained unstained. Similar sections when stained with phenol showed the middle lamella of the wood-fibres green, but the inner layers of the walls white and unstained; the wood-vessels and tracheides stained green. Again, in longitudinal section the borders of the pits were unstained. Phloroglucin stained precisely the same structures, and again the borders of the pits remained unstained.

Similar investigations with *Quercus*, *Alnus*, and *Ulmus* gave the same results. Fig. 8, showing a transverse section of *Quercus* after steaming, depicts a stage in which the progressive action of the delignification may be observed. The violet colour given by chlor-zinc-iodine is seen staining deeply around the lumen, and suffusing itself gradually outwards into the lignified layers.

The above experiments show that boiling water extracts some substance of the nature of lignin from the wood of all these trees and that sterilization by steaming would have a similar effect. To determine this point further some sawdust was obtained from a piece of *Aesculus* (diameter 3·5 cm., annual rings 19), covered with distilled water, and steamed for half an hour on one afternoon and again for an equal time next morning. The sawdust was then removed by filtration through an ordinary filter paper. A small portion of the watery extract thus obtained reacted very feebly to phloroglucin, but the colour was sufficient to indicate that some substance was present which gave the lignin reactions. The remainder of the filtrate was next extracted with ether, the water drawn off with a separating funnel, and the ether evaporated in a small white crucible on a water bath at 60° C. A brown deposit remained as a thin layer in the crucible after the ether had disappeared. This brown layer gave at once the characteristic red colour when a drop of phloroglucin was added.

In order to avoid any risk of minute fragments of wood passing through the filter paper and hence into the ether, or by any chance some substance being extracted from the filter paper which might react to phloroglucin, a similar watery extract of *Aesculus* sawdust was passed through a Kitasato tube, extracted with ether, and the ether evaporated. The result on addition of a drop of phloroglucin to the residue was the same, the red colour was instantly given. It only remains to say that the ether and distilled water alone, after the same treatment, gave no brown deposit, and not the slightest reaction to phloroglucin.

This shows conclusively that a substance which reacts to phloroglucin is extracted from *Aesculus* wood by boiling water, and confirms in a remarkable manner the reactions to the lignin and cellulose tests of the

boiled sections of this wood. In fact, the process of sterilization by steaming delignifies the xylem and produces appearances exactly similar to those described after the action of Fungi.

As a confirmation of these results with *Aesculus* it seemed worth while to try other woods, and pieces of Elm, Ash, Oak, and Scotch Pine were tested. From each of these, after removal of the bark, sawdust was obtained and placed in small flasks containing water. These were then steamed on three days for two hours, the decoctions filtered, extracted with ether and the ether evaporated. In all these cases a brown deposit was left after the evaporation of the ether which at once gave the characteristic red colour with phloroglucin.

The above experiments having so clearly shown the effect of boiling water in extracting from xylem a substance which reacts to phloroglucin and thallin sulphate, it seemed probable that cold water would have the same power if allowed to act for a longer period. From the stem of the forty years' Oak already mentioned small chips of alburnum and duramen were carefully made and five gram. of each placed in flasks containing 150 c.c. of distilled water and 5 c.c. of chloroform. The flasks so prepared will be referred to as the 'extract' of alburnum and duramen respectively. In all eight of these flasks were prepared, four containing alburnum and four duramen, which were then placed in an incubator at 28° C. After three days one flask of alburnum-extract was taken, the water filtered and extracted with ether, and the ether then divided into three portions in three porcelain crucibles; it was then evaporated at 60° C. A thin brown layer was deposited inside these crucibles which reacted at once, in one case to phloroglucin and in another to thallin sulphate (both of these being tests for vanilin), while the third gave no result with phenol, the test for coniferin. A flask of duramen-extract was similarly treated and gave precisely the same reactions.

These results show that a substance answering to the vanilin tests is dissolved out in cold water. Thus cold water has also an extractive power, and by continued soaking in water the xylem undergoes a partial delignification.

The effects of boiling and immersion in water here described were under the exaggerated conditions produced by the use of thin sections and fragments of wood, yet it must be allowed that some delignifying action would take place upon even small blocks of wood subjected to steaming in water for the purpose of sterilization and remaining under damp conditions for many months during cultural experiment.

It will be noted that the coniferin, or rather a substance answering to the test for coniferin, was apparently not extracted from the xylem. This result appears curious and somewhat at variance with the fact that the steamed sections, when treated with phenol-HCl, always gave an un-

coloured inner layer, which seemed to indicate that the 'coniferin' had been removed. Some explanation, however, was gained by a comparison with fresh sections treated in the same manner. These showed a very limited distribution of the 'coniferin,' it being found chiefly in the vessels, tracheides, and elements surrounding them, and appearing to be absent in a great measure from the fibre-walls. This suggests an interesting point touching upon the distribution of the vanilin and coniferin in woody stems, which remains to be followed up; and it appears to furnish a clue to a problem which presents itself. Would not the water passing up the stem as the transpiring current have a tendency to dissolve the 'lignin'? The walls of the vessels and other elements connected with the transmission of water are apparently impregnated with some substance not readily soluble in water.

To return to the alburnum and duramen 'extracts.' Of the eight flasks six remained in the incubator. After eight days another pair of these flasks was similarly treated and the ether extracted, divided into three porcelain crucibles, and evaporated as before. To my surprise the residue, after evaporation of the ether, gave only a faint reaction to phloroglucin and thallin sulphate in the case of the duramen, and the reaction with these stains was even less for the alburnum. This result at first seemed inexplicable, but I noticed that the extract in the flasks, especially that of the alburnum had become somewhat turbid. This seemed to indicate the presence of Bacteria, although it should be remarked that some of the chloroform still remained at the bottom of the flasks so that the water in which the Oak chips were immersed would be saturated with chloroform. However, as I have shown in a previous paper ('00), no reliance can be placed upon chloroform, thymol, &c. as antiseptics, as these substances do not necessarily prevent the growth of micro-organisms even when used in considerable strength.

To ascertain whether any micro-organisms were present stab-cultures from these flasks were made in sterile, plugged test-tubes containing beef-, Liebig-, and turnip-gelatine. These test-tubes were then incubated for five days, at the end of which time colonies of Bacteria were found in all the tubes. It may be mentioned here that stab-cultures subsequently taken from the remaining two pairs of flasks, after fourteen days and after thirty-two days, also developed colonies of Bacteria. The extracts from the flasks after these intervals gave much the same colour reactions as before, distinct though faint for the duramen and hardly perceptible in the case of the alburnum.

No attempt has been made at present to isolate and identify these Bacteria, but it may be remarked that the Bacteria from the alburnum invariably liquefied the gelatine, while in the tubes sown from the duramen the gelatine remained unliquefied.

The inoculation of the gelatine tubes from the cold-water extract of the Oak duramen and alburnum and the subsequent development of colonies clearly showed that Bacteria were present in these solutions, but further proof was needed to show whether or no these Bacteria could live upon and destroy the substance extracted from the wood by water. To determine this point strong decoctions were made from splinters of the duramen and alburnum of the same forty years' old stem. The splinters were boiled in water in two flasks, and after standing all night in the steamer were again boiled on the following morning. The decoctions thus obtained were freed by filtration from any particles of wood and drawn into smaller flasks, each containing 150 c.c. of decoction. In this manner eight flasks were prepared, four containing each 150 c.c. of alburnum decoction and four containing each 150 c.c. of duramen decoction. These will be referred to as alburnum- and duramen-'decoctions.' These eight flasks were then plugged with cotton wool and sterilized by discontinuous steaming. Of the eight flasks one of each kind was used for a control.

Of the other three flasks of alburnum-decoction one was sown with *Penicillium*, another with *Bacillus subtilis*—a pure culture obtained from Dr. Kral—and the third from the alburnum-extract in which the Bacteria had developed. Similarly one flask of duramen-decoction was sown with *Penicillium*, a second with *B. subtilis*, and the third from the duramen-extract in which Bacteria had developed. These flasks together with the controls were incubated at 28° C.

Flasks sown with B. subtilis. After twenty days stab-cultures were made on gelatine-tubes of Koch's beef-bouillon and Liebig-extract, and the flasks were then extracted with ether and tested as before. With phloroglucin the duramen-residue gave a most distinct red, but the colour was much fainter in the alburnum-residue. With thallin sulphate the colour was indicated in both cases but more faintly in the alburnum-residue, and with phenol-HCl no reaction could be detected in either case. In the stab-cultures, colonies developed from the alburnum-decoction, but not from the duramen. This experiment seems to indicate that *B. subtilis* can grow in the alburnum-decoction and destroy the substance extracted from the wood, while it is incapable of living in that obtained from the duramen.

Flasks sown from alburnum- and duramen-extracts. After twenty-one days stab-cultures were made from both flasks in tubes of beef-bouillon and Liebig-extract. The stabs from the alburnum-decoction developed colonies which quickly liquefied the gelatine, but the colonies from the duramen-decoction developed much more slowly and without liquefying the gelatine. The ether-extracts from the alburnum-decoction showed no colour with either phloroglucin or thallin sulphate, but in the case of the

duramen-decoction both phloroglucin and thallin sulphate gave the characteristic colours, but somewhat faintly. Phenol-HCl gave no colour-reaction in either case.

Flasks sown with Penicillium. After twenty-three days several colonies had developed in the alburnum-decoction and produced conidia, but in the duramen-decoction no signs of colonies could be detected. With phloroglucin and thallin sulphate the ether-extracts gave very distinct reactions in the case of the duramen-decoction, but with the alburnum the colour was very much fainter. Phenol-HCl gave no colour-reaction in either case.

The control flasks were next examined (after twenty-five days). The ether-extracts from both the alburnum- and duramen-decoctions gave the characteristic colours most distinctly with both phloroglucin and thallin sulphate, but not with phenol-HCl, again showing that the vanilin is extracted but not the coniferin.

This experiment was repeated with decoctions of the alburnum and duramen from the sixty years-old Warwickshire Oak, using 2 gram. of wood to each 100 c.c. of water. The results were in all respects confirmatory of those just described.

These experiments show very clearly that the substance extracted from the Oak-wood by water, which reacts to phloroglucin and thallin sulphate, is destroyed by certain Bacteria and in some measure by *Penicillium*. Both *B. subtilis* and the Bacteria which developed naturally in the extract, grew vigorously in the alburnum-decoction and destroyed the substance extracted from the wood. Bacteria being thus able to destroy the 'lignin' substances, this fact explains why the colour-reaction to phloroglucin was no longer given in the cold-water extract after eight days (p. 132).

It is further demonstrated that the alburnum is more readily acted upon by these organisms than the duramen. This is an important consideration, and suggests that the duramen contains substances not possessed by the alburnum which are unfavourable to the growth of Fungi or Bacteria.

These investigations throw some light upon the natural decay of timber, and suggest that one of the initial stages of decay is the extraction by water of some substance or substances from the xylem; by this process the cellulose is exposed, and it is then liable to be attacked by vegetable saprophytes. The fact that these organisms do not grow so readily in the watery-extract from heart-wood is suggested as a reason why the heart-wood is more durable.

In criticizing Singer's work Czapek considers his conclusions to be erroneous. He, however, makes no mention of repeating his experiments; while my results support Singer and prove that he was right in attributing

to boiling water the power of extracting certain substances from the xylem which give the so-called lignin reactions, and the extractive power would be greatly increased under Singer's conditions owing to the very long process of boiling to which his material was subjected. I have shown further that this delignification of the xylem may be accomplished by cold water. The relationship of the substance extracted by water to the hadromal which has been extracted by Czapek by means of a boiling solution of zinc chloride needs to be determined.

Czapek ('99) has isolated a delignifying enzyme, but the details given of his experiment are not very complete. He employed the mycelium of *Pleurotus pulmonarius* and *Merulius lacrymans*, obtained from wood decaying under the influence of these Fungi, presumably not pure cultures. From these a watery extract was obtained and to the filtrate a small quantity of wood-filings added, together with chloroform, and incubated at 28°C. The alcoholic-extract, tested with phloroglucin, after three days showed no reaction; after eight days a positive but weak reaction; and after fourteen days the reaction was tolerably strong, while the wood was coloured strongly violet with chlor-zinc-iodine.

In considering these experiments it would be useful to know from what tree the wood-filings were made, and again whether the wood-filings were examined previous to being subjected to the Fungus-extract, in order to determine whether any cellulose was present. The subsequent blue colour given by chlor-zinc-iodine cannot be accepted as evidence that the wood-filings had been delignified by the action of the Fungus-extract. Water alone extracts substances from wood which react to phloroglucin, and chloroform is not efficient in preventing the growth of Bacteria which would inevitably complicate the experiment. Positive evidence in favour of the hadromase is given by the fact that the Fungus-extract lost its wood-destroying properties when boiled, and that a white precipitate was thrown down by alcohol which had the same destructive action upon lignified cell-walls. It does not, however, afford absolute proof that the hadromase is a product solely of the Fungus, as, unless pure cultures were used under the strictest precautions to exclude Bacteria, the problem is complicated by the probable development of these organisms, which I have shown also possess the power of destroying lignin compounds.

The important papers by Marshall Ward upon the biology of *Stereum hirsutum*, and by Biffen upon the biology of *Bulgaria polymorpha*, which treat of the action of these Fungi upon the xylem, afford strong evidence in favour of a delignifying enzyme, these authors describing a gradually progressive delignifying action of the Fungus.

In the examination of his cultures of *Stereum hirsutum* upon *Aesculus* wood, with the reagents for differentiating lignified membranes from those

devoid of lignin, Marshall Ward finds 'during the first month no distinct reactions . . . and stains, such as Delafield's haematoxylin, do not colour the walls blue or purple, but merely brown or yellowish; but in some cases a thin lining layer is found to react in wood acted on by the Fungus for six weeks to a couple of months, and the altered layer gets more and more decided as the action progresses.' The unequal distribution of cellulose make it possible that its presence might have been overlooked in the first blocks examined, and no mention of any examination of the blocks prior to sterilization is made. I have not had the opportunity of studying a quite freshly cut stem of *Aesculus* of more than about three inches in diameter, but, judging from numerous sections of small branches and some much larger pieces of Oak, the distribution of cellulose is always somewhat irregular, and hence certain of the blocks cut from a good-sized stem might be entirely lignified, while in others lignification would be by no means complete. In *Aesculus* this partial lignification occurs more especially in the spring wood, and this may explain why it was found that 'in some transverse sections the spring wood is invaded much more rapidly than the autumn wood of the same annual ring' when attacked by *Stereum hirsutum*. Marshall Ward's figure 17 corresponds exactly with many sections I have seen of normal wood not attacked by any Fungus.

In his description of tangential longitudinal sections of cultures a month old which had been treated with gentian-violet and Congo-red, Biffen noted that it was easy to find, especially in the vessels, walls in which every pit was marked out by a well-defined, bright pink zone surrounding it, indicating that that portion of the wall had been delignified and a cellulose basis staining with Congo-red remained. It is significant that he observed, moreover, 'that no hyphae passed through the majority of these pits, so that one has to assume the secretion of a delignifying enzyme in quantity by the fungus into the wood-elements.' This appears to be an unnecessary assumption which cannot be allowed in face of the fact that this appearance is observed very commonly without any Fungus being present. That the margins of the bordered pits often remain unlignified, especially where the vessel crosses the medullary rays, has been demonstrated in the instances above quoted; and that it is no mere optical illusion is shown from the fact of the borders of the pits staining distinctly blue upon treatment with iodine followed by sulphuric acid, as in *Salix* (compare Fig. 4). Among herbaceous stems also I have observed this ring of cellulose very beautifully shown by chlor-zinc-iodine in the large wood-vessels of *Cucurbita*.

It has become clear that the presence of cellulose in the wood-fibres cannot be attributed entirely to the action of a delignifying enzyme, and it is now necessary that the enzyme in each case should be isolated.

Granted that the Fungus finds in the cellulose a necessary food-element and given the existence of layers of this substance in the wood-fibres, it follows that the hyphae would proceed in the direction of the source of supply, and the delignifying enzyme only comes into play where this supply is no longer available. The large amount of cellulose which is now shown to occur in the wood, at all stages, having been previously unrecognized, the conclusion becomes inevitable that much of the effect ascribed to the action of Fungus-hyphae is referable to conditions already present, and the action upon the xylem due to any process of sterilization must be taken into account when estimating the effect of the penetration of hyphae into the wood in culture experiments.

Without denying the existence of a delignifying enzyme, it is probable that the Fungus first attacks the elements where cellulose is already present, which would account for the direction in which the destruction of the wood sometimes advances. Thus in the formation of partridge-wood by *Telephora perdix* the Fungus attacks certain areas which eventually become hollow. Possibly these areas are those in which cellulose is present and which, therefore, succumb first, and the hyphae penetrate along the line where it is to be found, and the same reason may explain why *Polyporus sulphureus* extends in the direction of the annual rings.

In describing the process of decay in the Oak, due to *Polyporus dryadeus*, Fr., *Telephora perdix*, M., and *Stereum hirsutum*, Fr., Hartig distinguishes two methods of attack, one accompanied by delignification and another in which the conversion of lignin into cellulose does not take place. It is conceivable that Hartig may be describing the action of these Fungi upon elements already containing cellulose and upon those in which cellulose is absent. The fact that in the former method of attack the progress of decay is more rapid gives support to this suggestion.

It appears probable that the occurrence of cellulose in certain areas represents a stage of arrested development. The largest distribution of cellulose was very frequently observed in the wider annual rings, in which the wood appeared to have grown rapidly in a favourable season, and the direction of orientation evidently has its influence also. There may be some connexion with the phenomenon known to gardeners as the 'ripening' of the wood, and in certain seasons when the wood is said not to 'ripen' it may be an expression of the fact of an incomplete lignification. I may mention that I imagine this condition of partial lignification may be found to be generally prevalent. Among herbaceous plants, for instance in *Vicia faba* and *Oenothera biennis*, in the older stem-internodes just above the surface of the ground the cellulose lining of the wood-fibres is very beautifully shown.

Summary.

1. It is found that a gelatinous thickening layer which reacts at once to the various colour-tests for cellulose occurs very commonly, though very irregularly, in the fibre-walls of the xylem as a normal condition in a great number of perfectly healthy trees, in all localities and situations. It may have a very partial distribution, or may occur very generally and conspicuously through the stem, and may be present only in parts of the same annual ring. Sometimes this innermost layer is represented only by a thin lining, at other times by a very broad band which appears swollen and occupies a large part of the lumen.

A margin of cellulose is also often present round the bordered pits.

2. A delignification of the xylem is effected by the action of boiling water, which removes substances which impregnate the cellulose and react to the lignin stains, leaving a basis of cellulose as indicated by the reactions to the various colour-tests. This is shown by submitting thin sections of wood to the action of boiling water, and is confirmed by the fact that ether removes from the watery extract obtained from sawdust and fragments of wood a substance which reacts to lignin tests.

Further, cold water, operating for a longer period, has a similar power in extracting from the xylem a substance which reacts to phloroglucin and thallin sulphate, and thus by continued soaking in water wood undergoes a partial delignification.

A substance showing a blue-green colour with phenol-HCl, the test for coniferin, is apparently not extracted.

3. It is demonstrated that the 'lignin' substances extracted from the xylem by water are destroyed by certain micro-organisms, and that these flourish more vigorously in the sap-wood than in the heart-wood extracts. This latter point suggests that the heart-wood contains some substances not readily attacked by Fungi or Bacteria, and accounts for the fact of its greater durability.

4. The presence of this unlignified layer in the wood-fibres probably represents a stage of arrested development. Its general prevalence having been overlooked, the conclusion is inevitable that the occurrence of cellulose which has been attributed to the action of Fungi must to some extent be ascribed to conditions already present, and the effect of any method of sterilization must also be taken into account. The delignification cannot be entirely attributed to an enzyme secreted by Fungi.

Postscript.

Since the above was written I have examined the roots of *Lupinus*, *Phaseolus*, *Polygala Senega* and *Aesculus*, and have found the cellulose lining in the fibres very distinctly shown in all these cases. Doubtless fibres remaining partially unlignified are of quite common occurrence in roots.

In view of the examination of additional specimens of *Fraxinus* grown in a different situation, my statement with regard to the complete lignification of the fibres requires some modification. Recently I have had the opportunity of examining the wood of *Fraxinus* attacked by *Polyporus hirsutus*, and found that when the xylem was treated with chlor-zinc-iodine the fibre-walls became an intense blue. In accordance with the observations previously made, this seemed to indicate a delignification due to this parasite. But on examining perfectly healthy shoots from the same tree, and also others from trees in the same locality quite free from any fungoid attack, it was at once seen that in these cases many of the fibres were only partially lignified; hence the delignification was not primarily due to the action of this *Polyporus*. Local conditions of soil and climate seem in some cases to retard the complete development of the xylem, and thus render such trees constitutionally weak and very liable to attack. For instance, in the particular locality now under consideration very few of the trees were free from the infection of *Polyporus hirsutus*.

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EXPLANATION OF FIGURES IN PLATE VIII.

Illustrating Professor Potter's Paper on the Occurrence of Cellulose in Xylem.

Fig. 1. Transverse section of a young stem of *Quercus*, diameter 1·3 cm. The shading extending partially round the stem indicates the regions of cellulose distribution.

Fig. 2. Transverse section of wood-fibres from a stem of *Quercus*, cut when fresh and stained with Delafield's haematoxylin. The lignified layers are yellow and the enclosed gelatinous thickening layer violet. Zeiss D., Oc. 4.

Fig. 3. Portion of a transverse section from the stem of *Fagus*, cut when fresh, showing the inner gelatinous layer present in some wood-fibres, coloured violet. Zeiss D., Oc. 4.

Fig. 4. Longitudinal section from the wood of *Salix*, cut when fresh and stained with chlor-zinc-iodine. Showing the margins of the bordered pits stained blue.

Fig. 5. Transverse section of wood-fibres from the stem of *Fraxinus*, cut when fresh and stained with chlor-zinc-iodine. The yellow walls show a complete lignification with no internal cellulose layer.

Fig. 6. Similar section from the same stem of *Fraxinus*, after boiling on four consecutive days and staining with chlor-zinc-iodine. The lignified parts of the walls remain yellow, while the inner swollen layers are coloured violet, having undergone delignification.

Fig. 7. Transverse section from a stem of *Aesculus*, steamed for two hours on three consecutive days, stained with Congo-red, and mounted in Canada balsam. The inner delignified layer of the fibre-walls is stained red and lies detached, swollen, and crumpled in the lumen.

Fig. 8. Transverse section of the wood-fibres from a stem of *Quercus* after steaming three times, stained with chlor-zinc-iodine. Showing the advancing process of delignification due to the action of boiling water.

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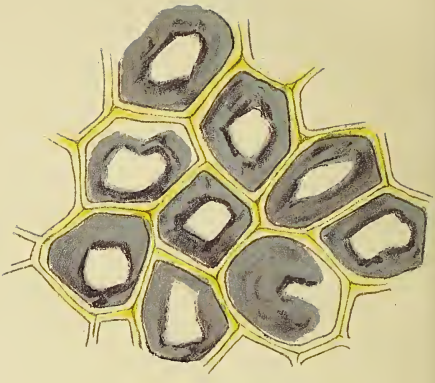

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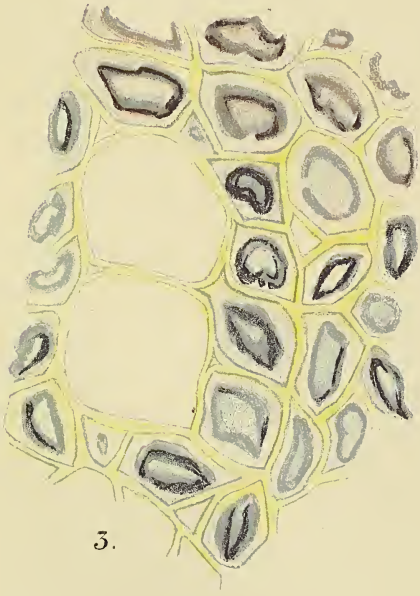

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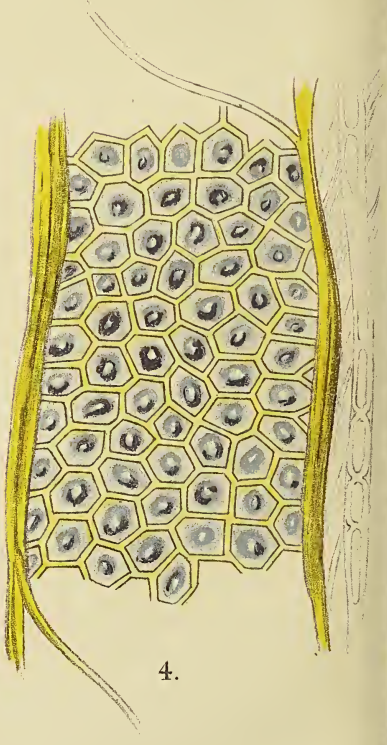

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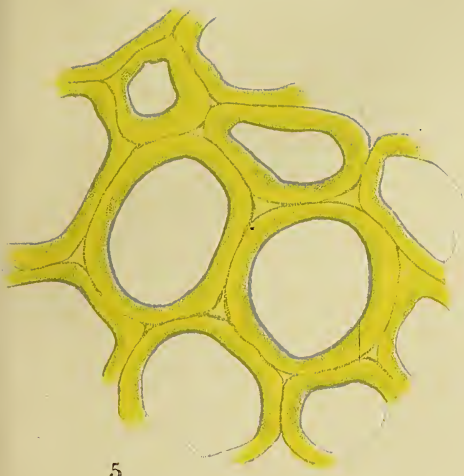
N. H. Potter, del.

POTTER. — CELLULOSE IN WOODY STEMS.



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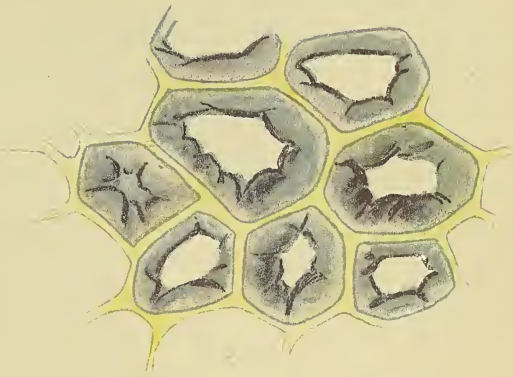

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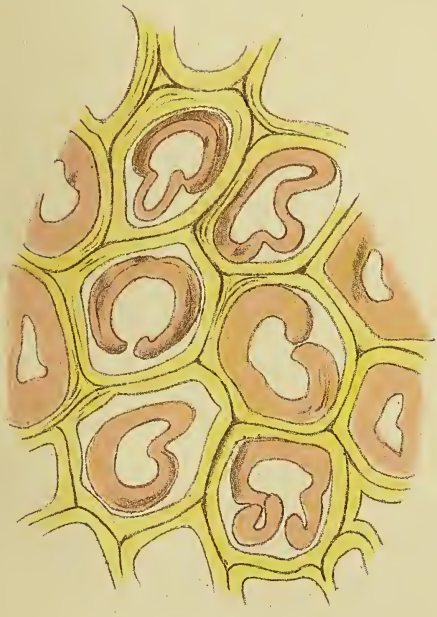

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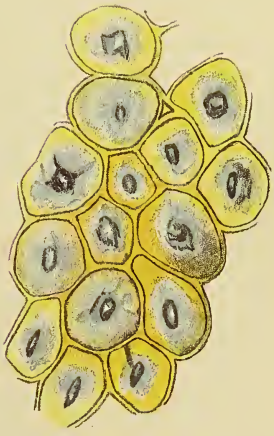

8.

University Press, Oxford.

Studies in the Dictyotaceae.

I. The Cytology of the Tetrasporangium and the Germinating Tetraspore.

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With Plates IX and X.

IN 1897 I published an account of the discovery of motile antherozoids in *Dictyota*[1]. At the Bristol meeting of the British Association (1898) the mode of fertilization was described. The cytology of the antheridia and oogonia and of the apical region of the tetraspore plant was also explained and illustrated by means of figures. It was found that the full number of chromosomes obtained in the apical nuclei of the asexual plants, while in the single division of the oogonium and in the early divisions of the antheridium the reduced number prevailed. This necessitated a more careful search for the actual reduction division. Ultimately it was found to be the first division in the tetraspore mother-cell. Meantime Mottier[2] in 1898 announced his discovery of centrosomes in *Dictyota*. Subsequently (in 1900[3]), he published a detailed description of the reduction division, as well as of the second division of the tetraspore mother-cell and of the vegetative mitoses in the thallus-cells. I have worked out the cytology of all the various kinds of cells in the three forms of *Dictyota*, male, female and asexual, and a considerable body of facts has been gathered relating to the natural history of the living plant, both under cultivation and in its natural habitat. As far as cytological evidence is concerned there seems to be no reason to doubt that we have here a clear case of alternation of generations. Publication of the results has hitherto been delayed in the hope of completing the evidence by actual cultivation of the plant from spore to spore. All the numerous attempts made to produce this result have hitherto failed, owing to the difficulty of getting the plant to fruit in cultivation, and it has been thought best not to further delay

[1] Williams, Annals of Botany, xi, 1897.

[2] Mottier, Ber. d. deutsch. Bot. Gesellsch., xvi, 1898.

[3] Mottier, Ann. of Bot., xiv, 1900. All subsequent references to Mottier will be to this paper.

[Annals of Botany, Vol. XVIII. No. LXIX. January, 1904.]

publication of the results hitherto obtained. The present paper deals with the sporophyte generation, and the first segmentation of the spore. The cytology of the gametophyte and of fertilization, the very remarkable phenomena associated with parthenogenesis, as well as the natural history of the plant, will be dealt with in subsequent papers.

Mottier has described and figured karyokinesis in the vegetative cells of the thallus. Our results being in general agreement it is not proposed to deal further with them. As, however, he has not described the stalk-cell division or the earlier stages of the reducing division, a detailed account of them will be given here.

The Stalk-cell Division.

One of the elongated rectangular cells of the thallus begins to swell in all directions, but chiefly outwards, in the direction of least resistance. At the same time the nucleus increases greatly in size. The nucleolus appears granulate, there is a fine reticulum uniformly distributed through the nuclear cavity, and upon it are numerous granules. The axis of the nucleus is as yet parallel to that of the thallus. At each pole there is a curved rod-like centrosome, and radiations extend into, and become merged in, the cytoplasmic reticulum. The chloroplasts stain uniformly; they are much paler than those of the vegetative cells, and at first they are uniformly distributed. Soon, however, the basal portion immersed in the surface layer becomes greatly vacuolated. In the vertical walls separating the cell from the neighbouring ones there are large pits and the vacuoles lie opposite them.

The lateral extension of the cell soon ceases, but it grows rapidly outwards until it is several times the depth of the cortical cell. In cross section the projecting part is nearly spherical, but a longitudinal section shows an elongated, dome-shaped mass. The dense cytoplasm, containing the bulk of the chloroplasts and the nucleus, is aggregated in the swollen, free portion. The axis of the nucleus has now swung round until it is vertical to the thallus, and the distal pole has a very distinct curved centrosome and beautiful radiations; the space occupied by the latter being quite free of chloroplasts (Pl. IX, Fig. 1). A cap of cytoplasm covers the basal pole. From this a cytoplasmic cord extends downwards into the vacuole, where it sometimes branches. The centrosome here is often only distinguished with difficulty and the radiations are completely absent. Stages where the axis of the nucleus is oblique to the thallus are very difficult to find, the change of position probably taking place very quickly. The nucleolus is now large and stains deeply. Sections through it show that it is uniformly fibrillate, or minutely vesicular in texture.

Before the spirem there is a stage where the nuclear reticulum shows

a tendency to draw away from the nuclear membrane (Fig. 1). It is true that this effect is probably due to the fixing reagent, but the readiness with which the effect is produced both in this and the corresponding stage of the next mitosis shows that the relation of the network to the membrane—probably to the cytoplasm—is much less intimate than it is during the spirem stage.

The spirem is very coarse, the chromatin granules being unequal in size and the staining not deep. It frequently seems polarized, but at this stage no signs of splitting can be seen. The nucleolus now becomes vacuolate. Frequently, but not as regularly as in the next division, there is a large central vacuole with deeply stained inclusions. Immediately before the segmentation of the thread (Fig. 2) the nucleolus becomes swollen, irregular in form, and distinctly fibrillar in texture,—an appearance which is also seen at this stage in most of the other mitoses. Soon the nucleolar membrane disappears and the free, fluffy ends of the fibrillae project from the general mass of nucleolar substance. This is identical with what occurs in the prophase of the next division (Fig. 19). At this time the nuclear cavity has no reticulum within it.

The chromosomes now begin to appear and are often disposed in the neighbourhood of the nucleolus. The latter loses its coherence and becomes an irregular mass of granules and curved fibrils, some of them looking not unlike minute chromosomes, and, unless very carefully counterstained, very similar in their staining reactions. Besides these, the nucleolar globule, which always accompanies the spindle, is already differentiated. This is much less deeply stained, and, except for a small vacuole which sometimes appears in it, is quite homogeneous. Two sheaves of fibres now project into the nuclear cavity, their extremities reaching about one-third of the way to the opposite pole, leaving the equatorial region free. Here are aggregated the fragmenting nucleolus and the chromosomes. The latter are considerably bigger than during the succeeding mature spindle stage; they are curved and distinctly split. Fig. 3 represents one of three oblique sections through such a nucleus. After making allowance for chromosomes that have been cut and consequently appear partly in this and partly in other sections of this nucleus, the number of chromosomes is from thirty to thirty-four.

The spindle when fully formed is intranuclear, the membrane persisting close up to the poles. The spindle proper is truncated at the poles, and in some cases (Fig. 4) is not greatly dilated at the equator. The space between the spindle and the membrane is occupied by a few mantle fibres and a large number of curved fibrils almost certainly derived from the disintegrating nucleolus. The nucleolar globule is also seen somewhere in the vicinity of the spindle—it now stains just like the cytoplasm. The chromosomes are uniformly distributed through the nuclear plate in a dense

flat disc. Those at the periphery are curved and have the free ends directed outwards. Careful countings of polar and other views give from twenty-seven to thirty-two chromosomes. The polar radiations and centrosomes are not nearly as clear as they are during the early prophase stage.

When the daughter chromosomes are halfway to the poles the nuclear membrane is still intact, and the centrosomes are visible at both poles. Between the chromatic discs and the poles the cones of fibres are clear of granules and the fibres are fine and close set, while the interzonal fibres are, as usual, fewer, far coarser, and the space between them invaded by granules and fibres. When the chromosomes reach the poles, they form two flat discs, the distal one being much greater in diameter than the basal. Very often a chromosome lags behind the others and projects towards the equator (Fig. 5). The membrane has now disappeared, so that the nucleolar fibrillae and globule are excluded from the chromatin discs (Figs. 5 and 6). The coarse connecting fibres still remain and the distal centrosome and radiations are distinct. Sometimes the basal centrosome can be made out, but there are no radiations. The lower half of the figure projects into the vacuolated region while the upper half is imbedded in the denser cytoplasm.

A membrane now surrounds each of the daughter nuclei, the diameter of which is much less than in either the preceding or succeeding stages. Instead of lying parallel in a flat disc, the chromosomes now form a tangled mass, but they are still distinct and preserve their curved form. In this condition it is often easier to estimate their number than during the late diaster stage. At this period nothing can be distinguished within the nuclear membrane besides the chromosomes. The chromosomes gradually fuse so as to form an irregular coil or reticulum with a very few meshes. This coil is very much thicker in the tetraspore mother-cell nucleus than it is in the sister nucleus. Each thick thread is at least double, the median line can frequently be seen to be lighter than the two edges (Fig. 6, upper nucleus). The strands of this irregular coil are often swollen at intervals, and there may be ten to sixteen of the swellings. Throughout this period of partial fusion the membrane of the upper nucleus, on the distal side, is very irregular, there being a marked projection towards the centrosome. A very faint reticulum now appears in the nucleus, and the condensation of the coil proceeds till very often it assumes the form of a thick open ring.

Ultimately the chromatin mass becomes spherical in form and uniformly granular or finely fibrillate in appearance, in fact it is the nucleolus. It is worthy of note that of the four mitoses described in this paper this is the only one in the telophase of which there is no differentiation of nucleolar and chromatin masses. The diameter of the nucleus is now much greater, and there is a fine but very faintly staining reticulum.

The septum dividing the stalk-cell from the sporangium proper soon makes its appearance, and the separation of the tetraspore mother-cell is completed.

The First, or Reducing Division of the Tetraspore Mother-cell.

Before the initiation of the spirem the nucleus passes through a similar condition to that shown in Fig. 1, where the reticulum easily separates from the nuclear membrane. Soon, however, there is a very thin convoluted spirem distributed through the nuclear cavity. The fine granules upon this stain but feebly as yet, but here and there a few larger, but still small, deeper-stained bodies appear between the threads (Fig. 7). The nucleolus preserves for a time the fibrillar appearance already described, but as the spirem develops a vacuole appears in the former with, at first, a few smaller ones. Soon, however, there is only one fairly large vacuole which has a firm, deeply staining outline, and there are darker staining granules within it. The axis of the nucleus is once more parallel with that of the thallus, but the poles can only be distinguished with great difficulty owing to the faintness of the radiations (Fig. 7), and the centrosomes, if they exist at all, are not distinguishable. No signs were seen at this stage of the division of the centrosome, nor could the change of position of the nuclear axis be followed. The spirem gradually becomes more prominent, and shows a tendency to aggregate near the poles. A deeply staining spherical or sometimes angular body, considerably larger than any of the granules hitherto described, now makes its first appearance. It is peculiar to this mitosis, and it persists till after the splitting of the spirem (Figs. 8–12). It is always present during these stages, and there is hardly ever more than one. Whether it directly separates from the nucleolus or is formed by the fusion of the smaller granules shown in Fig. 7 could not be decided. The body will be designated for the present the *chromophilous spherule*. It should not be confused with the nucleolar globule which appears during several of the mitoses in this plant. The faintly staining cloudy nucleoplasm which appears during the synapsis is absent from this stage.

The above condition leads directly to the very interesting *knot stage* or true *synapsis*, which forms a most striking feature in the cytology of the reduction stage, but appears nowhere else in the whole history of the plant. Mottier[1] says: 'During the prophase of both divisions in the tetraspore mother-cell the behaviour of the chromatin differs strikingly from that in the higher plants. There is not developed here a regular and continuous chromatin-spirem which segments into the chromosomes, but these arise as isolated masses often differing much in size. It gives the impression

[1] 1900, l.c., p. 188.

that the quantity of chromatin is not sufficient to form a continuous spirem.' This, however, is a mistake. It would be hard to find a more distinct spirem than this, or one more interesting in its development. Many hundreds of them have been studied, and in some material they are far more numerous than spindles, showing that they persist for a considerable time. The main features of the spirem stage in *Dictyota* are so constant that no one after seeing the synapsis could possibly call it an artefact. After completing the work on *Dictyota* I began to study the cytology of *Padina*. This plant was found to agree with *Dictyota* in all the principal points, while it presented the great advantage of having the various stages sorted out as it were. In *Dictyota* the sporangia are isolated and the various stages are generally mixed up in a haphazard fashion. Thus the finding of any particular stage is a matter of chance, and in order to fill up some of the gaps several thousand sections have been examined. In *Padina*, however, the sporangia are collected together in concentric bands on the fan-shaped thallus, and in these bands many hundreds are so closely crowded together as to be in actual contact. Furthermore, all the primary sporangia in any one band are approximately in the same stage. Thus the first band behind the apical region is frequently found to have all the nuclei in the spirem stage, while the older band behind this has the sporangia in various stages of division. In very old sori, where there has been liberation of spores, new sporangia are initiated; here the uniformity above spoken of does not obtain.

The failure to recognize this stage is probably due to the fact that the tetrasporangia in Mottier's plants were too old, for it is a striking peculiarity of *Dictyota* and *Padina* that the spirem appears as soon as the mother-cell has been separated and while the sporangium is still young and small; it then disappears, and the nucleus assumes the appearance of the resting stage. In the preparation for this mitosis there are then three well-marked stages: (1) A very precocious spirem, which lasts a considerable time and goes through several interesting changes. (2) A reticulum stage, which is exactly like that of rest. It is with this that Mottier's studies commence, and his Fig. 1 comes between Figs. 14 and 15 of this series. (3) A prophase stage which is passed through somewhat quickly.

The Synapsis Stage. The spirem is now very long, thin, and deeply stained. It is closely coiled up into one or two clumps of angular loops (Figs. 8 and 9). The two knots appear closely pressed against the nuclear membrane, one opposite each centrosphere. The centrosome and radiations are still very indistinct, those shown in Fig. 8 being the best observed. The nucleus is very large, but the membrane is thin and ill-defined. The knot is so pressed against the membrane that it is often difficult to decide whether the spirem is entirely within it, and it

frequently seems as if the thread were directly continuous with, or attached to some of the cytoplasmic fibres. Very often, also, the long angular loops of the spirem seem to radiate from the vicinity of the centrosome towards the nuclear cavity. Although generally in two knots, the spirem is continuous, one or several connecting threads stretching across the cavity from one knot to the other. These frequently pass over the surface of the nucleolus, and can be seen to be in contact with it (Fig. 8). The behaviour of the latter is interesting. It invariably becomes swollen, very irregular in shape, and greatly vacuolated, *but never fibrillar*. Many cases are seen where a portion is drawn out towards the spirem, to which the apex of the projection is attached (Figs. 9, 10). Sometimes big roundish lumps appear to be on the point of separating from it, and at others the whole nucleolus seems to be fragmenting. This, however, it never does; as soon as the synapsis is over the nucleolus resumes its spherical form. Fig. 9 shows a case where there is only one knot. It sometimes happens that the spirem is entirely on the basal side of the nucleus (i. e. on the side nearest the 'basal' or 'stalk-cell') and not in the vicinity of the poles. In *Padina* it is most frequently either on the basal or on the distal side, or both. This has probably some reference to the fact that here the sporangia are compressed laterally by contact with each other, so that the axis of the first division spindle is vertical to the surface of the thallus instead of parallel to it as in *Dictyota*.

The other constituents of the nucleus at this period are the spherule above described and a small quantity of cloudy nucleoplasm, which on closer examination resolves itself into a very fine reticulum which, however, stains but slightly.

The staining reactions at this stage are as follows. The spirem, spherule, and the physodes stain deeply with the chromatin stain, the two latter often showing different shades of colour. The nucleolus, chloroplasts, and cytoplasm take the basic stain, the two former more deeply than the latter.

It is very evident that there is an intimate relation between the spirem and the cytoplasm at this stage, and that communication between them is chiefly localized at the poles. The frequent connexion between the nucleolus and the spirem, as well as the poverty of the former in chromatin, both seem to indicate that much, if not all, of the chromatin comes out of the nucleolus.

The Beaded Spirem Stage. The thread now becomes thicker, and the chromatin discs can more easily be seen (Fig. 11). The knots loosen out and the thread becomes distributed more uniformly over the membrane on the basal side of the nucleus, more rarely over the upper side. The cloudy nucleoplasm is also generally aggregated along the basal side, so that the spirem seems embedded in it. The nucleolus gradually

becomes more spherical, and a large vacuole appears in it containing minute fibrils similar to those shown in Figs. 11 and 12. After a time the spirem is more uniformly distributed over the whole surface. Fig. 11 does not show this very clearly, as the section is a median one, the rest of the spirem being in other sections. The nucleoplasm begins to diminish in amount; some of it is seen as little fluffy threads attached to the spirem.

The staining reactions are peculiar. With brazilin and picric-Hoffman-blue, for instance (the former being the chromatin and the latter the plasma stain), the spirem and the nucleolus stain reddish brown, the spherule yellowish red, while everything else, including the fibrils in the nucleolar vacuole, stain blue. Later on the spirem loses its chromatin reaction and colours blue like the chloroplasts. No definite conclusion respecting the chemistry of the nucleus can be drawn from this, but it is interesting to note how the nucleolus and the spirem reverse their respective colour reactions in the knot and the later spirem stage.

The Split Spirem. The thread now instead of lying along the nuclear membrane becomes more evenly distributed through the cavity. Longitudinal fission can be seen here and there, and before long it is found to form two distinct threads which often twist round each other (Figs. 12 and 13). The granules are irregular in size and more distant from each other. As in the preceding stage they show no special affinity for chromatin stains. The nucleolus stains more deeply, and shows a very big vacuole with inclusions. The spherule is still present, and besides the nucleole it is the only nuclear structure that colours with chromatin stains. The nucleoplasm diminishes in amount and finally disappears, being probably incorporated in the reticulum formed out of the split spirem. The centrospheres are still very indistinct, the mother-cell is bigger and more vacuolated than in the preceding stage.

The 'Resting Stage.' The next stage in the history of the spirem is difficult to make out. Instead of a comparatively small number of threads which are split, one sees a large number of very fine threads with numerous granules, very variable in size and disposition, crossing the nuclear cavity in all directions, and in many places appearing to form a reticulum (Fig. 14). Whether this appearance is due to the mere separation of the halves of the split spirem, with a lengthening and thinning out of the threads, or of this with a second split superadded, it is very difficult to make out. The halves undoubtedly do separate widely, and the identity of the constituent threads is completely lost, but a careful examination of a very large number of examples failed to disclose any clear evidence of such a second split. On this account, and after a close study of the prophase stage (Figs. 17–19), I have come to the conclusion that there is no second split at all, and that the appearance seen in Figs. 14, 15, is due to an alveolation of the separated halves of the chromatin thread

and a joining together of the very fine reticulum thus produced by fine cross threads, so that the nucleus appears to be in a state of rest.

The spherule now stains paler than before, then breaks up into two or several smaller ones, and at the close of this stage completely disappears.

After a time the sporangium presents several striking features. The cytoplasmic constituents when seen in section are arranged in evident zones, so that the term 'zonated' might be appropriately applied to it. The centrosomes and radiations are now exceedingly distinct at both poles, and there is a narrow zone of kinoplasm extending round the sides of the nucleus. This area is quite clear of chloroplasts. Immediately outside, and in sharp contrast to it, there is a zone of dense protoplasm with crowded chloroplasts which stain very deeply, generally taking more or less of the chromatin stain (Fig. 15). The outer region is vacuolated, and here the chloroplasts generally stain like the cytoplasm. This is not included in the figure.

The nuclear membrane is firm and clear. At the poles it is generally very irregular; the example selected to draw Fig. 15 from does not show this very clearly. It generally forms projections towards the centrosomes, and this causes a number of folds and wrinkles. Frequently a conical extension of the polar membrane has its apex touching the middle of the rod-like centrosome, the two ends of which are free. Occasionally the projection is truncated at the apex, and is in contact with the rod along its whole length. When this is viewed from the side the centrosome looks almost like a fold of the nuclear membrane.

In *Padina* there is the same arrangement of the cytoplasm into concentric spheres, though perhaps not quite so pronounced as in *Dictyota*. The centrosome and radiations are very much less distinct.

The nucleolus is spherical and somewhat deeply stained, but spongy, with large and small vacuoles. The only other constituent is the reticulum already described.

The Prophase Stage. Very soon thick cloudy strands appear in the nucleus. There seems to be little, if any, diminution of the reticulum, so that it is not clear whether these are derived from a further condensation of the denser strands of the reticulum or not. As the cloudy bands assume a more definite form, a longitudinal split makes itself evident in them (Fig. 16). When these bodies have still further condensed, and assumed the forms of chromosomes, they are seen to be either closed rings or looped-up bands with their free ends crossing. This is well shown in Fig. 17 from *Taonia*. The next figure (18) represents three rather thick sections of a similar nucleus from *Padina*. These include all the chromosomes, and they are uncut with the exception of the one in the extreme left of *c*, the bend joining the two halves of which is shown in section *b*.

This shows clearly that the loop-figures have not been caused by cutting off the ends of elongated rings. The failure to find evidences of a second split of the chromatin thread, and a careful study of the development of the chromosomes, inclines me strongly to Farmer and Moore's view of the origin of these bodies, as recently advanced before the Royal Society[1]. According to this the space inside a ring-chromosome, or that between the two limbs of a loop-figure, does not represent the first split, but rather the space between the two constituents of a bivalent chromosome bent over on itself so that the two ends meet, or the two limbs cross, or some modification of these forms is brought about. The split itself (the only split according to this theory), though partly obliterated by the condensation of the chromosomes, is still evident in the free ends of several of the chromosomes in Figs. 17 and 18. During this stage the nuclear reticulum is somewhat dense and the nucleolus minutely vesicular.

By the time the spindle cones begin to develop the chromosomes have still further condensed until they are small and deep-staining, but the ring form is very prevalent (Fig. 19). Thus, although a long time has elapsed since the spirem stage, and in the meantime all traces of it have been lost in the apparently formless reticulum, yet the chromosomes make their appearance already segmented and longitudinally split. The length of the spirem period and the comparative shortness of the prophase irresistibly suggest that the arrangements for mitosis are completed, or nearly so, in the former; that the grouping of the various constituents is there determined, and however disguised in the reticulum of the post-spirem stage that it still exists. This is equivalent to saying that the spirem maintains its identity throughout, and that when the stimulus is given which produces the prophase there is a rapid condensation of the previously extended, unrecognizable spirem into compact, deeply stainable, split, bivalent chromosomes. This does not exclude the possibility of substance passing into the chromosomes from the nucleolus. From actual observation, however, we can determine that this must be small in amount, for the bulk of the nucleolar substance is otherwise accounted for. In Fig. 19 the nucleolar membrane has disappeared, and the contained fibrillae, as deeply staining as the chromosomes, lie together in an irregular mass, some of them with their free ends projecting. Other sections show, as in the case of the stalk-cell division, that the spherical globule subsequently seen near the spindle is also formed out of the disintegrating nucleolus.

The Spindle Stage. Of this a great many examples have been studied, and they all agree in their main features. The spindle is intranuclear, very narrow, and nearly iso-diametric. The nuclear membrane is intact excepting at the poles, where there are evident gaps nearly as

[1] Proc. Roy. Soc., vol. lxxii, p. 104, 1903.

wide as the spindle itself (Fig. 20). There are divergent mantle-fibres between the spindle and the membrane—these rarely reach as far as the equator. The radiations and centrosomes are much fainter than during the post-spirem stage. Fig. 22 shows a very good centrosome from an oblique section; there is a very similar one in the section containing the other pole.

In many of the figures the spindle-fibres seem tense and nearly straight, while the mantle-fibres are lax and wavy (Fig. 20). The former give one the impression of having contracted and shortened till in some cases (not so well shown in the one drawn) the polar edges of the membrane are curved inwards and the centrosome is sunk within the gap. Here, as in the preceding mitosis, there are nucleolar fibrillae, which, as observed by Mottier, often stain almost as deeply as the chromosomes, but when a deep counterstain has been employed there is no difficulty in differentiating them from the chromatin. The nucleolar globule is nearly always present, generally at the periphery in the equatorial region. It always stains like the cytoplasm.

The chromosomes, as already stated by Mottier, are sixteen in number, and decidedly heterotype in character. Figs. 20 and 21 show their form, and it seems hardly necessary to describe them in greater detail. Their small size makes it difficult to decide how they are placed on the spindle; and whether the division is such as to bring about true reduction or not is for the same reason equally difficult to decide.

In sections parallel to the surface of the thallus it happens occasionally that the nuclear figure is flattened so as to widen out considerably at the equator. In these cases the chromosomes are widely separated, and consequently more easily counted.

The Anaphase Stage presents no peculiar features—the description of the stalk-cell division anaphase would almost apply to this. Mottier, however, states that chromosomes on their way to the poles often fuse together into larger masses. I have seen no instances of this in healthy cells. From the post-spirem stage onwards a large number of tetrasporangia become arrested in their development, and frequently this takes place while the nucleus is in mitosis. In such cases the chromatin shows various abnormalities of structure. As a rule, however, one finds it easy to recognize such abnormal examples from the peculiar appearance of the cytoplasm. Occasionally, in the earlier stages of degeneration, there may be difficulty in deciding whether a phenomenon is normal or not. Here one has to depend entirely upon comparison of a large number of cases; from such a comparison I have come to the conclusion that the fusion of chromosomes above spoken of never takes place excepting in degenerating sporangia.

The Telophase Stage is strikingly different from that of the stalk-cell

division, and though more like that of the first segmentation of the spore there is a difference in the condition of the chromatin mass. Mottier has good figures of this stage (see his Figs. 8, 9). Instead of the chromosomes forming one large, deeply stained body, two, sometimes more, are formed. One is very large, irregular in form, and composed of fine fibrillae and granules, while the other is smaller, denser, and more suggestive of a nucleolus (Fig. 23). The former is undoubtedly the chromatin. In this condition it is exactly like a stage in the fragmentation of the nucleolus. A little later the chromatin fibrillae are seen to be more widely distributed through the nucleus (Fig. 24); eventually they disappear altogether. Coincidently with this the reticulum becomes more and more evident and the nucleus increases in size (Fig. 25).

The Second Mitosis in the Tetraspore Mother-Cell.

The axes of the two daughter-nuclei are now parallel to each other and to the surface of the thallus, but at right angles to that of the stalk-cell. Thus a section tangential to the thallus shows both nuclei longitudinally. In such a section a first division spindle would also be cut longitudinally but it would be parallel to the axis of the stalk-cell, whereas in the stalk-cell division the figure would be cut across so as to give a polar view. During the prophase stage the chloroplasts are densely aggregated in the vicinity of the two nuclei, while the median plane is sharply marked out by their absence or scarcity. The two nuclei are somewhat flattened on the outer surfaces, whence radiations curve round the ends of each nucleus towards those of the sister nucleus. The spirem is thick and irregular, and it soon segments, the nucleolus at the same time going through the usual process of disintegration into fibrillae (Fig. 26). In both nuclei 'kinoplasmic' activity is confined to the nuclear surfaces remote from each other—those nearest the periphery of the mother-cell. It follows from this that in the early spindle the cones of fibres are not in a straight line. This has been well shown by Mottier. In a nucleus similar to his Fig. 12 the scattered chromosomes have been counted without difficulty, and here again the reduced number has been found to obtain. The mature spindle (Fig. 27) is an interesting object, especially when viewed in profile. Owing to the apparent 'kinoplasmic' repulsion above described the spindle is placed against the outer side of the nucleus, the sister nucleus on the opposite side of the cell presenting the same features but reversed as to direction. Even at maturity there is frequently a slight curve in the figure. The centrosomes are not easy to make out, but the radiations are clear, and as before curved towards those of the sister nucleus. The spindle itself is elongated and narrow, the chromosomes are curved, and countings of both oblique and polar views leave no doubt that the number is the reduced one. The remaining constituents—

mantle-fibres, nucleolar globule, and fibrils—are shown in Fig. 27 and call for no special comment. Mottier says that 'the invariable tendency of the chromosomes to collect and fuse into larger masses has made it impossible to determine accurately their number in this division. In the equatorial plate often only two large chromatin masses are seen (Figs. 14, 15).' This description does not apply to the material examined by me, where the chromosomes remain distinct up to a late stage in metakinesis. Both in the prophase and spindle stages the chromosomes are clearly seen to be homotype in character.

The diaster stage is of the usual type. The figure becomes very narrow and elongated as a rule (Fig. 28). The membrane often persists until the daughter chromosomes are nearly at the poles. (Mottier says it 'disappears soon after the spindle is mature, generally before metakinesis.') The pale-staining nucleolar globule is still visible at this stage.

The formation of the daughter-nuclei shows the same phenomena as in the preceding division. Here, however, the nuclei are exceedingly small. The radiations completely disappear. In *Padina* the four spore-nuclei within the sporangium are bigger than those of *Dictyota*, their chromatin thread is prominent during the resting period, and the cytoplasm is differentiated into distinct concentric spheres.

Karyokinesis in the Germinating Tetraspore.

The tetraspore on being liberated, instead of assuming a spherical form as is the case with the oosphere, is nearly always elongated and almost cylindrical. The position of the nucleus is indicated by a lighter area. Division of the nucleus takes place in twelve to sixteen hours. The resting nucleus is much larger than it was in the tetrasporangium. The condition of the cytoplasm—the greater width of the meshes and the separation of the plastids—shows that this is due more to distension consequent on liberation from the confined space of the tetrasporangium than to growth. One consequence of this is that the parts of the mitotic figure are much clearer here than in the sporangium.

Before the development of the spirem the usual reticular structure is shown. At the same time the nucleolus has the fibrillar structure so frequently referred to already. The spirem (Fig. 30) is coarse and unevenly beaded like that of the stalk-cell division: in this case, however, it is accompanied by a considerable amount of fine reticulum.

The spindle (Fig. 31) is intranuclear like the preceding ones. It is, however, much broader at the equator and the centrosomes and radiations are more prominent, a result probably of diminished pressure in the cytoplasm. It is a rather striking fact that no nuclear globule has been observed at any stage of this mitosis.

The chromosomes have been counted in a great many figures, and they

are always about sixteen in number. Their form is curved just like those of the stalk-cell division. During the anaphase stage the membrane persists up to a very late period (Fig. 32). The mantle-fibres, centrosomes, and radiations are very distinct; in fact all the accessory structures are far more easily seen here than in the preceding mitoses.

The curved chromosomes aggregate at the poles in the usual way with their free ends towards the equator; a membrane is formed; then, as in the stalk-cell division, they lose their polarity but remain distinct enough to be countable for some time longer. Here (Fig. 33) again it is seen that the number is the reduced one. The centrosomes and radiations, together with the connecting fibres, are still clear. A striking feature of this and of the next stage (Fig. 35) is the projection of the nuclear membrane towards the centrosome, already seen in some previous stages.

Fig. 34 shows the chromosomes to be accompanied by a distinctively staining body which in all probability is the rudiment of the nucleolus.

The fusion of the chromosomes goes on at first somewhat like that of the stalk-cell division. Here, however, two masses are formed (Fig. 35), of which one is larger and more irregular in form than the other. A reticulum also appears which at first stains but faintly. Later on the smaller of the two masses dwindles in size and finally disappears, the reticulum at the same time becoming more deeply stained and the other body (the nucleolus) assuming a spherical form.

Abnormalities.

There are various abnormal developments here which it might be profitable to study in greater detail:—

(1) Undivided tetraspore mother-cells are sometimes liberated, and several instances have been observed of their nuclei in the prophase or even in the spindle stage. Most of these, however, were evidently arrested, and there is as yet no evidence to show whether karyokinesis was initiated before or after separation from the thallus.

(2) Several instances have been seen of liberated sporangia containing two spore-like bodies, each, however, being invested with a cell-wall. In one case the nuclei seemed to be in second division prophase, with spirem and fibrillar nucleolus. In the other the late anaphase stage was seen but the chromosomes could not be counted.

(3) This is an abnormality which is of greater interest, as it occurs more frequently and may possibly be of some utility to the plant.

Towards the close of the season, instead of dividing in the usual manner to form tetraspores, the mother-cell, without greatly increasing in size or taking on a deeper colour, divides into two and then into a small mass of parenchyma. Richards[1] observed this phenomenon in *D. ciliata*, but says:

[1] Proc. Amer. Acad., 1890, p. 83.

'The significance of this multiple division could not be explained with only alcohol material; it was too frequent, at least in the specimens I examined, to be an accident.'

The parenchymatous mass may later on develop an apical cell and grow out into an elongated germling-like branch, and, as will be shown in another paper, it may remain alive when the rest of the thallus has decayed.

The cytology of this stage has not been fully worked out, but what probably happens is that the conditions (temperature and light) being unfavourable to the development of the reduction stage (it is evidently very sensitive to external influence) the stalk-cell division is followed by ordinary vegetative divisions. As far as my observation goes this mode of cell-multiplication never follows the reduction division, and when this division is abnormal the death and disintegration of the cell inevitably ensues.

(4) In *Padina* 'twin' tetrasporangia are of very frequent occurrence. After separation from the stalk-cell the mother-cell nucleus instead of going into synapsis divides vegetatively, there is a longitudinal division of the cell, then the two nuclei go through the various phases of the reduction stage, but they lag somewhat behind the neighbouring sporangia, as if they had lost time by the extra division.

Conclusions.

1. Alternation of Generations.

It has now been shown that in the thallus- and in the stalk-cell divisions of the tetraspore plant the nucleus has about thirty-two chromosomes. The first division of the resulting mother-cell is different from all the others in its long preparation for division, its elaborate and distinctive spirem stages, the heterotype character of its chromosomes, and in the fact that here the number is reduced to sixteen. The succeeding division has the same number of chromosomes, and in the young plant produced from the tetraspore the earlier mitoses all show the reduced number.

As will be shown in detail in a succeeding paper the thallus-cells of both male and female plants are probably characterized by the reduced number: it is difficult to be absolutely certain where the nuclei are so small. In the oogonial and antheridial divisions, however, there is no doubt about the number. Furthermore, in all these various mitoses there is not one that resembles the reducing division in its distinctive characters.

In the interesting series of abnormal figures observed in the parthenogenesis of unfertilized eggs the chromosomes are always scattered, and consequently easily counted, and the number is invariably sixteen, whereas the fertilized oosphere in all its segmentations shows the full number. It

it difficult in the face of these facts to resist the conclusion that the germling produced from the tetraspore, with its sixteen chromosomes, is a young male or female plant, and that the segmenting oospore with thirty-two chromosomes to its nucleus is a young tetraspore plant.

There is a diffiulty which will have to be explained. In certain localities experience has shown that it is most difficult to find a sexual plant. There are extensive tracts along the coast where all the plants seem to be the form *intricata*, many of which have no reproductive cells of any kind, and the remainder are nearly always tetrasporic. *Taonia* has a similar reduction division to *Dictyota*, and from analogy one would expect to find alternation here. Since the year 1897, however, I have not succeeded in finding a single sexual plant, whereas the others are found without difficulty. This of course is merely negative evidence, and after all there may be a few individuals of the gametophyte generation even in these localities. Assuming that the above observation is correct, one has to account for two things—the failure of the tetraspores to produce sexual plants, and the perpetuation of the former in the absence of the latter. This cannot be fully discussed without going into the whole question of environment, which will be done in a later paper. It may, however, be suggested:—

1. That the same unfavourable conditions that give rise to the *intricata* form also account for the absence of sexual plants.

2. That one of the several modes of vegetative reproduction occasionally resorted to by the plant, including perhaps the curious abnormal development of the tetrasporangium-rudiment above described, may enable the asexual generation to perpetuate itself indefinitely.

Some hundreds of culture experiments have been tried in order to test the validity of the conclusions arrived at from cytological evidence. Plants reared from tetraspores and others from fertilized eggs have been kept alive for months, but owing to their sensitiveness to external conditions it has hitherto been found impossible to get them to develop reproductive cells. Improved methods are being tried at present, and it is hoped that these will be successful.

It may possibly be urged that the two generations in the higher plants are generally quite dissimilar in form and structure, whereas in this case they are identical in both respects. Where, however, the two generations, as in this case, are both strictly aquatic, there seems to be no inherent necessity for a difference of form or of structure.

II. The Nucleolus.

With regard to the nucleolus, it has been seen that in the earliest stage in all the mitoses this body is always fibrillar or granular. It is a suggestive fact that the same appearance is resumed during the prophase stage,

when it swells, becomes angular, and finally its membrane disappears and the fibrillae are allowed to disperse through the nuclear cavity.

The stages of the 'reduction' nucleolus are the following:—

1. The nucleolus is uniformly fibrillar, and probably contains the bulk of the chromatin.

2. It becomes vacuolate and the spherule forms.

3. It is much distorted and attached to the spirem; the staining power becomes gradually less.

4. A large vacuole appears, with fibrillar inclusions.

5. It becomes spongy and more deeply stained.

6. It swells, becomes angular and fibrillar.

7. It disintegrates into fibrillae and a nucleolar globule, which are both excluded from the daughter-nuclei.

The distorted nucleus accompanying the 'knotted' spirem, and its frequent actual attachment to the thread, irresistibly suggest that the nucleolus nourishes the spirem at this stage. This view is strengthened by the fact that about this time the nucleolus becomes less responsive to chromatin stains.

In the succeeding stage the very large vacuole occupying the greater part of the diameter seems to show that the nucleolus has been deprived of much of its substance. The significance of the included fibrils is not clear.

The spherule accompanying the reduction spirem is most probably a derivative of the nucleolus. It stains intensely with most chromatin stains, but shows some distinctive reactions, especially its retention of Carbol Fuchsin. Very often the spherule remains bright red when the colour has been extracted from all the other constituents. It is not homogeneous—very frequently deeper stained bodies can be seen within it. It shows no definite relation to other nuclear structures and its *rôle* cannot at present be explained.

The disintegration stage shows that the nucleolus contains two very different substances, the fibrillar chromatin-like constituent, and the globule —plasma-like in its colour reactions. If the former substance be not chromatin, then a third constituent must be added, for it is exceedingly probable that part of the chromatin is stored up in the nucleolus previous to karyokinesis.

It was shown that in the reconstruction of the daughter-nuclei the process in the stalk-cell division is different from that in all the others. In the tetrasporangium nucleus all the chromatin is at first stored up in the nucleolus, and there is no second body within it. This may be part of the elaborate preparation for the reduction of the chromosomes. In the telophase of the next division the chromatin is aggregated in a separate mass of fibrils which, however, become dispersed through the nuclear space and

then disappear. The study of these makes it clear that in the karyokinetic stage the bulk of the nucleolus is cast out into the cytoplasm in the form of fibrillae and globule, and it does not seem that either substance is used in the formation of the spindle, for they coexist with the mature spindle up to a late stage in metakinesis. The appearance of the new nucleolus follows so quickly upon the stage when the nuclear membrane encloses nothing but the dispirem, that we are forced to the conclusion that the substance separates from the chromosomes.

The centrosomes and other accessory structures will be discussed when the cytology of the sexual generation has been described.

With regard to the development of the chromosomes it is clear that if the description given above be correct, whatever the details of the metaphase stage may be, a transverse division of the chromosomes must occur somewhere. No other theory seems to account so satisfactorily for the facts as that of Farmer and Moore, already referred to; and I regard the figures of the prophase stage in the three Dictyotaceous genera examined as strongly confirmatory of the hypothesis.

EXPLANATION OF FIGURES IN PLATES IX AND X.

Illustrating Mr. Lloyd Williams' Studies in the Dictyotaceae.

All the figures have been drawn with the aid of the camera lucida and the apochromatic 4.0 mm., aperture 1·30 of Zeiss with ocular 6 (× 800). For convenience of comparison the same scale of magnification has been kept throughout, but a few of the figures having been drawn from tangential sections are apparently smaller than they ought to have been. Figures 2–19 have the stalk-cell on the lower side, and the terms 'basal' and 'distal' are used with reference to this cell.

Stalk-Cell Division.

Fig. 1. Tetrasporangium rudiment before stalk-cell division.

Fig. 2. Prophase of the stalk-cell division. The coarse spirem is beginning to segment and the nucleus is swollen and irregular in form.

Fig. 3. Early spindle formation. One of three sections, slightly oblique, showing a number of curved, split chromosomes, the fragmenting nucleolus with its globule, and the basal pole with a few spindle-fibres.

Fig. 4. Equatorial plate stage. The spindle is intranuclear. The remaining chromosomes are in another section. There is nucleolar globule on the spindle, and nucleolar fibrils between it and the membrane.

Fig. 5. Anaphase stage. The two centrosomes are visible, but radiations are confined to the distal pole. The nucleolar globule is on the connecting fibres.

Fig. 6. Telophase stage. The chromosomes form an irregular coil the strands of which in places appear double. The nucleolar globule is seen to the right. The tetraspore mother-cell nucleus is bigger than that of the stalk-cell, and its membrane is drawn out towards the distal centrosome.

The First or Reduction Division in the Tetraspore Mother-Cell.

Fig. 7. Early spirem. The nucleolus is vacuolated, and there are a number of small, deeply staining granules which probably fuse together to form the 'chromophilous spherule' peculiar to this mitosis. The radiations, to the right and left, are but faintly suggested, and no centrosomes can be distinguished.

Fig. 8. The 'knot' or 'Synapsis' stage. The spirem is in two knots near the very faint centrospheres. The spherule is seen to the left.

Fig. 9. Another example, showing the distortion of the nucleolus and the intimate connexion between it and the spirem.

Fig. 10. A slightly later stage. The spirem is spread out on the basal side. A strand is shown attached to the nucleolus. The axis of the nucleus is not median but nearer the basal side.

Fig. 11. The spirem is more evenly distributed over the membrane, and is distinctly beaded, with fine fluffy threads projecting laterally. The nucleolus has a large vacuole with included fibrils and granules.

Fig. 12. The spirem begins to split in two, and the staining is less deep. The section is transverse to the nuclear axis.

Fig. 13. A later stage in the splitting. The nucleolus and spherule appear in one of the other four sections through the nucleus. The reticulum becomes fainter.

Fig. 14. The identity of the chromatin thread has been lost, and the nucleus appears as if in a state of rest. The spherule is still present.

Fig. 15. A later stage. There are denser strands in the reticulum, the spherule has disappeared, the membrane, centrosomes, and radiations are more distinct, and the cytoplasm is differentiated into separate zones.

Fig. 16. Early prophase. Thick cloudy bands appear, with indications of longitudinal splitting. The section is not median, and consequently does not include the nucleolus and centrosomes.

Fig. 17. (*Taonia*). The chromosomes appear as loops with their limbs crossed, or as closed rings. The free ends of the chromosomes frequently appear split. The nucleolus is minutely vesicular.

Fig. 18, *a*, *b*, *c* (*Padina.*). Three sections of the same nucleus, showing the reduced number of chromosomes, all of which except one are uncut.

Fig. 19. The chromosomes reduced in size and ready for being placed on the spindle. A few spindle-fibres have already appeared, and the nucleolus is breaking up into fibrillae.

Fig. 20. Intranuclear spindle with mantle-fibres and nucleolar fibrillae. (The globule is in another section.) The chromosomes are heterotype in character.

Fig. 21. A slightly oblique section, showing more clearly the form of the chromosomes. The number is sixteen, but those in a lower focus have not been drawn.

Fig. 22. One of the poles of the preceding figure, showing the curved rod-like centrosome. The section containing the other pole is very similar.

Fig. 23. One of the two daughter-nuclei of the first division. There is a large mass of fibrillar chromatin and a small nucleolus.

Fig. 24. A later stage. The chromatin becomes distributed through the nucleus and the nucleolus increases in size.

Second Division in the Tetraspore Mother-Cell.

Fig. 25. Early prophase. Nucleolus swollen, irregular, and fibrillar. The section is transverse to the nuclear axis.

Fig. 26. A number of short curved chromosomes have appeared, and the nucleolus is irregular and fibrillar.

Fig. 27. Spindle. The polar radiations are directed towards those of the sister-nucleus, the membrane on the inner side is intact, the spindle being on the opposite side. A nucleolar globule is present.

Fig. 28. Anaphase stage. The nuclear cavity is much narrower and curved. The chromosomes though small show no signs of fusion.

Fig. 29. One of the four nuclei of the sporangium before spore differentiation.

The First Division in the Germinating Tetraspore.

Fig. 30. Prophase. Very coarse spirem and fibrillar nucleolus.

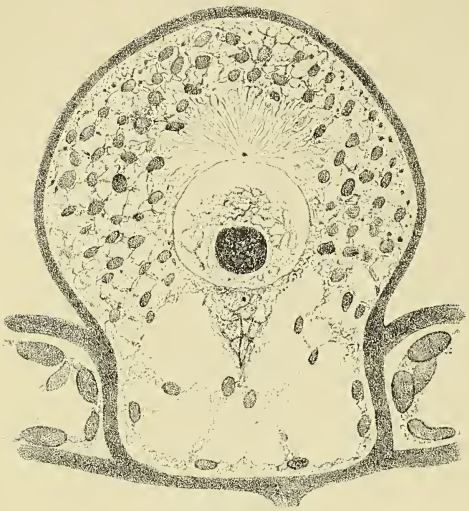
Fig. 31. Spindle with curved chromosomes (one has lagged behind). There are two sections of this, and the number of chromosomes in the two together is sixteen. The radiations are distinct; there is no globule.

Fig. 32. Anaphase stage. Membrane still intact at the sides.

Fig. 33. 'Dispirem' stage. The nucleolar membrane projects towards the very distinct centrosome.

Fig. 34. Polar view of daughter-nuclei before fusion of chromosomes. About sixteen may be counted. Near the bottom of the figure a small body staining differently to the chromosomes is seen. This is probably the beginning of the nucleolus.

Fig. 35. A later stage than Fig. 26, where a chromatin mass, a nucleolus, and a reticulum have been differentiated in each daughter-nucleus.



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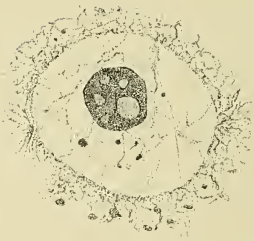

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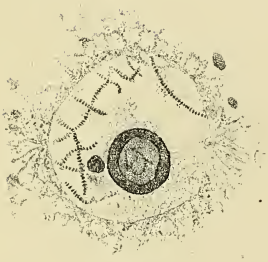

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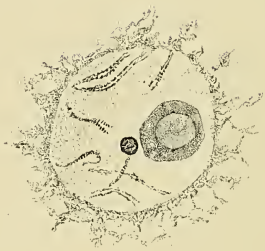

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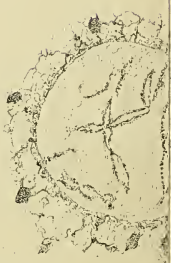

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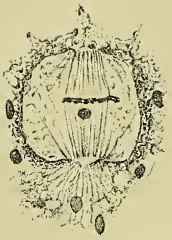

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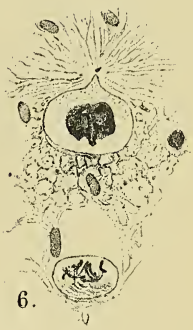

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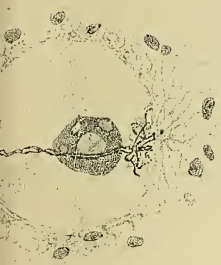

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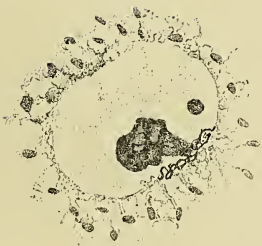

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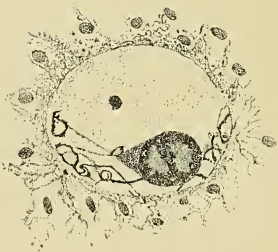

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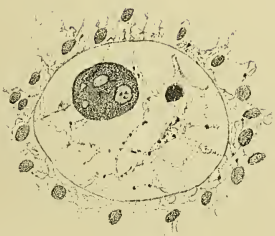

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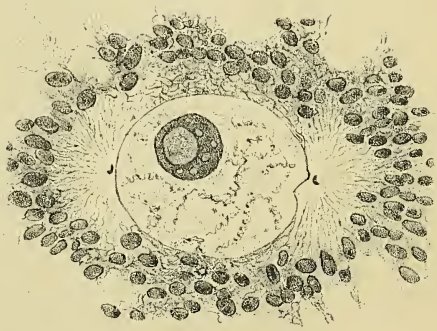

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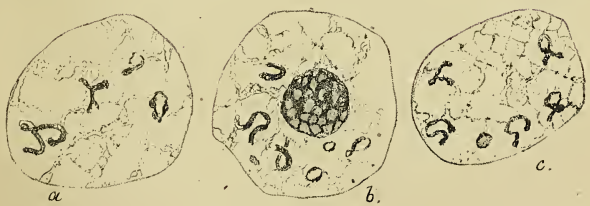

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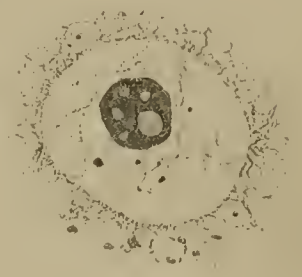

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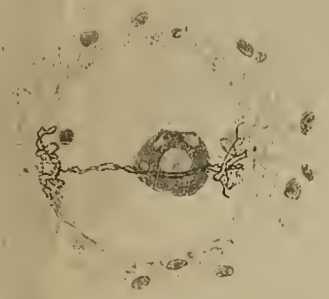

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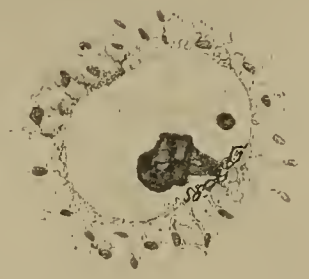

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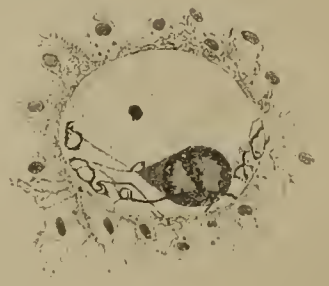

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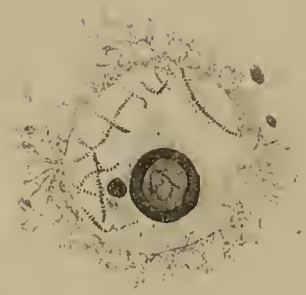

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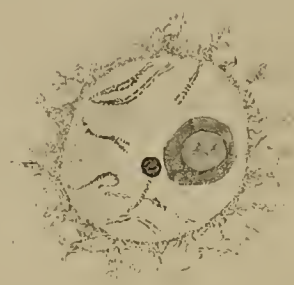

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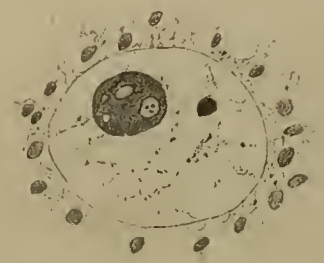

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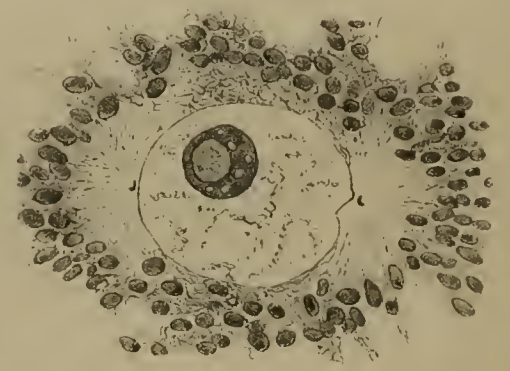

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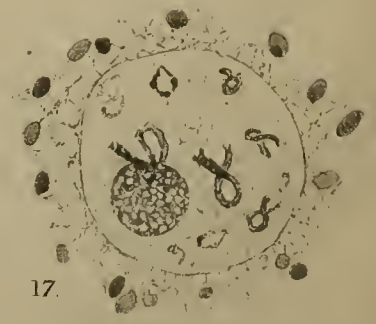

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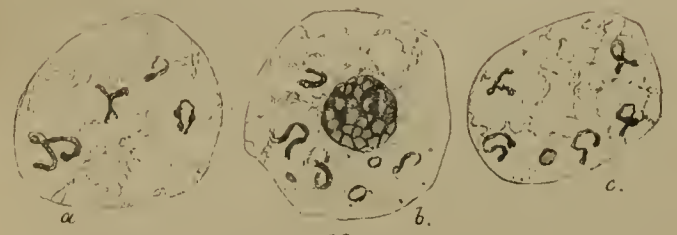

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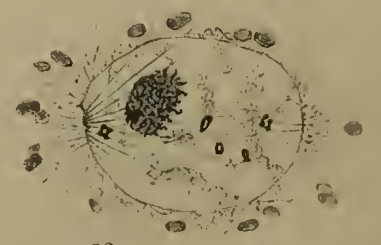

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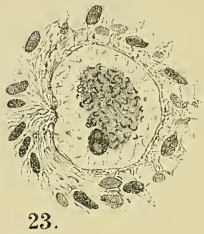

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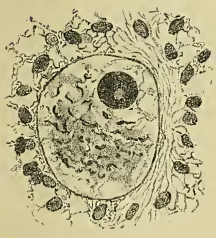

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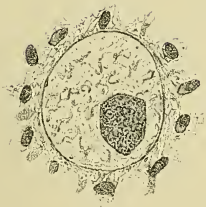

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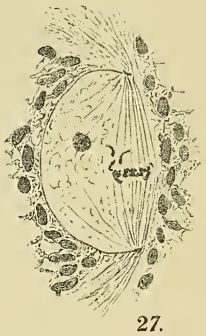

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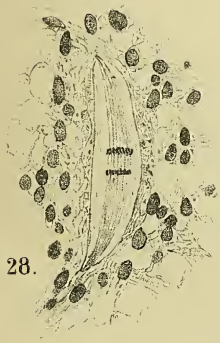

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LLOYD WILLIAMS, —— DICTYOTACEAE.

Telangium Scotti, a new Species of Telangium (Calymmatotheca) showing Structure.

BY

Miss M. Benson, D.Sc.,

Senior Lecturer in Botany at the Royal Holloway College, Englefield Green.

With Plate XI and a Figure in the Text.

AMONG the numerous plant remains preserved for us as impressions on the Palaeozoic rocks are some digitate clusters attached to branching petioles devoid of lamina, and associated with, and sometimes attached to, leaves of the *Sphenopteris* type.

They were first investigated and named by Dr. Stur [1]. The species *Calymmatotheca Stangeri*, Stur, may be taken as the type of these impressions. Dr. Stur regarded the constituent parts of the cluster as indusial valves, but they were differently interpreted by Renault, who figured them in his 'Cours Fossile' [2] as sporangia.

M. Zeiller [3] also supported the sporangial interpretation of the lobes. Several species of genuine sporangia have subsequently been included in the genus *Calymmatotheca*, e.g. *C. affinis* and *C. asteroides*. They were all founded on casts, however, and it was not until May, 1902, that petrifactions were obtained. Sections of coal nodules from the Gannister beds of Dulesgate and Hough Hill have recently been yielding a good many of these synangia, some of which have been beautifully cut in series by Mr. Lomax of Bolton.

This led to a re-investigation [4] of Stur's type-specimens, which has convinced me that he was right in his interpretation of his specimens, and that

[1] Die Culm-Flora, 1875–77.

[2] Cours d. Botan. Foss., troisième année, p. 198, 1883.

[3] Bassin houiller de Valenciennes. Flore Fossile, 1888, p. 34. Sur quelques Fougères houillères d'Asie Mineure. Bull. Soc. Bot. de France, tom. xliv, p. 199.

[4] Dr. Scott and Prof. Oliver tell me they have come to the same conclusion after a careful inspection of the specimens. The re-investigation was rendered possible by the kindness of the Director of the Geol. Reichsanstalt at Vienna, who, at the request of Dr. A. Smith Woodward, F.R.S., lent the valuable specimens in question to the Geological Department of the British Museum, so as to give English Palaeobotanists an opportunity of examining them.

[Annals of Botany, Vol. XVIII. No. LXIX. January, 1904.]

no sporangia can therefore be included in the genus *Calymmatotheca*, which he founded upon *C. Stangeri, C. Haueri, C. Schimperi*, and *C. minor*. I have founded therefore the form-genus *Telangium* for the reception of such specimens as can be diagnosed as follows:—Fertile and barren pinnae dissimilar; fertile pinnae represented by synangia only; synangia borne at the extremity of the ultimate ramifications of rachis, composed of 6–12 sporangia which taper to the apex and are united primarily for almost their whole length to form a body which is continued into a sterile base of decreasing diameter through which runs longitudinally a single vascular strand. Each sporange ultimately becomes almost free from the others by septicidal dehiscence and liberates large spores from a ventral suture. As I have not been able to identify these new specimens with any so far described species, I have much pleasure in calling it *Telangium Scotti*, after Dr. D. H. Scott, F.R.S., whose work on *Lyginodendron* has added to the interest in this type of fructification.

The first specimen that came into my hands was the longitudinal, tangential section represented in Pl. XI, Fig. 1.

The longitudinal dimensions of the sporange on the left are 3·2 mm., but the full length of the sporange was probably somewhat greater. If one compares Fig. 8 one sees that the sporange has really a free narrow apex which brings its length up to 3·8 mm.

The sterile base would probably have brought the length of the whole synangium up to at least 5 mm.

The width of the synangium averages a little under 3 mm. before dehiscence, if it be measured at the widest part.

Shortly after examining this preparation I was enabled by the kindness of Prof. F. W. Oliver to look through slides from a similar source that belonged to the Collection at University College, London. These yielded a beautiful series of four slides (C.N. K3 *a–d*), cut from a block containing three synangia, and Figs. 2 and 3 have been drawn from them. Mr. Lomax has recently cut another excellent series, which is now in Dr. Scott's Collection, and has been kindly lent to me with other slides for the purposes of this paper.

One slide from the Manchester Collection, which I owe to the kindness of Prof. Weiss, has also been of service and is shown in Fig. 9.

It will be seen from the drawings that the synangium has eight sporangial chambers arranged in two rows. If we take the transverse sections in the order of their position, beginning at the base, we should first examine Fig. 4 A, which is a transverse section of a synangium immediately below the insertion of the sporangia.

The long diameter of the ellipse is 1·7 mm., and the short diameter is ·9 mm. in length.

This section shows the vascular bundle in transverse section, the

outline of which is not very clearly defined, but can be seen to contain tracheides of narrow lumen, *v.* Fig. 4 C. These are best seen rather to one side of the section, and possibly occupy one arm of a V-shaped strand.

The whole section is limited by the large-celled epidermis with blackened contents ; within this are groups of thin-walled cells which have broken down, and at *l* the section has passed through the lacunar tissue, which is visible again in Fig. 2 and partially in Fig. 1.

Fig. 2 represents a section of another synangium at a level just above that of Fig. 4 A. We see that the dimensions have much increased. Two of the sporangia have been injured. The walls are thicker than they are nearer the apex, and show at least seven layers of cells. The epidermis is supported by a hypoderm which is not so regular as is the case at a higher level. The lacunar tissue appears at *l* and l_1. I have been unable to trace any vascular strand at this level.

Fig. 4 B is a section at a slightly higher level. The septa have given way at two places which probably represent the basal parts of the fissures shown in Fig. 5 B, which is another section of the same synangium. Fig. 4 B shows the hypoderm to consist at this level of an interrupted layer of cells empty of contents. In many cases they show scalariform marking. This can be best seen in Figs. 1 and 2. They are elongated in the long dimension of the sporange, and are probably not continuous with the vascular strand but simply hypodermal cells differentiated for some special function. It is of course possible they were of use at first as water-conducting elements, but it seems more probable that their chief function was to secure dehiscence. They may be regarded as physiologically analogous with the fibrous layer in the wall of the pollen-sac of Angiosperms. If we refer to Fig. 5 *h h'* we shall see that on the outer wall between each sporange there is a group of thin-walled cells which tear on dehiscence. This would be brought about by the contraction of the convex, free portion of the sporangial wall. This contraction may very well have been due to the hygroscopic structure of the membrane of these hypodermal cells.

At intervals the living parenchyma interrupts these cells. This may be seen well in Fig. 3 and in 5 A, at *x* and x^1, and it is possible that contraction may have been aided by loss of turgidity in these cells.

The sections taken at a higher level where the sporangia have separated from one another are represented in Figs. 3, 5 B, 7 B, and 9. It is easily seen that part of the wall of each sporangium is composed of a segment of the peripheral wall and a thinner part derived from the partition which splits longitudinally, reminding one of the septicidal dehiscence of a syncarpous fruit. There is, however, one exception to this, for the epidermis is complete all round the extreme apices which appear to be free primarily,

v. Fig. 5 B, *ep.* This should be compared with Fig. 8, *ep.*, where the free apex may be seen still containing spores.

The spores seem to have been ripe, and in most cases have been partially shed. In one sporange in Slide C.N. . M . 21 (*a*) from Sharney Ford, kindly lent me by Prof. Oliver, there are numerous spores. Fig. 6, *a*, *b*, *c*, *d*, are drawings of such spores made with the help of Zeiss's Abbé Camera, and show the form and characteristic wall of the spore. The spores vary slightly in size and form. Many are somewhat elliptical, measuring from 5 to 6 μ in the longer dimension, and from 4 to 3·5 μ in the shorter. Many look circular, but this may of course be due to the elliptical forms being looked at end-on. They are reticulately marked, the ridges meeting at an angle of about 120°, and the thinner areas are approximately hexagonal.

In form and in the character of the wall these spores agree very closely with the pollen-grains in the pollen-chamber of *Lagenostoma ovoides*, which are drawn in Fig. 6, *e*, *f*, *g*. The size of the latter is, however, slightly greater. Those in the pollen-chamber of a *Lagenostoma ovoides* in my collection measure 6·75, and 7·2 μ in the longer dimension and 5 μ in the shorter. An increase in size of a pollen-grain after entering the pollen-chamber is known to occur. In the interesting parallel case recorded by Renault of the pollen-grains of *Cordaianthus* the shagreen-like coats of the spores were similar, but the size of those in the pollen-chamber showed a marked increase. We are now in a position to construct a diagrammatic figure if we superpose these sections upon one another, and the result is given to scale in Fig. 10. Septicidal dehiscence has advanced almost to the base of the synangium.

Fig. 33. ×4.

It will be seen how closely this resembles some of the *Calymmatotheca* impressions, which are reproduced for comparison (Figs. 11 and 12 and Text-Fig. 33). Stur's drawings of *Calymmatotheca Stangeri* (Fig. 11) show a form very similar to that of *C. Scotti*, except that the former is distinctly larger, measuring 6 to 8 mm. in length, whereas *C. Scotti*, even if we take account of the sterile base, can hardly have reached more than 5·5 mm.

But the chief difference is due to the absence of relief in the impression, which in this respect offers a sharp contrast to the form reproduced photographically in Text-Fig. 33. The many specimens of the latter I have seen lead me to wholly agree with Mr. Kidston's interpretation of it as sporangial, and I shall, for convenience, therefore refer to this species as *Telangium affine*. The same may be said of the other British species which will be referred to under the names *Telangium asteroides* and *T. bifidum*.

Count Solms-Laubach, in a recent review[1] of my preliminary note to this paper in the Annals of Botany, 1902, stated that the agreement between the new species and *C. Stangeri* is not perfect owing to the absence of thorn-like emergences on the back of the sporangia in the new species. This was in reference to Stur's Plate VIII, figs. 5 and 6, which show emergences on the lobes reminding one of the glands on the newly discovered outer envelope of *Lagenostoma Lomaxi*; and it is of course possible that this may be shown by Drs. Oliver and Scott to be their nature. This valuable criticism is met by my present action in withdrawing the new synangium altogether from Stur's genus and founding a new one.

In 1877 a paper[2] by the late Mr. C. W. Peach was read before the Geological Society describing some beautiful casts[3] of a smaller form of *Telangium*, hitherto known as *C. affinis* (see Fig. 12). He found them attached to fronds of *Sphenopteris affinis*, and suggested that they were parasitic upon them. 'Each flower-like form' (Mr. Peach's expression for the synangium) 'is about $\frac{1}{8}$ in. over and fully that in height.'

Mr. Peach compared his specimen with that of *C. minor* as figured by Dr. Stur, p. 237 of his 'Culm Flora,' and it is not impossible that *C. minor* may be a *Telangium*.

By the kind permission of the Council of the Geological Society I have been enabled to reproduce two of Mr. Peach's figures (Fig. 12 *a* and *b*).

The form of *T. affine* is very like that of *T. Scotti*, but it is distinctly smaller. The dimensions as kindly given me by Mr. Kidston are as follows:—

Length, 2·5 —3·5 mm.
Breadth, 2·75—3 „ (after dehiscence).

Those of *T. Scotti* are as follows:—

Length, 4·5—5·5 mm.
Breadth, 3 „ (before dehiscence).

The figure in the text is reproduced from a photograph kindly made expressly for this paper by Mr. Kidston of a specimen of *T. affine* in his possession. It is noticeable that the synangia of *T. affine* are represented sometimes in approximation and in planes parallel to one another (Fig. 12 *a*). This seems to have been the case also in *T. Scotti*, as is shown in Figs. 3, 4, and 5. These specimens of *T. affine* were found by Mr. Peach in the Calciferous Sandstone rocks of North Britain, and the two species thus belong to different horizons.

Another British species the description of which we owe to Mr. Kidston[4]

[1] Bot. Zeitung, 60, Dec. 1902.
[2] Quarterly Journal of the Geol. Soc. of London, vol. xxxiv, p. 131.
[3] Admirable specimens of these are preserved in the British Museum.
[4] Trans. of the R. S. Edin., vol. xxxiii, p. 140.

is *T. bifidum*. The synangia are still more markedly aggregated than those of *T. affine*. A careful examination of Mr. Kidston's Figs. 1–6, Plate VIII, would lead me to conjecture that the synangium is composed of not more than ten or twelve sporangia, and that the appearance of a greater number is due to the shortness of the ultimate ramifications and hence the almost capitate condition of the fructification. The dimensions of the synangium of *T. bifidum* are given by Mr. Kidston as follow:—

Length, 6·5 —6·7 mm.
Breadth, 3·75—4 „

Mr. Kidston's Fig. 6 *a*, Plate VIII, depicts one synangium in which bipartition is very noticeable, and may be compared with Fig. 7, which represents a synangium of *T. Scotti* showing the same tendency.

We see from this review that, as respects size, *T. Scotti* is intermediate between *T. affine* and *T. bifidum*, and that it shows many features in common with both species. The only species of *Telangium* recorded so far from the Upper Carboniferous[1] is *T. asteroides*. This is generally represented as having had but six sporangia in its synangium, but I have not been able to confirm this from the specimens preserved in the British Museum. The longitudinal dimension of the synangium is a little over 3 mm. The synangia are borne on branching petioles like those of other species. Owing perhaps to its imperfect preservation it does not seem to be so near *T. Scotti*, the new Upper Carboniferous form, as do several of the species already referred to, which belong to the Lower Carboniferous.

As the attribution of *Telangium Scotti* to *Lyginodendron* was at the time[2] partly based upon what Dr. Scott and I now consider to be a misinterpretation of Stur's type specimens of *Calymmatotheca Stangeri*, it remains for me to discuss what evidence is still available in support of the view adopted in the preliminary note [2] to the present paper.

Not only is internal evidence available owing to the preservation of the tissue of *Telangium Scotti*, but the recent announcement[3] on the part of Messrs. Oliver and Scott that the seed *Lagenostoma Lomaxi* grows attached to an envelope showing characteristic structural features of *Lyginodendron Oldhamium* has given unexpected opportunity for further comparison.

The evidence may now be summarized under the following headings:—

1. Association and character of impressions or casts.
2. Association of petrifactions.
3. Character of tissue.
4. Correspondence between the spores of *Telangium Scotti* and the

[1] Potonié's statement in Engler's Pflanzenfamilien, Teil I, 4. Abteilung, p. 449, that *Calymmatotheca* belongs to the 'Ober-Carbon' seems due to an error, as he does not refer to *C. asteroides*.

[2] Benson, The Fructification of *Lyginodendron* (note), Annals of Botany, xvi, 1902.

[3] Proc. R. S., vol. lxxi.

pollen-grains germinating in the pollen-chamber of *Lagenostoma Lomaxi* and *L. ovoides*.

5. Correspondence in certain morphological characters between the synangium of *Telangium Scotti* and the seed *Lagenostoma*.

We will deal with these subjects in succession, and the last will be found to involve a wholly new theory of the phylogeny of the inner integument.

Firstly, association and character of impressions. Those who have had the pleasure of studying the numerous and beautiful plates the late Dr. Stur included in his 'Culm Flora' and 'Carbon Flora' cannot but be impressed with the family likeness which seems to reign among the fronds, whether they are called *Calymmatotheca*, *Diplothmema*, or *Sphenopteris*. Zeiller has expressed the view that they all belong to stems of the *Lyginodendron* type. The branching of the leaves may be dichotomous, or pinnate, or various combinations of both systems. These leaves are in one case found associated with one species of *Telangium* fructification. Thus *T. minor* is found associated with *Sphenopteris* (*Diplothmema*) *patentissima*, and also with indusiate seeds which Stur calls *Rhabdocarpus conchaeformis*.

Turning to records of British impressions of *Telangium* we have three—*T. affine* and *T. bifidum* from the Lower Carboniferous, and *T. asteroides* from the Upper, i. e. the Lower Coal Measures.

T. affine is not only associated with but attached to leaves of *Sphenopteris affinis*, so much so, indeed, that Mr. Peach in the description of his beautiful specimens suggests that they were parasitic upon the leaf. The frond in this case, which is familiar to many as represented in the frontispiece of Hugh Miller's 'Testimony of the Rocks,' dichotomizes freely, and thus exhibits a type of branching also found in *Sphenopteris elegans*, the leaf of *Heterangium*. *T. bifidum* is also found growing on leaves very similar in character to those of *Sphenopteris affinis*, as bifurcation is frequent. There is no reason to expect in such an advanced type as *Lyginodendron* an exact correspondence in size and form between the microsporophyll and the sterile frond, and with this interpretation of such fronds in view it is interesting to note in this latter British species the appearance of the synangia only on the more basal part of the leaf.

Secondly, the association of the petrifaction *T. Scotti* with *Lyginodendron* in the coal-nodules of the Gannister beds of Lancashire. Not much weight can be attached to the fact of the association with fragments of *Lyginodendron* owing to the great abundance of the latter in these nodules. But the value of the association is augmented by the fact that *T. Scotti* appears in sections of a nodule from Sharney Ford which is otherwise almost purely composed of the vegetative organs of *Lyginodendron*. It may also be stated that in several of the slides containing sections of *T. Scotti* there are also *Lagenostoma* seeds. Their close approximation

is shown in one case in Fig. 9, which is from a slide kindly lent by Professor Weiss from the Collection of the Manchester Museum, Owens College.

Thirdly, the character of the tissue. The tissue of the lower part of the synangium has much in common with the familiar sterile pinnae of *Lyginodendron*. We have a well-developed epidermis, a definite hypoderm, and lacunar tissue which is indistinguishable from the corresponding tissue of the sterile pinna. The vascular strand of the pedicel is composed of tracheïdes of very narrow lumen, and thus resembles those of the petiole of *Lyginodendron*. The preservation of the tissue is unfortunately not good enough to show the form of the strand clearly. The group of tracheïdes which is preserved (Fig. 4 C) may be the whole, but it is possible that another corresponding group may have occupied the other arm of a V-shaped strand, but has become opaque owing to the minuteness of the lumen of the tracheïdes.

Fourthly, correspondence between the spores of *Telangium Scotti* and the pollen-grains germinating in the pollen-chamber of *Lagenostoma Lomaxi* and *ovoides*. Ripe spores occur in five of the synangia already to hand, and have been measured by Prof. Oliver and myself. As already pointed out they agree with considerable exactness in form and in the character of the wall with the pollen-grains in the pollen-chamber of *Lagenostoma,* but the latter slightly exceed them in size. This comparison, already found of value in the magnificent work of Renault on *Cordaianthus*, is of great interest. The spores of *Telangium* average 5·5 μ in their longer and 3·7 μ in their shorter dimension. The spores when they are germinating, apparently in the very act of yielding antherozoids like those of Cycads and *Ginkgo*, measure 7×5 μ. The wall of both is thick and has thinner areolae, and thus may be described as reticulate, *v.* Fig. 6.

Fifthly, correspondence of *Telangium Scotti* in certain morphological characters with the seed *Lagenostoma*. The seed *Lagenostoma* (the three species of which were first named and partially described by Williamson) has since received a searching investigation at the hands of Prof. F. W. Oliver[1]. The connexion of one species, *L. Lomaxi*, with *Lyginodendron* has recently been announced[2] by him and Dr. Scott. To quote from their account of this species: 'In the most general relations of its organization the seed approaches the Gymnosperm type in that the integument and nucellus are distinct from one another in the apical region only, whilst the body of the seed which contains the large single macrospore shows complete fusion of the integument and nucellar tissues. But in other respects the seed is remarkable. The integument, which is a simple shell where fused with the nucellus, becomes massive and com-

[1] See 'Oliver, The Ovules of the older Gymnosperms,' Annals of Botany, xvii, 1903, Pl. XXIV. Fig. 9.

[2] Proc. R. S., vol. lxxi.

plicated in its free part which corresponds to the upper fifth of the seed. In this region it is usually composed of nine chambers radially disposed around the micropyle. The whole structure from within is like a fluted dome or canopy, the convexities of which correspond to the chambers. The vascular system of the seed enters as a single supply bundle at the chalazal papilla and branches a little below the base of the macrospore into nine radially-running bundles. Each of these bundles passes without further branching to the apex of the seed, running outside the macrospore and a little distance below the surface. At the canopy the bundles enter the chambers and end at the tips.'

A somewhat lengthy quotation has been made, as it is necessary to understand the structure of the seed if the comparison with the microsporangial sorus is to be appreciated. The transverse section[1] of the seed, if taken in the plane of the canopy, somewhat resembles a cartwheel, in which the nucellar apex forms the axle, the radial walls between the chambers the spokes, and the peripheral walls of the chambers the rim of the wheel. The comparison does not hold good, however, in well-preserved sections, as the chambers are seen each to contain large, thin-walled cells which support the delicate branch of the vascular bundle that is contributed to each.

The correspondence which must have already suggested itself to the reader is between such a seed as *Lagenostoma* and such a synangium as *Telangium Scotti*. The chambers surrounding the nucellus seem to represent its sister sporangia, which have become sterile, the large-celled, thin-walled tissue and delicate vascular strand being all that represents the ancestral sporogenous tissue; while the micropyle corresponds with the original space between the tips of the sporangia. The seed in fact is assumed to be a synangium in which all but one of the sporangia are sterile, and form an integument to the one fertile sporange which has become a megasporange with one large megaspore. In *Lagenostoma physoides*[2] the integumental ridges are continued into tapering tentacles around the micropyle, and this still further accentuates the resemblance to a sorus. In *L. ovoides* the number of chambers is often seven instead of nine. Hence we have only to imagine that one of the sporangia of a sorus of eight or ten sporangia gradually evolved megaspory, and that the remaining seven or nine sporangia became a sterile envelope,—a correlation in development which has many analogies in the animal and vegetable kingdoms. As soon as one of the sporangia became a megasporange the symmetrical arrangement of the sister sporangia would become an advantage and naturally follow. At the remote period of time at which the seed was

[1] Oliver, loc. cit., p. 461.

[2] See Williamson, Phil. Trans., vol. clxvii, 1877, Pl. XI, Fig. 77. A full account by Prof. Oliver of this seed will appear shortly. It should be compared with *Telangium bifidum*.

evolved, a period probably anterior to the Carboniferous epoch, it may be conjectured that the arrangement of the sporangia in the sorus was irregular, and that the more centrally placed sporange with its better vascular supply may have gradually attained the megasporangial condition. In *Gleichenia* and *Oligocarpia* some sori have, and others have not, a central sporange. As respects the vascular supply in the centre of each compartment of the integument, it is well known that in many of the Permo-Carboniferous seeds a vascular bundle entered the base of the nucellus, even passing from the chalaza to the pollen-chamber[1], and it is hence easy to conceive of a vascular strand having early entered its sister sporangia. Again, if we take an example from a seed of very remote affinity, we find that in *Castanea* a vascular strand may be demonstrated running up the whole length of the nucellus, and is especially well developed in nucelli whose embryo-sacs have long remained unfertilized.

I will now proceed to show that this interpretation of the integument of *Lagenostoma* is helpful in clearing away many of the difficulties that have beset the general problem of the integument hitherto. The more generally accepted interpretation of the inner integument is that it is due to a special development of the indusium. We are compelled to regard the integument of *Lagenostoma* as a single integument, firstly because of the primitive character of the seed, and secondly because of the existence in *L. Lomaxi* of an exterior envelope. Hence it is probably safe to regard it as homologous with the inner integument, and consequently as hitherto accounted for merely by Čelakovský's theory of the indusium, or by another theory to which I will allude later. But the cohesion of integument and nucellus which we know to be characteristic of the Cycadean seed receives no explanation on the indusial theory, whereas on the synangial theory the cohesion is seen to be due to the origin of the seed from structures already coherent.

Moreover, as it is generally agreed that the heterosporous habit arose from the homosporous, it is *a priori* probable that there should be a correspondence between the microsporangial sorus and the primitive seed, and this correspondence seems best obtained by harmonizing the seed and the synangium.

If it should be shown conclusively that *T. Scotti* is the microsporangial organ of *Lyginodendron* the homologizing of *Lagenostoma* with its ♂ synangium would simplify the problem of the integument in that we should then have but one envelope to account for in the seed over and above what was present in the male sorus.

I will now refer shortly to another widely accepted view, which has been adopted by Strasburger, Treub, and Dr. Lang. Though their views

[1] Oliver, loc. cit., p. 454.

vary as to the homologies of the seed as a whole they agree in regarding the integument as a new formation.

Dr. Lang's conclusions are based on his own investigation into the morphology of the sporangia of *Stangeria*[1], and on the results of work by Warming and Treub on other genera of Cycadaceae. He points out that 'with regard to the development considerable correspondence between the ovule and the sorus can be traced in the early stages. The differences between the development of the sorus of microsporangia and the ovule only become pronounced when active growth becomes localized around each archesporial group[2].' He therefore homologizes the sorus and the ovule at the outset, but looks upon the ovular sorus as monosporangiate and the integument 'as an annular upgrowth, around the apex of the nucellus, of the bulky sporangial wall or, which comes to the same thing, of the edge of the receptacle which had kept pace with the single sporangium.'

Thus it would appear that owing to the relatively advanced type of seed investigated, Dr. Lang could not homologize the upgrowing 'edge' of the receptacle with sterilized sister-sporangia of the nucellus. He adds that his view is only put forward as a provisional statement, which will have to be tested 'in the light of the evidence obtainable from extinct forms.' It is in the light of these extinct forms that the new theory of the integument is now being put forward.

Whether *T. Scotti* be ultimately proved to belong to *Lyginodendron* or not, we may well bear in mind that the synangium is a very ancient type of fern fructification, for from the Culm onwards we have numerous examples of it recorded. Where the individual sporangia are not entirely coherent they generally form a sorus of bulky sporangia like those of the Filicinean class 'Simplices' suggested by Professor Bower. The ancient sporange was very rarely solitary, and we have already undoubted evidence in *Cycadeoidea* of a seed-plant having synangia for its microsporangial organ.

Among synangia which are found associated with Cycadofilicinean seeds are *Hawlea* and *Scolecopteris*[3]. The latter I will shortly describe, as I believe a reference to it may make the comparison of seed and synangium more clear.

Scolecopteris is a form-genus including several species of sorus, which have been described by a succession of palaeobotanists[4]. It is sufficient

[1] Lang, Annals of Botany, xiv, 1900.

[2] Note the support that these observations give to the soral theory of the seed.

[3] Kidston, 'On the Fossil Flora of the Radstock Series of the Somerset and Bristol Coalfield.' Trans. R. S. Edin., 1888. Also, 'On the Fructification of Carboniferous Ferns,' Trans. Geol. Soc. Glasgow, vol. ix, 1889, Plates II and III. Further announcements bearing on this subject will shortly be made by Mr. Kidston.

[4] Strasburger, 'Scolecopteris elegans, Zenk.,' Jenaer Zeitschrift für Naturw., vol. viii, 1874.

for our purpose to refer to the drawings of *Scolecopteris polymorpha* in Engler and Prantl, Teil I, Abt. 4, p. 440. It will be seen that the sorus as a whole somewhat resembles *T. Scotti*, but the four or five sporangia, which here constitute the sorus, are inserted around a pedicel along which runs a vascular strand. If this were to become continuous with a strand of tracheïdes developed in the sporogenous tissue, we should obtain the vascular supply which characterizes *Lagenostoma*.

The beautiful plates in Brongniart's 'Recherches sur les graines fossiles silicifiées' afford many opportunities of applying and testing the new theory, and amongst others I would suggest a reference to the following:—

Plate IX, Fig. 4, showing a vascular bundle entering the nucellus in *Rhabdocarpus subtunicatus*.

Plate XIII, Figs. 6, 7, 17, 19, showing the contrasted tissue-systems of the integument of *Sarcotaxus avellana* and its septicidal dehiscence.

Plate IV, Figs. 1 and 3, showing the sporangial appearance of the *inner* integument continued to the base of the nucellus in *Cyclocarpus nummularis*. (These figures should be compared with *Telangium Scotti*, Fig. 8.)

Plate C, Fig. 9, in which the seed *Codonospermum* is shown to present a striking external resemblance to such a synangium as *Asterotheca*.

The similarity of the inner integument of *Pachytesta* to that of *Lagenostoma* has been recently pointed out by Professor Oliver[1], and a transverse section has been constructed which exhibits its compartmental nature at a level much lower than that in which it can be demonstrated in *Lagenostoma*. Professor Oliver adds: 'The presence of vascular strands in the chambers of *Lagenostoma* is the most important difference.'

Much fuller details are to hand of another seed which seems to bear out this theory. I refer to *Bennettites Morierei*, Sap. and Mar. (spec.), which has been admirably worked out by Professor Lignier[2]. This fructification, as is well known, belongs to a much later horizon, i. e. Mesozoic, and shows Cycadean affinities.

If one consults Lignier's Plate III, Figs. 35 and 37, one sees transverse sections of the upper part of the seed, showing the thick integument divided up into four compartments by radiating vertical walls of flattened cells, very comparable to those which separate the constituent members of a synangium.

The interior of each compartment is described as succulent tissue, but offers an abrupt contrast to the walls. Plate III, Fig. 38, shows

[1] Oliver, 'On some Points of apparent Resemblance in certain Fossil and recent Gymnospermous Seeds.' New Phytologist, vol. i, p. 150, Text-figure 5.

[2] O. Lignier, 'Structure et Affinités du *Bennettites Morierei*, Sap. and Mar. (sp.).' *Végétaux fossiles de Normandie*. Caen, 1894.

the constitution of the integument at a lower level. Here we find the peripheral epidermis of the integument lined as in *Telangium* and other synangia, with a layer of reticulately thickened cells within which lie the large thin-walled cells which seem to correspond with the sporogenous tissue, and this is limited internally by thick-walled fibres. Plate IV, Fig. 45, shows also on a smaller scale the compartmental structure of the integument.

It is interesting to note that *Bennettites Morierei* is in some respects evidently less reduced than *Bennettites Gibsonianus*, in which, as Dr. Scott says in his 'Studies,' the structure of the pericarp is a matter of inference. Nor is there any possibility of avoiding the conclusion that the inner tube of the micropyle is nucellar in origin if we accept the diagrams Prof. Lignier gives.

I cannot but regard this example as very strongly confirmatory of the homology of the seed with the synangium. If we compare the peripheral epidermis of the integument with that of the microsporangial sorus of *Cycadeoidea* we obtain a possible explanation of the radially elongated epidermal cells[1] of the sunken seed. Is it possible to call in here the aid of a wholly hypothetical indusium and invest it in turn with so many points of similarity to the sister sporangia of the nucellus, sporangia which it cannot but be granted originally surrounded the ancestor of the megasporange? Or, on the other hand, can we, with others, call in a 'new formation' to account for an integument so obviously compartmental?

Thirdly, I wish to refer to the seeds which somewhat unfortunately go by the name of *Gnetopsis elliptica*[2], Ren.

Although they are not yet worked out with the same detail as *Lagenostoma* and *Bennettites Morierei* there is considerable internal evidence in support of their synangial origin.

They are figured (after Saporta and Marion) in the English edition of Solms-Laubach's Fossil Botany on page 128, and come from the Upper Coal Measures of Grand' Croix. The ovules occur in one or more pairs in the hollow of a cup-like envelope which bears long hairs.

For convenience I will quote from Solms-Laubach's description of this most interesting type: 'That portion of the integument which encloses the apex of the nucellus behaves in a very peculiar manner, and may be compared perhaps with *Lagenostoma*, Will. It attains a considerable thickness and separates (*sic*) into a compact outer lamina and a similar inner lamina, while the cell-layer between the two is formed of extended filaments which represent so many cells and traverse a broad intercellular space at some distance from each other. This looser tissue ceases of course at the micropylar canal, where the outer and inner layer are in connexion with one

[1] Cf. Figs. 99, 101, and 102 in Coulter and Chamberlain's 'Gymnosperms.'

[2] Renault, 'Cours de Bot. Fossile,' T. 4, p. 179, Plates 20–22.

another. The margin also of the orifice of the micropyle is formed of a cup-shaped expansion which is seen to be drawn out at two points into long filiform appendages. A vascular bundle enters at the base of the ovule and splits into four branches.' If this account were translated into the language of this new theory we should say that each of the four abortive integumental sporangia contains loose elongated cells in its upper part, and that their extreme apices are prolonged much as in *Lag. physoides*, only that they remain adherent in pairs. The other two species of *Gnetopsis*, *G. trigona* and *G. hexagona*, are known only as impressions, and show four or five tentacles around the apex [1].

If it should be contended that in the case of *Lagenostoma* and *Gnetopsis* this special development of the inner integument is merely of biological significance, I would point out that it is difficult to see then why this should also occur in a seed outtopped by interseminal bracts as e.g. *Bennettites Morierei*. Nor does this explain the form of the section of the seed—triangular, hexagonal, &c.—nor the radiating vertical walls dividing the integument into compartments.

If, however, such internal evidence as I have brought forward appears inconclusive, it is satisfactory to find that there is a record in the literature of an exactly comparable transformation occurring in the sorus of a very ancient monostelic fern stock.

I refer to the fact that Renault in his Autun Flora describes a specimen of *Botryopteris* sporangia in which a group was found to be surrounded by an envelope formed of *sterile and highly modified sporangia* [2]. Renault figures some of these sterile sporangia in his 'Flore fossile d'Autun et d'Épinac.'

When we consider that on anatomical grounds it has long seemed probable that the Cycadofilices arose from some ancestral Filicinean group such as the *Botryopterideae*, we see that such a case as Renault cites is peculiarly significant in any discussion as to the phylogenetic origin of the integument of the seed. Hence any further confirmation of Renault's observation would lend a strong support to the new theory.

I will now refer to a few analogous cases which lend a general support to the claim for the sterilization of certain sporangia in a sorus during the evolution of the Seed.

In *Azolla* I believe most morphologists would admit that the microsporangial and megasporangial sori were originally similar, and that the megasporangial has gradually lost by abortion a number of sporangia, retaining only one. If the development of the megasporange in *Azolla* involved the total loss of its free sister sporangia, are we claiming too much

[1] Zeiller, Eléments de Paléobotanique, p. 224.

[2] Renault, Bassin houiller d'Autun et d'Épinac. Flore Fossile, ii, p. 54.

if we conjecture that in another Fern the sister sporangia, which were already adherent, were retained as a sterile envelope?

Turning to the Angiosperms, the modification and abortion of flowers in an inflorescence to construct the biologically interesting 'flag apparatus' is exceedingly common. The peripheral flowers in the capitulum of the *Cynareae*, in the thyrsus of *Viburnum Opulus* and *Hydrangea*, are among the most familiar examples. In *Muscari comosum* (var. *racemosissimum*) a very remarkable modification follows the sterilization of the central flowers. In *Rhus cotinus* De Candolle noted an increased growth of trichomes on the pedicels of the sterile flowers, and it has hence become a classical example of what he meant by the expression 'correlation of growth.'

Passing from flower to sporophyll we have no need to mention any of the innumerable instances of the change from stamen to sheathing organ which occurs commonly in Ranunculaceae, Scitamineae, &c. In *Salvia* we find that half the anther is sterilized to provide the lever which is to assist in the process of cross-fertilization. If a part of a sporophyll can be sterilized and adapted for an accessory function, why should not some members of a synangium?

Summary of evidence in support of the view that a seed is a synangium in which the peripheral sporangia are sterilized and specialized as an inner integument:—

1. Ontogeny. It is shown that wholly independent testimony is borne to the fact that in the most primitive of existing Spermophyta, the Cycadaceae, a correspondence obtains both in position and development between the microsporangial sorus and the seed.

2. Phylogeny. General considerations would lead us to expect comparable characters in the microsporangial sorus and the primitive seed. A synangium is the only form of microsporangial sorus so far known among the Cycadofilices, and it is found also in *Cycadeoidea*.

A special case is cited of sterilized sporangia in the tufted sori of *Botryopteris*.

3. Suggestions of sporangial origin in the inner integument of primitive seeds:—

1. It is frequently compartmental.

2. Each compartment contains large thin-walled cells as contrasted with the firmer peripheral layers.

3. The peripheral wall is constructed of the same characteristic layers as are met with in many synangia.

4. The form of the base and apex of each compartment is often very similar to those of members of a synangium.

5. In some cases there is considerable freedom between the constituent compartments whose apices form the so-called tentacles around the micropyle.

6. The compartments are comparable in size with the nucellus.

7. The compartments vary in number in the same way as the members of many Palaeozoic synangia.

8. The integument of many of the seeds undergoes septicidal dehiscence like a synangium.

9. The integument is generally as concrescent with the nucellus as the members of a synangium are with one another.

In conclusion, I may add that though I regard the theory as of wide-reaching importance, I am only concerned at present to put it forward as probably the true interpretation of the canopy of *Lagenostoma*, and hence as adding support to the view that *Telangium Scotti* is the microsporangial sorus of *Lyginodendron*. The full exposition of the seed which we await from Prof. Oliver will be based on wider researches into primitive types and a more intimate acquaintance with the difficulties of the problem.

I cannot conclude without expressing my indebtedness to Dr. Scott and Prof. F. W. Oliver. The stimulus of their example and criticism and their kindness in lending valuable slides have been most helpful.

EXPLANATION OF THE FIGURES IN PLATE XI.

Illustrating Miss Benson's paper on *Telangium.*

Telangium Scotti, figs. 1–10 inclusive.

h, *h'* = groups of cells (which tear on dehiscence), as seen in transverse section between the sporangia.

l, *l'* = lacunar tissue.

s, *s'* = fusiform empty cells with scalariform marking, which form the hypoderm or fibrous layer.

x, *x'* = parenchyma cells interrupting here and there the otherwise uniform fibrous layer.

cp = free apex.

Fig. 1. Longitudinal, somewhat tangential section of the synangium. × 33. A few spores can be seen in the sporange on the left. Slide M.B. Coll. 71.

Fig. 2. Transverse section of the synangium but little above the insertion of the sporangia. Two of the latter are injured. The fibrous layer does not extend over the whole surface at this level. Slide lent by Professor Oliver. U.C.L. Coll. K 3 (*b*).

Fig. 3. Transverse sections of two synangia at a higher level. × 50. The fibrous layer is now complete. The group of cells (*h*) remain undehisced. This preparation also shows the extension of the parenchyma to the surface at *x* and *x'*. Slide lent by Professor Oliver. U.C.L. Coll. K 3 (*d*).

Fig. 4. *A* and *B* are transverse sections of two nearly approximating synangia which are taken at different levels. × 33.

Fig. 4. *A* is taken below the level of insertion of the sporangia and shows a vascular strand *v*, composed of very small elements. These are reproduced in a larger scale in Fig. 4 *C*. × 50. Slide lent by Dr. Scott, C.N. 1803.

Fig. 5. *A* and *B* are transverse sections of the same two synangia represented in Fig. 4 *A* and *B*, but at a higher level. Some of the spores are well preserved. Slide lent by Dr. Scott, C.N. 1804.

Fig. 6. Spores. × 260. Fig. 6, *a*, *b*, *c*, *d*, are taken from the sections of *Scotti*, and fig. 6, *e*, *f*, *g*, are taken from the pollen-grains in the pollen-chamber of *Lagenostoma ovoides*. In *a* and *g* can be seen well the reticulation of the coat. U.C.L. Coll. M 21 (*a*); also M.B. Coll. 62, 63, 70.

Fig. 7. × 10. Transverse sections at different levels taken from a Hough Hill specimen. The dotted lines are merely intended to suggest the relation of the sections. M.B. Coll. 74 *a*, 74 *b*.

Fig. 8. This is an admirable longitudinal section of a portion of a synangium showing apex and base of one sporangium. × 33. M.B. Coll. 70.

Fig. 9. A transverse section through *Telangium Scotti* and *Lagenostoma Lomaxi* to show their close association as petrifactions. *c* = canopy. *n* = nucellus. Slide 603, from the Collection of the Manchester Museum, Owens College.

Fig. 10. Diagram constructed by superposing the different transverse sections. × 4.

Fig. 11. *Calymmatotheca Stangeri* (natural size), showing three terminal clusters and thorn-like emergences. *e* = emergences (after Stur).

Fig. 12. *Telangium affine*. × 4 (after Peach). *a*, shows a pair of synangia growing closely adpressed in parallel planes. *b*, a single synangium.

Fig. 12 *a*, explains the relative position of pedicel and synangium in Fig. 4.

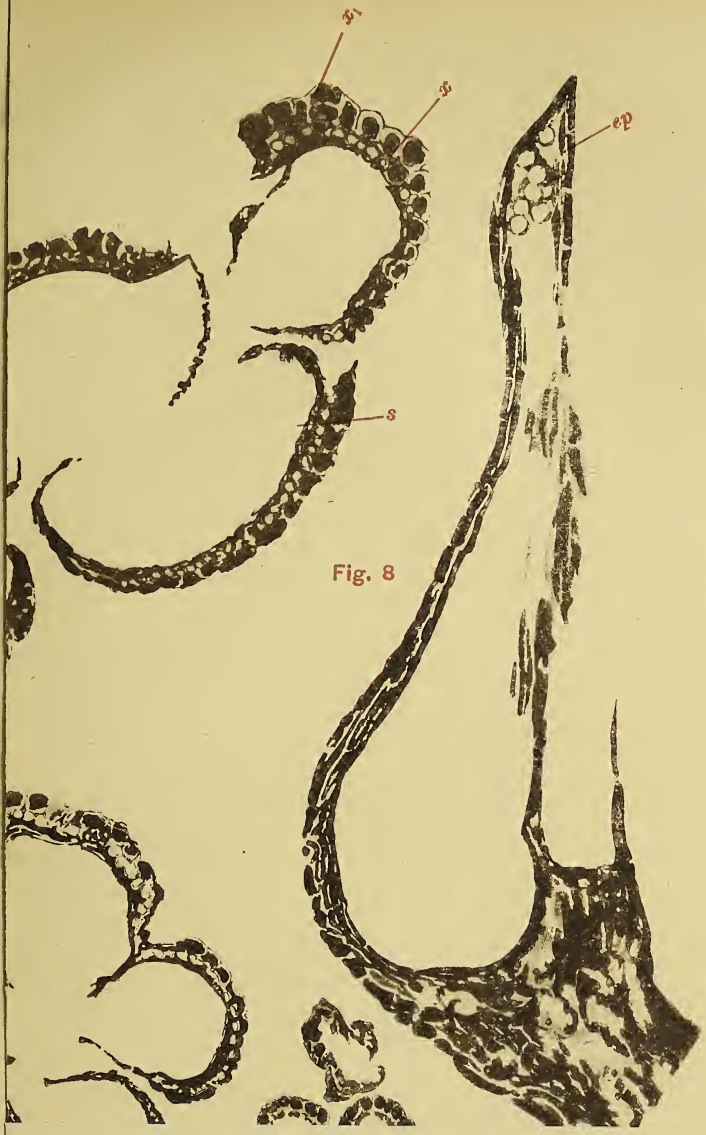

Fig. 8

Fig. 1

Fig. 3

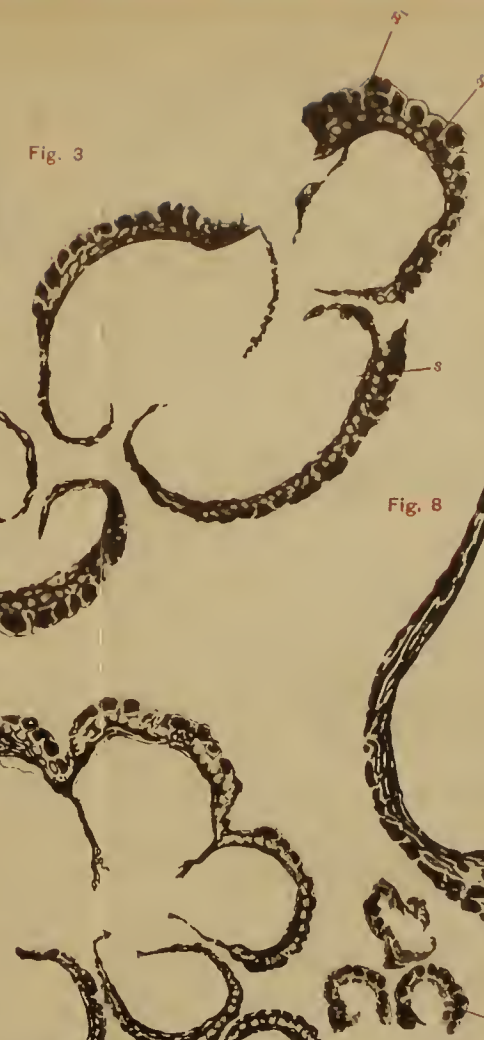

Fig. 8

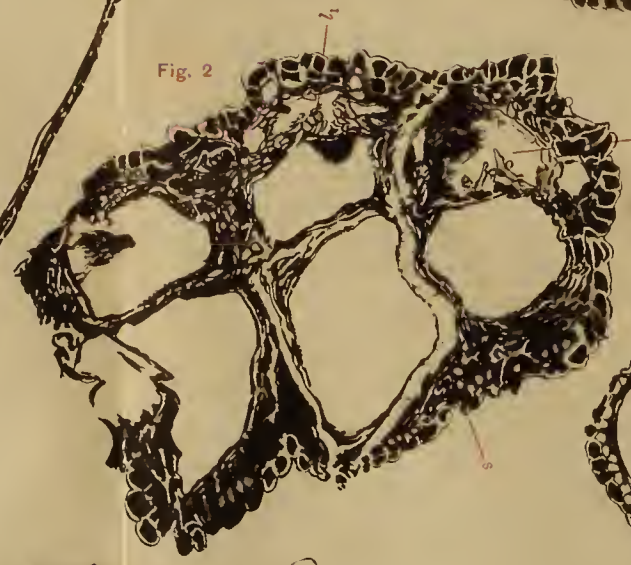

Fig. 2

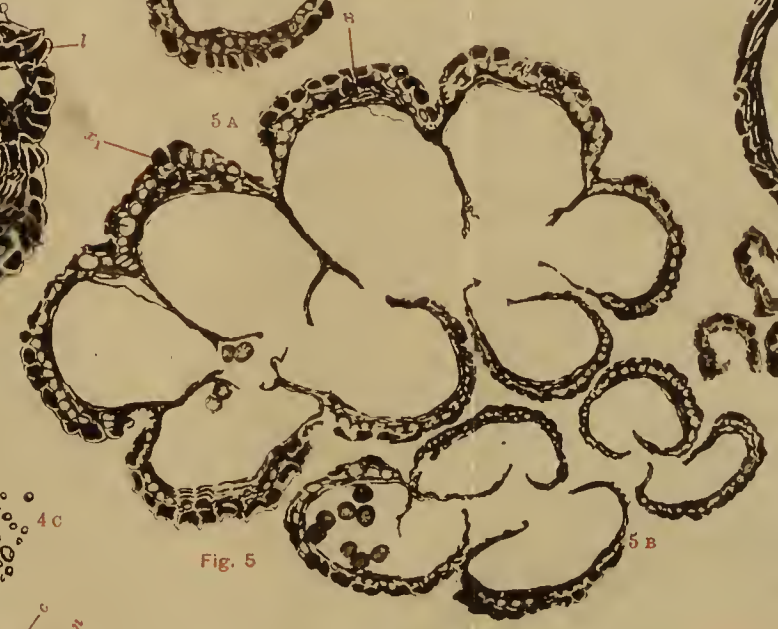

Fig. 5

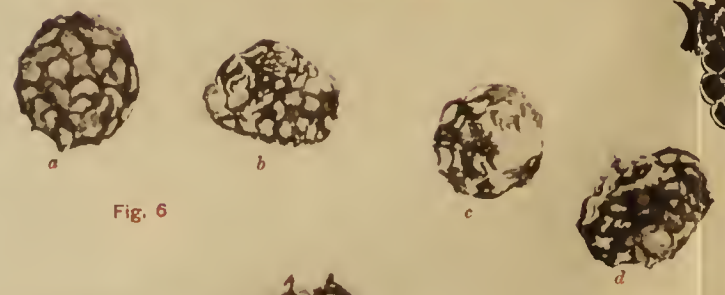

Fig. 6

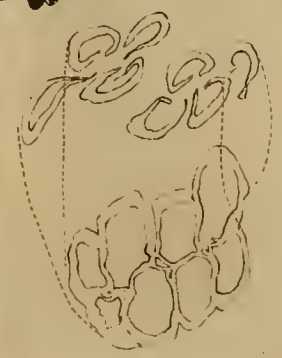

Fig. 7

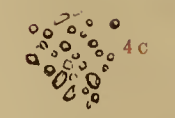

Fig. 9

Fig. 10

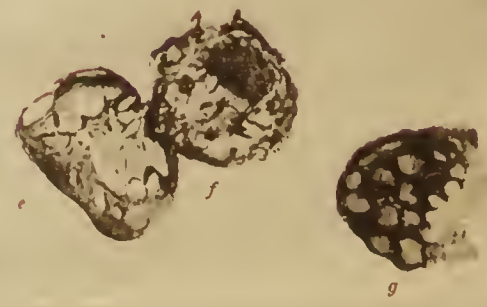

Fig. 11

Fig. 12

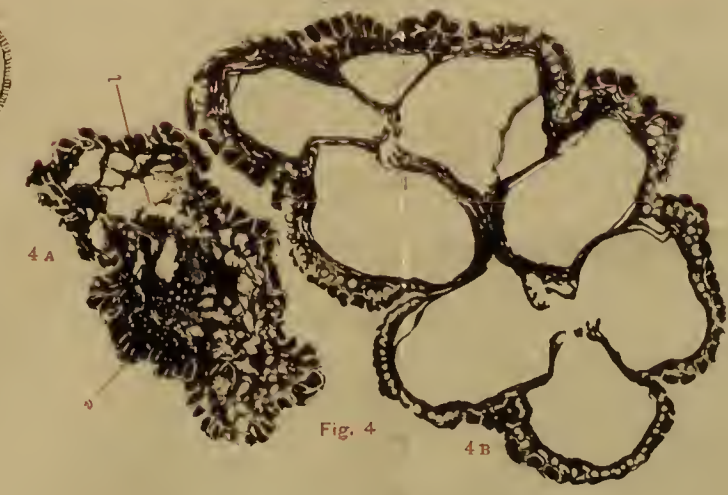

Fig. 4

NOTES.

ON THE GENUS CORYNOCARPUS, FORST. SUPPLEMENTARY NOTE. —I much regret that both Dr. Fritsch and I overlooked a paper[1] by Professor P. Van Tieghem, in which he discusses the anatomy, floral structure and systematic position of *Corynocarpus*. The Author courteously sent me a copy of the paper in question, and I hasten to make some amends for the omission of all reference to it in my account of the genus[2] by giving here the principal results of his investigations. His description of the anatomy of the stem and leaf agrees in the main with that of Dr. Fritsch; but the latter omitted to mention the isolated crystals, which one cannot possibly overlook in a longitudinal section. His part, however, was hurriedly done just before his departure for Ceylon. I wished merely to show that there are no resin-ducts.

Van Tieghem agrees with Engler in treating *Corynocarpus* as the type of a natural order or rather 'famille autonome,' as he designates it; but his views on the immediate affinities of this genus are wholly different. He lays great stress on the structure of the ovule in classification, and ranges *Corynocarpus* 'dans l'ordre des Pernucellées bitegminées . . . qui a pour famille type les Géraniacées.' I do not underestimate the value of anatomical characters, but I do not attach so much importance to them for purposes of classification as the learned author, mainly because it leads to refinements and generic subdivision impracticable in applied or daily botany. I would none the less recommend a perusal of his interesting paper[3].

Van Tieghem describes the ovule of *Corynocarpus* as having a large, persistent nucellus, surrounded by two integuments. The external integument is very thick, consisting of from twenty to thirty layers of cells; whilst the internal is thin, having only three layers of cells, of which the outer has larger cells elongated radially. At the micropyle the internal integument extends up the exostome but not above it, and the nucellus also projects into the endostome 'so that the pollen-tube comes directly upon the nucellus, without having to traverse the micropyle, as directly, indeed, as though the two integuments did not exist.'

I must confess that I do not quite understand this description, because the pollen-tube must traverse the micropyle, whether open or narrow, unless the nucellus actually projects to the very top of the combined endostome and exostome.

All the writers whom I have consulted, except Van Tieghem, describe the leaves of *Corynocarpus* as exstipulate. He says they are furnished with broad, caducous stipules. In flowering or fruiting herbarium specimens, no stipules are

[1] Sur le genre Corynocarpe, considéré comme type d'une famille distincte, les Corynocarpacées. *Journal de Botanique*, xiv (Juillet, 1900), pp. 193–7.

[2] *Annals of Botany*, xvii (1903), pp. 743–60, pl. 36.

[3] See also his paper: L'Oeuf des Plantes considéré comme base de leur classification, *Ann. Sc. Nat.*, 8*me.* série, xiv (1901), pp. 213–390.

to be found, but there are lines running from the base of the petioles, partly round the branches, which might be taken for the scars of fallen stipules or the decurrent margins of the petiole.

Fortunately I have been able to examine young shoots from a plant growing at Kew, and I suspect that the organs termed stipules by Van Tieghem are really bud-scales, but I have not been able to examine them so thoroughly as to give a positive opinion, though possibly he has. They are deltoid, acute bodies 2–3 lines long, situated more or less within the axils of the leaves, but falling away as the leaves above them develop. Occasionally they lodge in the axil of a leaf long after organic connexion has ceased to exist, sometimes even until the leaf above is fully developed.

I may add here that Mr. T. F. Cheeseman, Curator of the Museum at Auckland, New Zealand, has obligingly sent to Kew several flowering specimens and a quantity of flowers in alcohol of *Corynocarpus laevigata*, in order to give me further opportunities of examining the staminodes. It may not be remembered, perhaps, that there was a doubtful point in this connexion. I explained in my former paper, p. 744 (and Fig. 7, Pl. 46), that Banks and Solander described and figured the staminodes as three-toothed at the apex, whereas all the staminodes of *C. laevigata* examined by myself and others, including Cheeseman, were irregularly and minutely toothed or fringed from about the middle upwards and around the top. I have examined a number of the flowers sent by Mr. Cheeseman and they all presented the same kind of staminode, except that the toothing or fringe in some instances extended almost to the base. But in none of these did I find a second carpel, or traces of a second, though there was a slight obliquity in the one present.

With regard to the Maori name *karaka*, I have the authority of Mr. Cheeseman[1] that it is applied to *Elaeocarpus rarotongensis*, Hemsl., by the natives of Rarotonga. What connexion there may be, I cannot even suggest, but the foliage is somewhat similar to that of *Corynocarpus* and the fruit is a drupe. As mentioned before, some writers have endeavoured to prove that the Maoris migrated from 'Hawaiki' by way of Rarotonga to New Zealand.

W. BOTTING HEMSLEY, Kew.

THE VASCULAR SUPPLY OF STIGMARIAN ROOTLETS.—In Vol. XVI of these Annals I described the course and termination of certain vascular branches of Stigmarian rootlets, which had first been observed but wrongly interpreted by M. Renault. Instead of supplying lateral roots as Renault had supposed, the vascular branches terminate in the outer cortex in wide spirally thickened cells, resembling in appearance the transfusion-cells of leaves. These cells I figured in transverse and longitudinal sections of rootlets (Pl. XXVI, Figs. 4 and 5), and also in surface view (Fig. 2 c). But this latter figure was not very clear, as the section was somewhat oblique and slightly compressed at c, and, as I stated in my paper, 'it is difficult to ascertain what was the size and distribution of these patches

[1] *Trans. Linn. Soc.*, 2nd series, Bot. vi, p. 275, t. 31.

of spirally marked cells of the outer cortex.' The patches were apparently of some breadth, but only a tangential section near the surface of the rootlet could show clearly the extent and arrangement of these cells. Such a section I have found on a slide (prepared by the late Mr. J. Spencer) kindly lent me by Dr. Scott from his collection. Its cabinet number is 1527. This slide has two rootlets cut tangentially through the outer cortex, and from the better one of the two the accompanying drawing has been made with the camera lucida. The rootlet could hardly have been cut in a better direction or at a better depth for revealing the details of the vascular

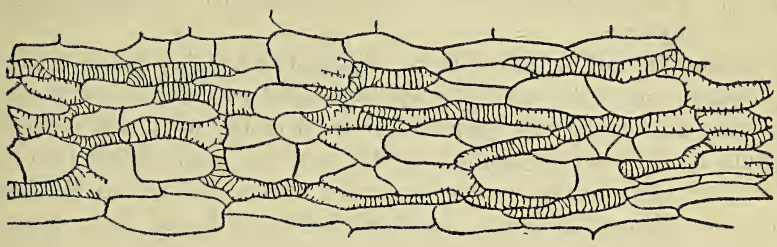

FIG. 34.

elements of the cortex. As will be seen from the Fig. 34, they form a complete network, over a width of six or seven cells, and greatly resemble the termination of the vascular bundles in the leaf. Between the spirally thickened cells are found wide thin-walled elements from which water could readily pass into the spiral elements and thence through the vascular branch into the stele of the rootlet.

At one or two points where the spiral elements are shown in the drawing in an incomplete condition, it is obvious from the difference in focussing that the spiral cells were there connected with a vascular branch lying at a different level and not in the plane of the section. In the other rootlet seen on the slide there seemed to be a slight difference between the cells at the ends of the ramifications and the connecting tracheids. The latter were slightly narrower and had a closer spiral marking than the former which were of greater width.

I must express my indebtedness to Dr. Scott, who has placed at my disposal this excellent preparation which throws further light on the curious vascular supply of the Stigmarian rootlets.

F. E. WEISS.

OWENS COLLEGE, MANCHESTER.

ROOT-PRESSURE IN TREES.—The following observations made upon the Wych Elm (*Ulmus montana*) appear to be of some interest. The tree used was over thirty feet high, and branched at the base into two main trunks.

Feb. 20. The larger trunk was sawn across. No bleeding now or subsequently.

Mar. 15. The second trunk was ringed, from eight to ten annual rings of wood being removed, which formed one half of the alburnum. Flowering and foliation in April were hardly at all delayed.

Ap. 19. A root 2·5 cm. diameter was cut through and mercury manometers attached to both ends. A rapid escape of sap took place from the end attached to the stem, the pressure varying from ten to fifteen feet of water until the fourth day when it began to fall. Practically no escape of sap took place from the end of the severed portion on the first day, but on the second, pressures equivalent to between two and three feet of water were shown, rising on the fourth day to nearly six feet, but distinctly falling on the sixth and seventh days.

Ap. 27. The second trunk was cut across completely. No bleeding now or at any time.

Two anomalies will be noticed here. Firstly, that a higher pressure was shown by the attached end of the root[1] than by the severed portion, as though the 'root-pressure' were driving the sap downwards instead of upwards; and secondly, that the pressure in the attached portion of the root was much higher than was required to raise water to the cut surface of the stump, and yet no bleeding took place from it, although an active exudation was shown by the root. Even when the second trunk was cut and covered with indiarubber no actual drops of water exuded, although both the duramen and the remaining alburnum were quite moist.

The explanation appears to be that, early in the year, the wood of the intact trunk offers a higher resistance to the passage of water than it does later on, although to obtain direct evidence of this is by no means easy. Furthermore, different portions of the root-system appear to awaken to active absorption at dissimilar times. Even although the pressure in the intact root-system was nearly uniform throughout, the maximal pressures shown by manometers attached to severed portions of it might vary considerably, according to the amount of absorbing surface and the relative activity of absorption. This is because the maximal pressure in a severed root always decreases sooner or later, so that the height of the pressure shown by an attached manometer will depend upon the rapidity with which the maximal pressure is attained, which again depends upon the rapidity of escape of sap, and this upon the activity of absorption. It would, in fact, be more accurate to test the pressure of absorption in a severed root by applying increasing pressures of Mercury until sap neither escapes nor is driven backwards.

To perform an extended series of observations of this kind, each of which demands the sacrifice of a large tree, is however possible only to a privileged few. The above observations are therefore merely put forward as suggesting the need of solving the following questions:—(1) Does the total resistance to the flow of water in the trunk of a deciduous tree vary and show an annual rhythm or periodicity? (2) Is the root-pressure comparatively constant throughout large root-systems, and do all regions of such systems awaken to active absorption at the same period of time?

ALFRED J. EWART.

[1] The root was subsequently traced to its junction with the parent-tree.

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NOTES.

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Studies in the Dictyotaceae.

II. The Cytology of the Gametophyte Generation.

BY

J. LLOYD WILLIAMS,

Assistant Lecturer in Botany, University College, Bangor.

With Plates XII, XIII, and XIV.

IN a previous paper the development, germination and cytology of the tetraspore in *Dictyota* and other members of the group were described. In the present paper it is proposed to deal with the development of the oosphere and antherozoid, the fertilization of the ovum and its subsequent segmentation, together with the parthenogenesis of unfertilized eggs—the observations in this case applying to *Dictyota dichotoma* only.

In this plant the male and female gametes are borne upon different individuals. Excepting so far as the reproductive cells themselves are concerned, there is no difference either of form or of structure between the tetrasporic plants on the one hand and the sexual ones on the other. It is true that the latter generally have the branches of the thallus broader than those of the former, but to this rule there are frequent exceptions.

As already described, the discovery of motile antherozoids, first made in 1896, was not confirmed till nearly a twelvemonth after. Subsequently the astonishing fact was established that, unlike the tetraspores, both the oogonia and antheridia are developed simultaneously in fortnightly crops, each crop being initiated a little before the lowest neap tide, and arriving at maturity about the period of the highest succeeding spring tide. The gametes thus produced are liberated during two or three days while the high tides are on the wane. A regular succession of crops continues thus from July to the end of October. So far as I am aware nothing like this remarkable periodicity has hitherto been observed in the case of any other Alga. During the last six summers careful records have been kept of the appearance of these crops, and their relation to the conditions of their environment. The data thus collected, together with a discussion of the factors concerned, will be published in a separate paper. These observations only apply to the plant as growing on the North Wales coast; it

[Annals of Botany, Vol. XVIII. No. LXX, April, 1904.]

would be instructive to know whether they hold good for other localities also.

As the sexual cells pass through the various stages of development at approximately the same dates, it is far easier to find any required mitosis here than in the gametangia of the Fucaceae or in the tetrasporangia of the Dictyotaceae. After the preliminary trials one has only to consult the tide tables for the particular locality to know within two or three days when certain stages may be found.

Although Thuret and Reinke have given excellent figures of the sexual cells of *Dictyota* no description of their cytology has as yet been published.

I. The Development of the Oogonia.

The oogonia instead of being isolated, as is the case with the tetrasporangia, are grouped together in sori, each sorus consisting of twenty-five to fifty oogonia, closely packed together without paraphyses or any other accessory cells. In the case of weak plants, or under adverse conditions, the number may be reduced to half a dozen or fewer. The sori are scattered all over the two surfaces except the basal portion, the apices, and a very narrow band at the margins of the thallus. The sori of the new crop appear between the scars of the older ones, and, in the case of an elongating plant, in acropetal succession on some of the surface cells which have sufficiently matured since the preceding crop. This process may go on till the whole of the available surface has been used up, then the plant dies. In no case are gametangia produced by the stalk-cells left by preceding sori.

As in the case of the tetrasporangium the rudiment of the oogonium is one of the small cells of the assimilating layer, which increases in dimensions till the free part is about three or four times the height of the original cell (Pl. XII, Fig. 1). The nucleus then divides, and a stalk-cell is cut off. The oogonium mother-cell increases somewhat in size, but it undergoes no further division, the nucleus becoming that of the ovum without an additional mitosis. This is essentially different from the state of affairs in the oogonium of the Fucaceae, where eight nuclei are produced even in the cases where only one egg ultimately matures. In the Fucaceae also, where there is no asexual generation, reduction takes place in the first of the three oogonial mitoses; in *Dictyota*, as shown in the preceding paper, it is brought about in the first of the two tetrasporic divisions. In a few days after the separation of the stalk-cell the apex of the oogonium ruptures and the naked oosphere is liberated into the water, where fertilization takes place.

The stalk-cell mitosis is accomplished from one to four days before the highest spring tide, the variation in the time being dependent on the length of the interval between two spring tides, the conditions with respect

to temperature and light prevailing at the time, and the period of the year. The whole process is gone through with considerable rapidity, and all the stages may be found in a single sorus. In the case of the tetrasporangium the stalk-cell separates while the rudiment is still comparatively small; here, however, it is delayed till the cell is nearly fully grown, and liberation follows in about three to five days.

Of the oogonia in a sorus the central ones only are vertical to the thallus; in consequence of their great increase in diameter as compared with the stalk-cells the others lean outwards, the angle being greater as the periphery is approached; this makes it difficult to obtain median sections through the dividing nuclei.

It is generally stated that there are no borders of elongated sterile cells to the oogonial sori, as there are to the antheridial ones. This, however, is not strictly correct; borders, partial or complete, are frequently found and may arise in one of two ways.

1. Some of the outer cells of a sorus may at a comparatively early stage in their development be compressed by the more actively growing inner ones, and prevented from developing further. These are visible in sections as narrow elongated cells, but in a surface view are completely hidden by the outermost of the fertile oogonia.

2. Towards the close of the season, when unfavourable conditions supervene, most of the outermost oogonia fail to mature even after attaining their full size; the cytoplasm is smaller in amount and the nucleus does not divide. These facts suggest the mode in which the antheridial borders may have arisen, and the manner in which borders may also in the course of time be acquired by the oogonial sori. In this connexion it is instructive to notice that the central portions of both male and female sori are more active than the peripheral ones; in the early stages the nuclei are more advanced, liberation generally starts in the middle of the sorus, and sterilization, although it sometimes occurs there, is not nearly as frequent as it is in the marginal regions of the sori.

Fig. 1 shows the structure of the oogonium-rudiment shortly before the separation of the stalk-cell, as seen in a section vertical to the surface of the thallus and transverse to the branch. As in the case of the tetrasporangium-rudiment the basal part of the cell has very large vacuoles, while the remainder is occupied by denser cytoplasm, in which the chloroplasts are much more crowded. The curved, rod-like centrosomes and radiations are very distinct, not only at the distal pole but most commonly at the basal pole as well. The physodes as usual are chiefly located in the peripheral layers of the free surface.

The early prophase stage is very much like that of the stalk-cell division in the tetrasporangium; there is the same coarse chromatin thread, irregular in the arrangement and thickness of its granules, and soon

segmenting into the chromosomes. When, however, the latter make their appearance, they exhibit the longitudinal split far more clearly than do the chromosomes in any other division (Figs. 2, 3). At first the nucleolus has one or more fairly large vacuoles with a number of smaller ones; soon, however, the nucleolar membrane disappears, and the bulk of the nucleolus is seen to consist of a large number of deeply staining fibrillae, together with a single, slightly staining globule (Fig. 3). During this period the amount of nucleoplasm is very small, and the centrospheres are less distinct.

The spindle (Fig. 4) is intranuclear and narrow. There are a few mantle-fibres extending outwards, the spaces between which are occupied by a large number of the deeply stained fibrillae derived from the disintegrating nucleolus. The globule is generally present either on the spindle-fibres or in the immediate vicinity.

The polar view represented in Fig. 5 shows the chromosomes to be curved in form and clearly sixteen in number. The nucleus at this stage is somewhat nearer the base of the cell, and the cordate appearance shown in Fig. 4 is very frequently met with; it is almost certainly due to a contraction of the spindle after fixation. In preparations of segmenting eggs depressions are frequently found at both poles of the nucleus. These facts confirm the observation that the spindle-fibres at this period are in a state of tension and that they contract on fixation.

During the anaphase stage the spindle is still narrow, but the nuclear cavity is wide and the membrane is intact except at the poles. The centrosomes are clearer than during the preceding stage, and in most cases the nucleolar globule is present. When the chromosomes reach the poles of the spindle the membrane disappears, the lower nuclear mass remains in the same position or descends slightly while the upper ascends, thus causing the whole figure to become greatly elongated. In the telophase stage (Fig. 6) of the oogonial nucleus the chromosomes, now greatly increased in size, but still preserving their curved form, are seen to be quite separate from each other and sixteen in number. When they fuse they frequently form a thick deep-staining irregular ring in which at one particular stage of the fusion about eight deeply stained masses may be distinguished (Fig. 7). Something similar was described for the stalk-cell division of the tetrasporangium, but was not observed in any of the other mitoses. The two divisions are strikingly similar also in the fact that only one deeply staining mass is formed in both cases, which ultimately becomes the nucleolus.

If Figs. 1, 10–13 be compared certain peculiarities of cell-wall structure may be observed. Although the drawings are made from preparations fixed and stained by different methods they all agree in showing a distinct difference between the membranes of the parts of the cells embedded in

the thallus and those of the free, reproductive portions. For want of space this topic must be deferred for discussion in another paper. It may be pointed out that we have a provision here for the rapid elongation of the gametangial walls, for the early splitting of the lateral walls, and for their dissolution when the gametes arrive at maturity. It is evident that the basal cells have their walls of different chemical composition, and of more enduring character.

When quite mature the degeneration of the apical walls and the increasing turgescence of the ovum causes the former to burst and to liberate the oosphere, which now lies a naked spherical mass of protoplasm. Fig. 19 shows a section through such an ovum. The nucleus now occupies nearly one-third of the diameter of the egg. It is surrounded by a zone of 'kinoplasm,' outside of which is the somewhat vacuolated cytoplasm with its numerous chloroplasts. In well fixed material these are generally oval or rounded, but when much contraction has taken place they are very narrow and elongated. At the periphery of the egg there is a zone of granular cytoplasm in which are embedded numerous intensely staining physodes.

Very soon after liberation of the oospheres in a sorus the oogonial walls are completely dissolved, leaving only the basal cells, which are marked from the other surface cells of the thallus by their slightly greater width, and particularly by their paler colour. The latter peculiarity is due to their having fewer and paler chloroplasts. As soon as they are exposed to the light by the liberation of the overlying eggs, the chloroplasts begin to multiply and acquire a deeper colour, but the scars of two or three of the most recent crops can be easily distinguished from each other by their different shades of colour.

II. The Development of the Antheridium.

If a fresh plant of *Dictyota* be examined one to five days before the lowest neap tide the rudiments of the antheridia may be distinguished by their slight increase in diameter and their deeper colour, but chiefly by the pale circular area in the centre, indicating the position of the nucleus. Material fixed about this period would show the various stages in the stalk-cell mitosis, and perhaps also in the first division of the mother-cell of the antheridium.

The antheridia, like the oogonia, are arranged in sori, but they are much more numerous than the former, the number in a fair-sized sorus being from one to two hundred. Each sorus has always a well-defined border of sterile cells consisting of three or more rows, the innermost of which are of about the same length as the antheridium, while the others successively diminish in height (Figs. 12–14). Some time after the liberation of the antherozoids the innermost border-cell cuts off a cell near the

top; occasionally this happens also in the case of some of the cells of the next row.

The stalk-cell division takes place when the cell is still very small, consequently the nucleus, though much larger than that of one of the vegetative cells, is considerably smaller than the nucleus of the tetrasporangium or of the oogonium. The result is that the various processes are more difficult to follow, the counting of the chromosomes being in some stages particularly difficult. This has necessitated the comparison of a very large number of figures. It is found that the mode of division closely resembles that which obtains in the stalk divisions already described. While the increase in size is as yet exceedingly slight and there has been but little accumulation of protoplasm, the nucleus and nucleoli are greatly enlarged though the nuclear network is still inconspicuous. Even at this stage the centrosomes are perfectly clear at both poles, and their form is that already described in other cases. As usual the distal pole is distinguished by beautiful radiations, which are nearly always absent from the other pole. There are no new points to be noted with regard to the spirem, the chromosomes, or the spindle. In many instances the lower pole seems to project into the vacuolated part of the cell. Sometimes a few strands of protoplasm bridge over the space, and connect the nucleus with the thin lining of protoplasm at the base. Frequently thick sections show strands that end blindly in the vacuole. These recall the appearance of the cords of protoplasm described by Phillips and seen in the living cells of certain Florideae, and which are in a state of continual motion within the vacuole, either extending or retracting or bending upon themselves. The absence of radiations and the position of the lower pole with regard to the vacuole preclude the possibility of a pull being exerted by any kinoplasmic structures at the base.

During the metaphase and anaphase stages the nuclear membrane is present, in fact it persists in some cases till the very commencement of the telophase stage, then, as in the oogonium, the membrane disappears and the lower nucleus descends into the middle of the vacuolated region.

The reconstruction of the nucleolus sometimes follows the same course as in the two stalk-cell divisions already described. In other cases several masses may be seen which differ greatly in size. These soon coalesce to form the spherical nucleolus, which at first presents a curious appearance, as if it consisted of a globule of pale-staining substance with a number of chromosome-like bodies embedded, chiefly though not solely at the periphery. This appearance, together with the very scanty, slightly staining nuclear network, confirms the idea that in the daughter-nuclei resulting from these three stalk-cell mitoses the whole of the chromatin is located in the nucleolus.

The partitioning of the antheridium into antherozoid mother-cells

is completed a few days before liberation, the whole process occupying from six to ten days. Particular attention was paid to the later cell-divisions to see if any evidence of amitosis could be observed. As far as can be seen, however, all the usual stages of karyokinesis are repeated at each step, and after each cell-division the nucleus assumes the appearance of the resting condition. Several reliable countings of chromosomes were obtained in the earlier mitoses, both in the prophase stage and in polar views of the equatorial plate stage (Fig. 11). All these agreed in giving the number as sixteen. Occasionally a larger number was indicated, but in all such cases it was easy to see that some of the bent chromosomes had their angles cut off, thus giving two chromatic segments instead of one. The first division-wall of the antheridium mother-cell is vertical to the thallus and at right angles to the long axis of the branch, the second is also vertical but parallel to the axis, so that in surface view four cells are seen. The succeeding divisions are not so regular, but a number of tangential division-walls appear before any further vertical ones are formed. Ultimately the antheridium in surface view is seen to consist of sixty-four cells arranged in four groups of sixteen. In vertical section there is great divergence between different antheridia, the number of tiers in some cases being twenty-four (Fig. 14), in others about twenty. The number of successive nuclear divisions must be about twelve, but it is clear that many of the cells fail to divide and others abort. It is instructive to note in this connexion that in many cases when the circumstances are unfavourable it is the lowermost cells in an antheridium that are first retarded; this fact will be referred to again when discussing the importance of light as a factor in the development of the gametangia.

Careful countings of the antherozoid mother-cells in a large number of antheridia show that the average number of sperms produced in an antheridium is about 1,500. It has already been shown that the number of oogonia in a sorus ranges from about twenty-five to fifty, while the number of antheridia is generally from 100 to 300. Taking the lower number in each case we see that for every egg in an oogonial sorus there will be 6,000 antherozoids in a sorus of antheridia. In order to form an approximate idea of the number of gametes produced by a single plant the sori were counted on a specimen twelve inches long. They were found to be over 3,500 in number. Allowing an average of 1,500 antherozoids for every antheridium this would give us a total of over 500 millions for the whole plant, a number which is certainly much too low for a vigorous, full-grown plant. If we consider a single locality, the Menai Straits for instance, where *Dictyota* flourishes in great abundance, and the vast multitude of sexual plants which on certain days every fortnight throughout the summer months fill the water in their vicinity with untold myriads of swarming gametes, we cannot but be filled with astonishment at the

prodigious numbers produced, and yet so far as the production of new plants is concerned nearly the whole of the energy expended and the elaborate mechanism employed has been wasted. This, of course, is an old story, but every new exemplification of it comes with a fresh surprise.

The antherozoids were described by me in a short paper published in 1897. Since then I have had many opportunities of examining these bodies in the active condition, of fixing and staining them by better methods, and of studying their structure by means of superior objectives. Some of the conclusions first arrived at are now found to be incorrect in certain particulars, and several points which were then obscure have now been satisfactorily cleared up.

Taking first the antherozoid as it appears in the living condition, we find that it is pear-shaped while active, but spherical when it comes to rest (Fig. 18). At the broader, posterior end there is a globular colourless part which with chromatin stains takes on an intense colouration, showing it to be the nucleus. The pointed end is occupied by granular protoplasm in which there is always a very minute red eyespot, sometimes two as in the lowest example in the figure. Instead of being situated close to the attachment of the cilium as is usual in other antherozoids and in zoospores, the eyespot here is always at a distance from it, frequently at the pointed anterior end. The cilium is not terminal as was stated in the '97 paper, but lateral as in other Phaeophyceae ; this fact can be clearly seen only while the antherozoid is in motion. The cilium is attached at the posterior edge of the cytoplasmic cone, near its junction with the nucleus. Very often there seems to be a slight thickening at the very base, but on this point it is not easy to speak with certainty.

Of the fixed preparations one of the most successful is that shown in Fig. 15, fixed with potassium-iodide iodine in sea-water ; this preserves the form and structure very faithfully. Those in Fig. 16 were fixed in dilute Flemming solution, and stained by the iron-alum method. Here the anterior part is much wrinkled, but the nuclei and physodes are very distinct. When fixed with dilute Hermann's solution (Fig. 17) and stained with gentian violet the nuclei stain very deeply, the cytoplasm slightly, and occasional physodes are seen. At the upper right-hand corner is shown a monstrous antherozoid with two nuclei. If doubly stained with brazilin and Hoffmann's blue the nucleus takes on the red colour, while the anterior part stains blue.

The number of physodes present varies greatly, as also does their reaction to a solution of vanillin in HCl. When fresh plants are tested with the above reagent the sori frequently give no trace of the red colour (phloroglucin ?) usually given by the physodes of mature cells. At other times every sorus is coloured a deep red ; this happens generally when there has been some delay in the liberation of the antherozoids. When

the reaction is partial it generally appears in the central antheridia only, an additional confirmation of the greater vigour of this region.

Much time and trouble have been expended in trying to find whether there is a second cilium or not. In the Fucaceae, as is well known, the posterior cilium is very long but very much thinner than the anterior one. The possibility had to be considered that in *Dictyota* the second cilium is so reduced in length as to be quite rudimentary, or that it is attenuated to such an extent as to be indistinguishable. In the upper left-hand corner of Fig. 17 is represented an apparently biciliate antherozoid, and several more such cases have been observed. After a careful comparison with fixed and stained antherozoids of the Fucaceae I have failed to satisfy myself that these appearances are not due to strands of mucilage. In the living condition I have only once seen anything suggestive of a second cilium. On one occasion, while examining a number of antherozoids by means of Zeiss's D* water-immersion objective, one was observed which had got stuck fast at the bottom of the dish. In almost all cases of the kind it is the end of the long cilium that becomes attached; in this instance, however, the cilium was quite free, and it could be clearly seen violently lashing about in the water. Careful observation showed that the antherozoid was attached to the glass by a filament of much greater tenuity than the cilium, and of about the same length as the body of the sperm. This is the strongest evidence yet obtained for a second cilium, but even this is quite insufficient to enable one to come to a positive decision on the point.

III. The Fertilization of the Egg.

Thuret, Reinke, and other investigators tried to solve the question of the mode of fertilization in *Dictyota*. Their failure to obtain active antherozoids for their experiments prevented them from arriving at a correct solution of the problem. It was further complicated by the fact that many of the eggs segmented in the total absence of antherozoids. Among the various suggestions advanced from time to time in explanation of the difficulty were the following :—

(1) That fertilization is effected before the liberation of the eggs from the oogonia ; this seemed to explain the apparent germination of liberated oospheres in the absence of sperms.

(2) That there are two kinds of asexual spores.

(3) That the antherozoids have become functionless and that the eggs are habitually parthenogenetic. To Johnson, in his study of *Dictyopteris*, belongs the credit of having first pointed out the true answer to the question, though his somewhat tentative pronouncements were never confirmed by him.

The present investigation has now conclusively demonstrated that

fertilization is entirely external as it is in the Fucaceae, that unfertilized eggs will pass through a few of the earlier segmentation stages by an abnormal process of parthenogenesis, but that normal germlings can only be produced from fertilized eggs.

The following is the method employed for securing material for this stage. Oogonial and antheridial plants are collected when the sori are quite mature, if possible on the evening preceding the day of maximum liberation. The plants are kept separate in moist chambers till the following morning, pieces are then immersed in glass dishes containing clean sea-water. The oospheres slowly burst through the walls of the oogonia and gradually sink to the bottom of the vessel. Before a sufficient number have been liberated to make it profitable to experiment with, half an hour or more will have elapsed. When the antheridial plants are immersed the antherozoids come out of the restraining cells very quickly, and soon the water is cloudy with swarming sperms. If now some of the latter are added to the oospheres by means of a pipette, it will be seen that instead of impartially surrounding all the eggs, as do *Fucus* antherozoids, many of the eggs are here passed over and entirely ignored, whereas others are covered with antherozoids, which in some cases lie three to twelve layers deep. If the same material be fixed after an interval of twenty-four hours and subsequently sectioned it will be found that the antherozoid-covered eggs have segmented in a perfectly normal manner, whereas the others show the abnormal mode of division described below, a method which is characteristic of the so-called parthenogenesis already referred to.

When pieces of oogonial plants have been immersed for about an hour the eggs set free will have been liberated at various intervals of time, some nearly an hour, others only a few minutes. If now instead of adding antherozoids we test the eggs with distilled water, we find that those that have been liberated for half an hour or more have already acquired walls. This partly explains why they are no longer capable of attracting the antherozoids, and why they cannot possibly be fertilized. From this circumstance it is very difficult to get uniformity of stages in cultures of *Dictyota* oospheres.

Although the mode of fertilization in *Dictyota* is very similar to that in *Fucus* there are important differences. In the former the liberation of the gametes is simultaneous; this tends to give it a great advantage over *Fucus*, where there is no regular periodicity in the production of the sexual cells. On the other hand, the eggs in *Dictyota* very soon lose their capacity for fertilization, whereas those of *Fucus* may retain it for days. This would seem to act as a disability, and so to neutralize the advantage obtained by the simultaneous emission of gametes.

As already explained it is my intention to deal with physiological considerations in a succeeding paper; two points, however, demand mention here.

The first is the relation of the antherozoids to light. It was stated in a former paper that light is essential to their mobility. It is true that light has a very great influence upon the antherozoids, as will be shown again, but if they are really ready for liberation they will on immersion display their activity at any hour, night or day.

The second point relates to chemotaxis in fertilization. Buller has recently performed some experiments on the eggs of certain animals, which lead him to infer that in external fertilization there is no such thing as chemotactic attraction of the spermatozoids. He seems inclined to follow Bordet, and to extend this generalization so as to include the Fucaceae. Thus he says in explaining Bordet's conclusions: 'According to this observer it is simply the ability of the spermatozoa to adhere to surfaces by the tip of one of the cilia which leads to their collection upon an egg, while their meeting with it is a matter of chance. A few observations of my own at Naples upon the fertilization of *Cystoseira barbata* did not reveal to me any certain attraction of the spermatozoids from a distance, but the collection of the spermatozoids upon the eggs in consequence of their ability to cling to surfaces was clearly seen. Nevertheless in view of the positive statement of Strasburger a careful re-investigation of the question seems to be desirable.' It is not my intention at present to discuss the question of chemotaxis in the Fucaceae. As regards *Dictyota*, however, it would be difficult to find anywhere a stronger suggestion of attraction at a distance than is offered by the phenomenon described above, where, out of several hundred eggs crowded together in a small dish, a few here and there are surrounded by several layers of spermatozoids, while the great majority of the eggs have none attached to them. Nor can it be said that in the former case the crowd of antherozoids are entangled in a mucilaginous envelope, for in the first place their movements are absolutely unrestrained, and furthermore, staining with gentian violet fails to demonstrate the presence of any such substance.

During the process of fertilization the eggs of *Dictyota* do not revolve as do those of *Fucus*, but the antherozoids, unless too crowded, slowly gyrate with the end of the cilium resting upon the surface of the egg. At the same time the body of the antherozoid displays a rapid vibratory motion. Frequently the cilium, instead of being straight, presents a wavy appearance (figured in the '97 paper). When the sperm is becoming less active waves can be seen travelling along the length of the cilium.

It has been far more difficult to trace by means of sections the path of the spermatozoid through the cytoplasm to the nucleus than it was in *Fucus*, and the reasons for this are obvious from the description given above. The examples observed are too few in number to enable one to base any conclusions upon them. The case shown in Fig. 20 is interesting for various reasons. The dark object seen to the right

of the egg-nucleus is almost certainly an antherozoid. When compared with those that are lying outside the membrane it is seen to have greatly increased in size and to be somewhat fibrillar in texture. The egg-nucleus is in a prophase stage, but its appearance is more like that of parthenogenesis than of normal segmentation (see Figs. 22, 23). For some reason or other this particular spermatozoid, although it has entered the egg, has failed to reach the nucleus. Fig. 29 is still more curious. Here I regard the egg-nucleus, the one to the right, as having divided parthenogenetically, while the left-hand nucleus is probably an antherozoid nucleus which has failed to fuse with the former, but, at the expense of the egg cytoplasm, has greatly increased in size. On this view this is a stage further than the preceding one.

IV. The Segmentation of the Fertilized Egg.

The first reliable evidence of fertilization having been accomplished is the presence in the egg-nucleus of a second nucleolus, which is nearly always smaller than the original one (Figs. 21, 22, 23); this undoubtedly represents the chromatin brought in by the spermatozoid. Very soon the nucleoli present an appearance suggestive of their containing a number of deeply stained granules or chromosome-like masses. At the same time the chromatin thread is very fine and distributed through the whole of the nuclear space. After this the chromosomes are seen as thick curved rods, coarsely and irregularly beaded (Fig. 22). These after a slight increase in size are longitudinally split, and the chromatin disks become more distinct. The crowded appearance of the chromosomes at once suggests that they are more numerous than in the preceding stages: when they are counted the number in each case is found to be thirty-two. The nucleoli at this period are very irregular in form, but though they seem as if going to fragment they keep their coherence till the spindle stage.

Returning now to a consideration of the extra-nuclear structures we find that in the unfertilized newly liberated egg, there is a zone of 'kinoplasm' surrounding the egg, and that outside this the chloroplasts tend to place their axes radially. At this period it is most difficult to find any recognizable centrosomes. In the stage represented by Fig. 21 there is a centrosome and radiations on one side of the nucleus; everywhere else the chloroplasts seem to be disposed without definite order, and in many instances they abut directly on the nuclear membrane. It is difficult to be quite certain about the number of centrospheres, but from all I have hitherto seen I am strongly inclined to think that there is only one.

Fig. 24 shows an appearance which is so common as to lead me to think that it represents the normal mode of development of the seg-

mentation spindle, as it undoubtedly does also in the second division of the tetraspore mother-cell. Two sheaves of fibres make their appearance at points in the periphery not far from each other; the whole figure forming a kind of angular spindle with the fibres not continuous. At the ends of the fibres are the chromosomes and a nucleolar mass. The spindle gradually straightens out till it assumes the form of a normal spindle (Fig. 25). If this view is correct then it furnishes a corroboration of the theory that the spermatozoid brings into the egg something (the centrosome, or the influence which produces the centrosome) that determines the polarity of the spindle.

First we have the newly liberated egg without centrosphere or any evidence of polarity. From the description given below we find that in parthenogenetic figures there is a total absence of directive influence, the figures are always multipolar, and very irregular. In the prophase stage of the fertilized egg there is a single centrosphere which divides into two; as the two separate the angular spindle straightens out until the two poles are exactly opposite each other.

Of the mature spindle it is unnecessary to speak at any length. It presents the usual features, as may be seen from Fig. 25, excepting that there is always less nucleolar matter. An interesting question, which I have not yet been able to solve, is the fate of the male nucleolus; is it all used up in chromosome-formation or does it ultimately fuse with the other nucleolar mass? I have up to the present been unable to identify the two masses during the spindle stage. The next figure shows a good polar view of the equatorial plate, which conclusively shows the chromosomes to be thirty-two in number.

The telophase stage (Fig. 27) is much like that of tetraspore-segmentation, but there is more frequently the rudiment of a nucleolus present; sometimes two may be distinguished. Later on the daughter-nuclei seem to contain several nucleolar masses. Very soon, however, the nuclei assume the appearance of the resting stage, in which there are most frequently two nucleoli. These appear very similar to those shown in Fig. 35 of the tetraspore paper; there, however, only one of the two bodies represents the true nucleolus, the other disappears soon after; here the two bodies are almost certainly the male and female nucleoli.

The centrosomes and radiations are very clear at this stage, and they remain distinct during the early stages of the succeeding division. Many of the later mitoses have been studied, but they present no new features, so it is useless to describe them.

The time occupied by the segmentation process in *Dictyota* is much less than it is in the Fucaceae. In the latter it averages sixteen to twenty-four hours: if fertilized eggs of *Dictyota* be left for this length of time it will be found that many of the germlings are already two to

four-celled. In material which was left for nine hours after the addition of antherozoids the following stages were found:

(1) Nuclei with two nucleoli and spirem.
(2) Nuclei with two nucleoli and chromatin segments.
(3) Spindles.
(4) Diasters.
(5) A few binucleate stages.

In addition to these, however, there was a large number of eggs which had been liberated from half an hour to an hour before the addition of antherozoids, and so had become 'stale' and incapable of being fertilized; these showed various stages of parthenogenesis.

V. The Parthenogenesis of Unfertilized Eggs.

A very large number of germination experiments was carried out upon the oospheres of *Dictyota*. Most frequently antherozoids were added, but control experiments were also performed with the antherozoids omitted. In all the former cases there was a mixture of germlings which had followed the normal course of segmentation as detailed in the preceding section, together with others which showed the characteristic phenomena of parthenogenesis described below. There is no difficulty in distinguishing between the two sets of figures. Those of the fertilized eggs can easily be recognized by their resemblance to ordinary karyokinetic figures; those of the parthenogenesis are quite abnormal in appearance. That the latter really are parthenogenetic figures is confirmed by the fact that they are very numerous in the control cultures, to which no sperms were added, and that these never show any signs of normal mitotic figures.

Similar irregular multipolar figures are obtained in certain animal eggs, chiefly as the result of artificial stimulation or of polyspermy, and also in malignant tumours. The cases described by Loeb, Morgan, the Hertwigs, Wilson, Galeotti, and a great many others are too well known to need recapitulation. It is important, however, to bear in mind that parthenogenesis in *Dictyota* is not the result of any such unusual conditions; the very fact that they occur side by side with perfectly normal karyokinetic figures is sufficient to exclude any such supposition.

As it is very important to ascertain the exact number of chromosomes in the figures, and to know whether two nucleoli are present or only one, it is evidently essential to compare together all the sections of a nucleus before drawing any conclusions. I have throughout adhered rigidly to this practice, but in dealing with cultures of oospores and tetraspores there are certain difficulties which do not confront one when sectioning the reproductive cells on the plant itself. It is very difficult to get cultures of *Dictyota* eggs free from sand, diatoms, &c., as in

order to get a sufficient number of the ova large pieces of the plants have to be placed in the culture dishes. When such siliceous particles are present they often interfere with the microtoming, and cause the tearing of the ribbons and the consequent loss of sections. Even when the cutting has been successful there are so many eggs in each slice that the identification of the successive sections of any particular one is often a matter of some difficulty. To obviate this trouble it is often a convenient practice to include in the material some other easily cut, and sufficiently distinctive, object to serve as 'landmark' so to speak, by reference to which to locate the others.

There are two ways in which an unfertilized egg-nucleus of *Dictyota* may initiate its division. In both cases the single nucleolus increases in size, and shows a tendency to break up into chromosome-like masses. In the mode shown in Fig. 30 the nuclear membrane disappears, though the limits of the nuclear cavity are still recognizable. A globular mass is found in the centre, which consists probably of both nucleoplasm and nucleolar substance. This is the least common method, but a fair number of examples has been met with. Fig. 31 shows a far more frequent case; here the membrane is still intact, but the nucleolus seems to be fragmenting. The considerations that incline me to the conclusion that the nucleolus breaks up directly into chromosomes are the following:

(*a*) In many hundreds of cases examined I have never yet seen a spirem.

(*b*) As soon as the chromosomes are formed the nucleolus completely disappears; neither nucleolar globule nor chromatin fibrillae remain.

While the change in the nucleolus is going on the nuclear network preserves its usual appearance. Outside the nucleus there is nothing suggestive of either centrosome or polar radiation, while the fertilized egg-nucleus on the other hand has a distinct centrosphere (Fig. 21). The cytoplasm and chloroplasts frequently retain the faintly radiate structure shown in Fig. 19. The stage where the chromosomes have been differentiated, and the nucleolus has very nearly disappeared, is seen in Figs. 20 and 32. Not only is this stage distinguishable by the absence of nucleoli, but the chromosomes are only sixteen in number, and they are different from those of fertilized eggs in being thinner and less regular in form.

Fig. 32 shows the only case observed where any fibres had appeared before the dissolution of the nuclear membrane. It is not unlike Fig. 24, but the very rudimentary appearance of the figure, and the fact that there are only sixteen chromosomes in the three sections of the figure, prove that it is merely an exceptionally regular case of parthenogenesis.

It is a very striking fact that while in nearly all the normal mitoses described in these two papers the nuclear membrane persists until a very

late period of the anaphase stage, in the division figures of parthenogenetic nuclei the *membrane is invariably absent*. The disappearance must be very rapid, for in all the hundreds of cases examined I have never met with any where the dissolution was partial. Coincidently with the disappearance of the membrane the nuclear space, now sharply delimited by the crowded chloroplasts and coarser reticulation, is seen to be occupied with very fine meshed or finely fibrillar 'kinoplasm,' in which long fibres can be distinguished. No two figures are alike in the arrangement of the threads. Some of the most regular are shown in Figs. 33–36. In many the longer threads cross each other in inextricable confusion, so that no two can be seen to converge. In such cases it almost seems an abuse of language to call them spindle-fibres.

A point in which these figures present a sharp contrast to the artificially produced multipolar figures of animal eggs is the total absence of both centrosomes and radiations. The figure is entirely confined to the nuclear space, and no cytasters or any other radiate structures are seen outside. This is the more remarkable as centrospheres are such prominent features in normal mitoses.

The subsequent history of the dividing nucleus is rather peculiar. In the dense 'kinoplasmic' mass a number of approximately spherical lighter areas appear, and in each area one or several curved chromosomes. Very soon after differentiation each area is delimited by a membrane, and the interior of the dense felt of kinoplasm is occupied by a cluster of nuclei, some large and some small, the size generally depending upon the number of the contained chromosomes (Figs. 37, 39). I have been unable as yet to decide whether as a preparation for this stage there is a division of chromosomes. I am inclined to think there is, but the supposed instances of it are not clear enough to enable one to come to a final decision. There is undoubtedly a subsequent increase in the number of nuclei, but not by karyokinesis.

The number of eggs in a group varies : comparing the several sections of a germling together, I found it to contain twenty nuclei ; in others the number would not be more than five or six.

After a time the several chromosomes in a nucleus become spherical in form, then fuse together to form a nucleolus ; at the same time a faint reticulum appears (Fig. 40), which very often contracts away from the membrane.

It was stated above that no cytoplasmic radiations were ever associated with the multipolar spindles described in the preceding section. Fig. 41 represents a later stage in which radiations occurred, and where also one of the nuclei had a well-formed centrosome and the usual distortion of the membrane opposite to it. Whether this is an instance of the formation of these structures *de novo*, or whether we have here one of those not

infrequent cases of polyspermy already referred to, could not be decided, as I have not yet seen the phenomenon in any of the control cultures.

After a time the nuclei separate into two or more groups. As a general rule there is nothing more to be seen than a constriction of the kinoplasmic mass containing the nuclei, followed by the appearance of two groups instead of one; these groups taking up the positions which in a normal segmentation would have been occupied by the two daughter-nuclei (Fig. 42). The plane of division of the cell becomes almost clear of chloroplasts, and after a time the dividing wall makes its appearance; the early stage of this process is shown in Fig. 42.

In a solitary instance nuclei were observed with connecting fibres between them (Fig. 38); these presented the additional peculiarity of being in the dispirem stage. Although it is very evident that the nuclei increase in number, the chromatin does not increase proportionately in amount. There is always a great difficulty in staining the nuclei, and in the later stages many of them are undistinguishable from cytoplasmic structures, excepting by their form. The multiplication of nuclei must be accomplished by direct division as, after the original multipolar figures, neither chromosomes, fibres, nor any other signs of mitosis are ever seen.

Instead of first dividing into two cells each with its group of nuclei, the original cluster in rare cases separates into three or even four groups. The largest number of cells seen in a germling was six; they were very unequal in size, but every cell contained a cluster of nuclei. After a few divisions the germlings invariably died. It may be suggested that some of them may develop into mature plants in their natural habitats; this is very unlikely, for the fertilized eggs in the same culture grow vigorously.

General Considerations.

A few general questions suggest themselves for consideration. At the present stage I do not intend to discuss them fully; this will be done when the investigation of other Dictyotaceae has been completed.

1. *The Nucleolus.* That this structure in all the cases discussed contains the bulk of the chromatin is fairly obvious. In most cases there is a quantity of substance which is not employed in building up the chromosomes. How is it that in unfertilized eggs the nucleolus breaks up directly into chromosomes, and how is it that no residual nucleolar substance is ever left over after the differentiation of the chromosomes? Can it be that in the mature egg the nucleolus has lost some of its capacity for metabolism, and that this can only be restored by the advent of the antherozoid?

2. *The centrosome and radiations.* It is clear from the foregoing description that, while the centrosome cannot be regarded as a mere condensation point at the focus of the system of radiations—its peculiar

curved rod-like form precludes that possibility—it cannot, on the other hand, be regarded as a permanent cell-organ, for at several stages it disappears completely. At the same time, the comparison of normal with parthenogenetic segmentation strongly supports the idea that the egg after maturation is far less capable of giving rise to a new centrosphere than before. The unfertilized egg shows no indication of its presence, while in the fertilized ovum there is apparently only one, which subsequently divides to form the two centrosomes of the angular spindle. It is difficult to resist the conclusion that the antherozoid introduces into the egg something which enables it to form a centrosphere afresh. If this be correct, the imported substance can hardly be a centrosome, for no such structure can be recognized in the antherozoid before entry.

With regard to the division of centrosomes, Mottier has described and figured the phenomenon in the early prophase of the second division of the tetrasporangium. I have never been able to satisfy myself that I have actually observed the splitting in the way that he figures it. In certain stages it is quite common to see the two chromosomes close to each other, and to find that during the later stages they travel farther from each other until the spindle, if already initiated, from being angular as at first, becomes quite straight or only very slightly curved. This occurs in the prophase of the first mitosis of the fertilized egg, in the newly formed daughter-cells of both tetraspore and oospore segmentation, as well as in the case described by Mottier, but not in the newly formed nucleus of the tetrasporangium or in the first division of the tetraspore.

The peculiarly irregular appearance of the membrane in the neighbourhood of the centrosome in some stages tempts one to subscribe to the idea advanced by Mathews and others, that the centrosome is a liquefying enzyme, and that in virtue of this characteristic it may thus act not only upon the cytoplasm but upon the polar regions of the membrane also. This, however, is exceedingly doubtful, for not only have investigators failed to extract an enzyme from cells containing centrosomes, but in the parthenogenesis of eggs of *Dictyota* it has been shown that where the centrosome is absent the dissolution of the membrane is sudden and complete, and takes place at an early stage.

3. *The nuclear membrane.* Whatever may be the structure and consistency of the membrane the phenomena just described support the hypothesis that its formation is determined by the metabolic processes going on in the chromatic mass. Evidently the reason for the formation of a number of nuclei in the parthenogenesis here described is that the chromosomes are too widely scattered, so that when a chromosome is isolated it directly or indirectly initiates the formation of a membrane round itself. Juel, in his paper on *Hemerocallis*, describes a stray chromo-

some surrounding itself with a membrane. It is a curious fact that in *Dictyota* a stage like Fig. 5 in the Tetraspore paper (Pl. IX) is followed by one similar to Fig. 33 of the same series, or Figs. 6, 27 accompanying the present paper, where the chromosomes are less crowded than before. Whatever be the cause of the former condition, it probably facilitates the formation of a common membrane, and so prevents the separation of the chromatic elements.

It has been shown that the thickness of the membrane varies considerably in different stages: we find it to be thinnest, for instance, during the synapsis stage of the tetrasporangium nucleus. This has probably some significance from the point of view of nutrition. The further fact that at certain periods, in some newly formed nuclei for example, the nuclear network contracts away from the membrane far more easily than at other stages, also suggests that the relation of the network to the membrane, and probably also to the cytoplasm, varies from time to time.

4. *Segmentation in parthenogenetic germlings.* Is the separation of the nuclei into two groups due to inherent repulsion between the nuclei of the separating groups, or is it due entirely to the action of the cytoplasm? If the former, then there must have been some sort of division of the chromosomes, or, failing that, subsequent differentiation in the characters of the nuclei must have taken place. At present the matter is obscure, and must be left over for further investigation.

Summary.

1. The sexual cells, unlike the tetraspores, are produced and liberated simultaneously in fortnightly crops. Fertilization is external. Eggs not fertilized within about half or three-quarters of an hour after liberation become invested with walls and germinate parthenogenetically. Freshly liberated oospheres strongly attract the antherozoids, are fertilized and segment in a normal manner.

2. The oogonium and antheridium are produced by the increased growth of surface cells, which, after cutting off a stalk-cell, form respectively a single egg, or over 1,500 antherozoids.

3. There is no division of the nucleus in the oogonium as there is in that of *Fucus*. All the divisions of the antheridium as well as the stalk-cell division of the oogonium are homotype, and are very similar to the stalk-cell division of the tetraspore, except for the fact that there are only sixteen chromosomes.

4. The antherozoid has the cilium lateral. There may be a second very much reduced cilium, but this is difficult to demonstrate. The nucleus is in the thicker end of the pear-shaped antherozoid; the eye-spot is very small, and instead of being at the base of the cilium is generally near the anterior end of the 'beak.' The antherozoids crowd

round the newly liberated eggs in preference to the others, probably in consequence of a chemotactic attraction.

5. When an egg has been fertilized the nucleus generally has within it a second smaller (male) nucleolus. Later on thirty-two chromosomes appear, but the two nucleoli are still present. There is at first a single centrosome which divides into two; as the two separate the two spindle-cones also diverge, till ultimately they form a normal spindle.

6. When an egg has not been fertilized the nucleolus breaks up into chromosomes; leaving no residual nucleolar matter to be extruded into the cytoplasm as occurs in other mitoses. The mitotic figure is very irregular and multipolar; there is no nuclear membrane, and a cluster of nuclei is formed each containing sometimes one, sometimes several chromosomes. These separate into two or more groups, and partition walls are formed between them. The process may go on a little further, but very soon it stops and the germlings die.

7. When normal germination is compared with parthenogenesis we find that the entry of the antherozoid into an egg produces the following results:—

(*a*) It causes a centrosome and radiations to appear in the cytoplasm.

(*b*) Sixteen additional chromosomes are introduced into the nucleus.

(*c*) The metabolism of the nucleus is far more active, as is shown by the greater amount of nucleolar matter and the increased size of the chromosomes in the prophase stage.

(*d*) In the mitosis it introduces a directive influence which is completely absent from the parthenogenetic figure. This prevents the scattering of the chromosomes and the consequent formation of a large number of nuclei.

(*e*) It prevents the early disappearance of the nuclear membrane.

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EXPLANATION OF FIGURES IN PLATES XII, XIII, AND XIV.

Illustrating Mr. Lloyd Williams's paper on the Dictyotaceae.

For convenience of comparison the same scale of magnification has been preserved throughout, all the figures having been drawn with the camera lucida under the Zeiss apochromatic oil-immersion objective 3·0 mm., aperture 1·40, with ocular 8.

The development of the Oogonium.

Fig. 1. The rudiment of the oogonium before stalk-cell division.

Fig. 2. Prophase. The spirem has segmented, and the nucleolus is becoming vacuolated.

Fig. 3. A stage further; the chromosomes are longitudinally split, and the nucleolus is breaking up into fibrillae and the usual globule.

Fig. 4. Stalk-cell division spindle. The cordate form is due to contraction, the nucleolar globule and fibrillae are present.

Fig. 5. Polar view of the equatorial plate, showing the curved form of the chromosomes; the number is sixteen.

Fig. 6. Dispirem of the nucleolus of the oogonium, showing the increase in size of the chromosomes before fusion.

Fig. 7. Telophase stage of the nucleus of the oogonium, showing a number of deeply stained masses embedded in lighter staining substance.

The development of the Antheridium.

Fig. 8. Rudiment of the antheridium, the nucleus in the prophase of stalk-cell division.

Fig. 9. Telophase of the stalk-cell division; the stalk-cell nucleus has descended into the vacuolated basal region.

Fig. 10. Vertical section parallel to the long axis of the thallus. The antheridium has already divided transversely.

Fig. 11. Vertical section transverse to the axis of the thallus. First division of the antheridium, polar view of the nuclear plate, showing the number of chromosomes.

Fig. 12. Vertical section showing border-cells.

Fig. 13. Vertical section transverse to the thallus. Division more advanced. The inner border-cell is seen to the left.

Fig. 14. Vertical section through a mature antheridium; the inner border-cell is still undivided.

Fig. 15. Antherozoids fixed with potassium-iodide iodine in sea-water and stained with methylene blue. Nuclei and physodes shown.

Fig. 16. Antherozoids fixed with dilute Flemming's mixture and stained by the iron-alum-haematoxylin method.

Fig. 17. Antherozoids fixed with dilute Hermann's solution and stained with gentian violet. At the top left-hand corner is an apparently biciliate, and at the right-hand side of the figure a binucleate antherozoid. In Figs. 15–17 the small black dots are physodes.

Fig. 18. Antherozoids drawn while motile. The small black dot is the red eye-spot, the colourless part is the nucleus; some show the presence of physodes.

Fertilization and segmentation of the Egg.

Fig. 19. Section of newly liberated egg fixed with dilute Flemming. There is a zone of 'kinoplasm' round the nucleus, and a radiate arrangement of the chloroplasts. The physodes are at the surface of the egg. Of the numerous antherozoids in the neighbourhood three have been drawn.

Fig. 20. Delayed fertilization. The antherozoid, greatly increased in size, is seen to the right of the nucleus, a few are shown outside the membrane. The egg-nucleus is in the prophase of parthenogenesis.

Fig. 21. Fertilized egg-nucleus with male and female nucleoli and a single centrosphere.

Fig. 22. Prophase of the first segmentation mitosis; the chromosomes are thirty-two in number, the remainder being in two other sections.

Fig. 23. A later stage; the chromosomes are split and the nucleoli very irregular in form.

Fig. 24. Early spindle showing two cones at an angle with each other. The faint radiations are better seen in the succeeding section.

Fig. 25. The completed spindle.

Fig. 26. Polar view of the nuclear plate, showing the thirty-two chromosomes.

Fig. 27. The two daughter-nuclei in the dispirem stage; the nucleoli beginning to differentiate.

Fig. 28. The daughter-nuclei completed; each has two nucleoli as in the prophase of the preceding mitosis.

Fig. 29. Abnormal fertilization; a stage later than Fig. 20.

Parthenogenesis of unfertilized Eggs.

Figs. 30, 31. Two modes of initiating the parthenogenetic figure. In both the nucleolus seems to be directly converted into chromosomes.

Fig. 32. An exceptionally regular early spindle. Here again the chromosomes seem to be directly converted into chromosomes, of which there are eleven in this section and five in the next.

Figs. 33–36. Four examples of the parthenogenetic 'spindle.' It is very multipolar and exceedingly irregular, there is no trace of nuclear membrane or of nucleolar substance, and the chromosomes are invariably sixteen in number.

Fig. 37. Vesicles (nuclei) containing one or several chromosomes beginning to differentiate in the kinoplasmic mass.

Fig. 38. An exceptional figure showing the separation of two groups of nuclei with a few connecting fibres.

Fig. 39. Newly formed nuclei with chromosomes.

Fig. 40. A few nuclei out of a group of twenty; nucleoli beginning to appear.

Fig. 41. The only instance observed of a group of nuclei with centrosome and radiations.

Fig. 42. Parthenogenetic germling in which the nuclei have separated into two groups; a septum is beginning to form between the two halves of the cell.

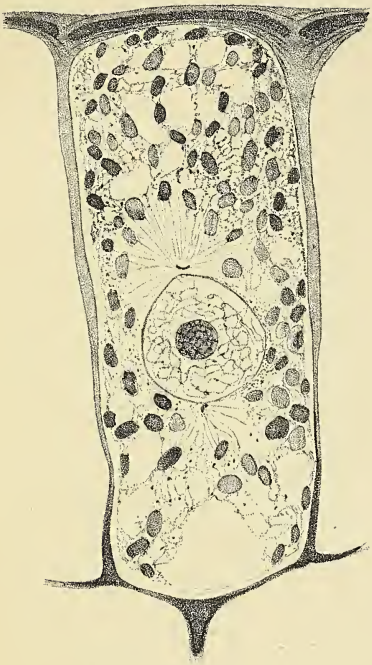

Fig. 1.

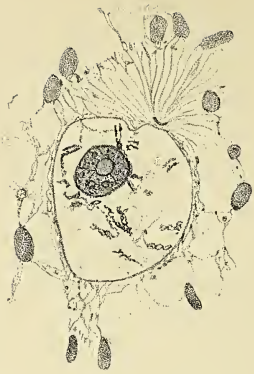

Fig. 2.

Fig. 3.

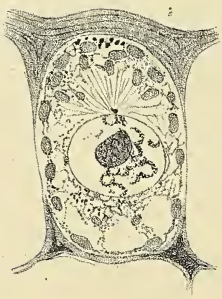

Fig. 8.

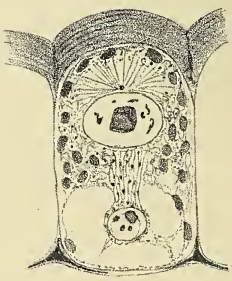

Fig. 9.

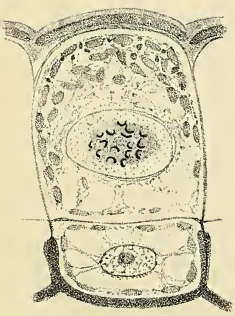

Fig. 11.

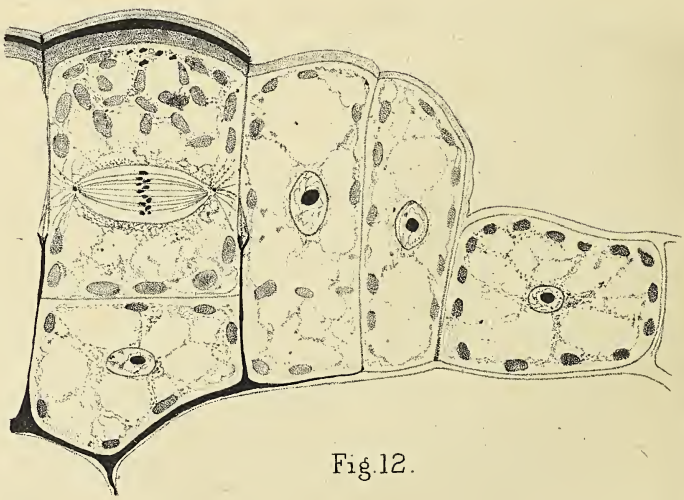

Fig. 12.

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Fig. 4.

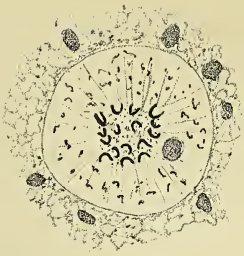

Fig. 5.

Fig. 6.

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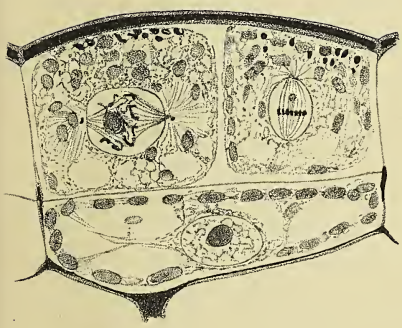

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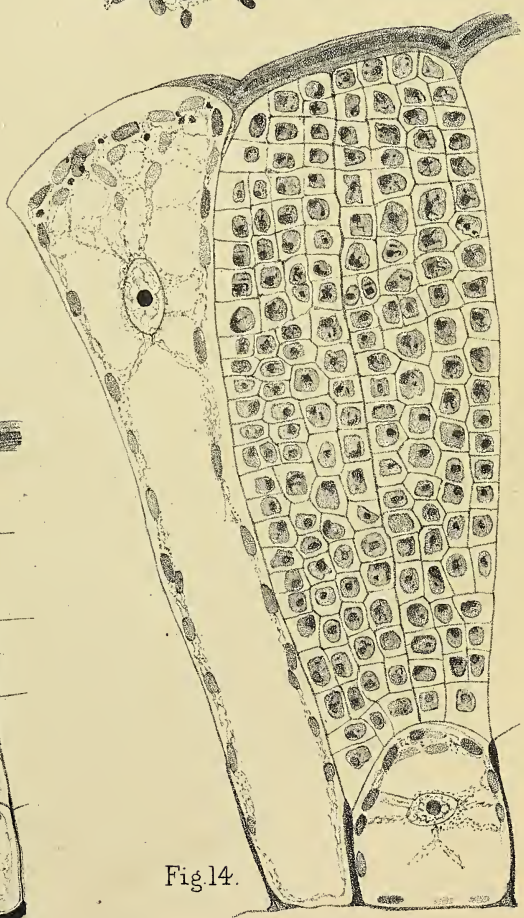

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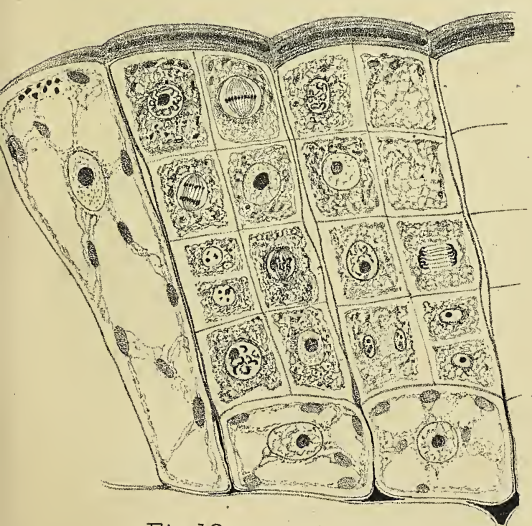

Fig. 13.

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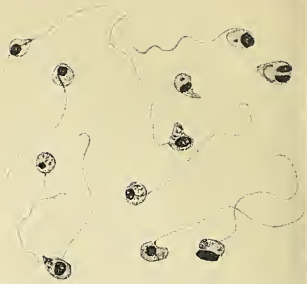
Fig.15.

Fig.16.



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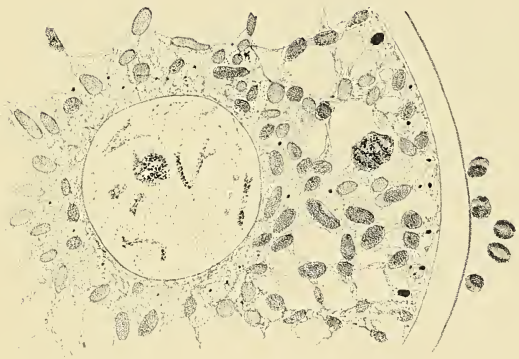


Fig.20.

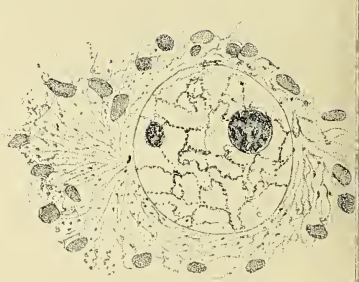
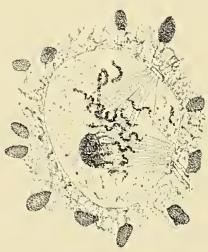


Fig.21.

Fig.23.



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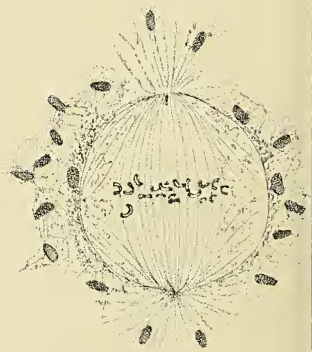

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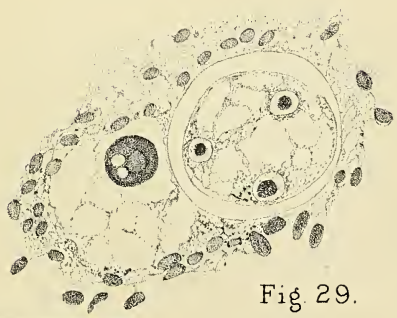

Fig.29.

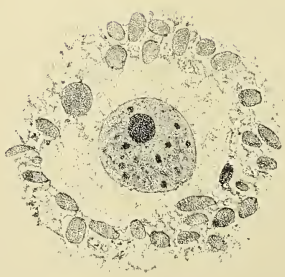

Fig.30.

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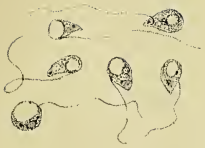

Fig. 18.

Fig. 19.

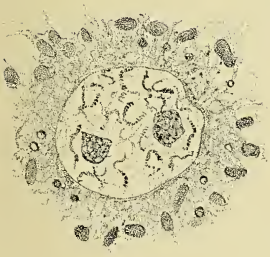

Fig. 22.

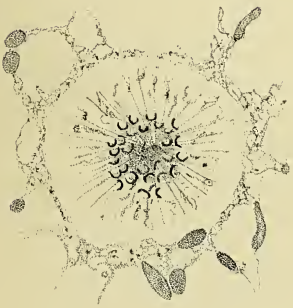

Fig. 26.

Fig. 27.

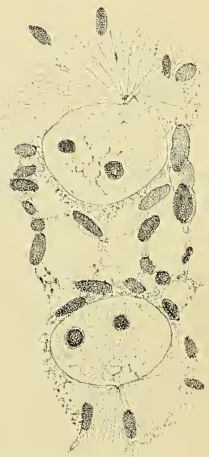

Fig. 28.

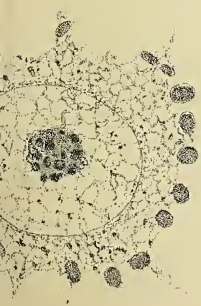

Fig. 31.

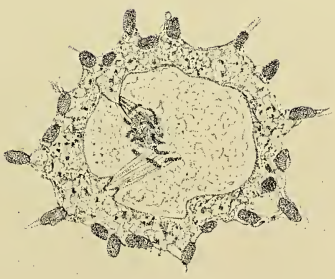

Fig. 32.

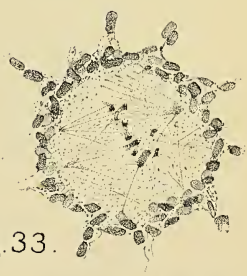

Fig. 33.

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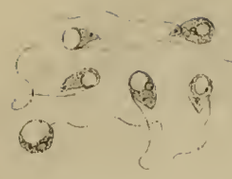
Fig.15.

Fig.16.

Fig.17.



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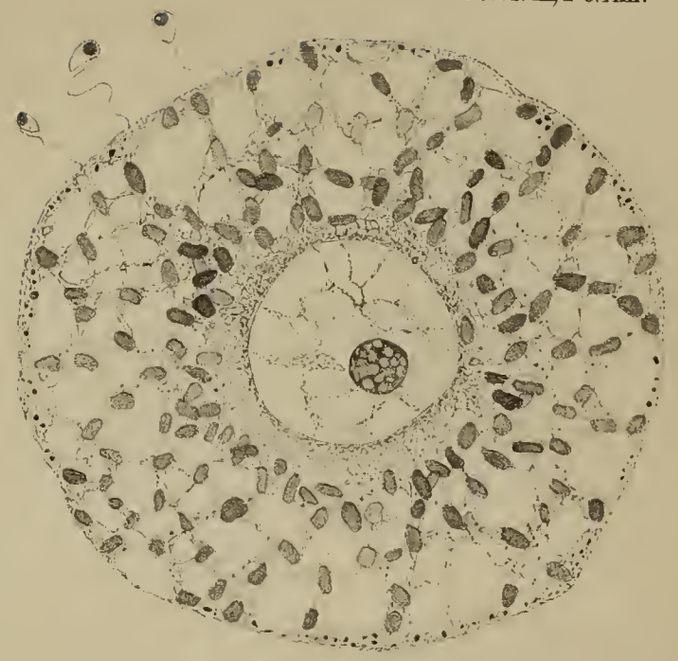

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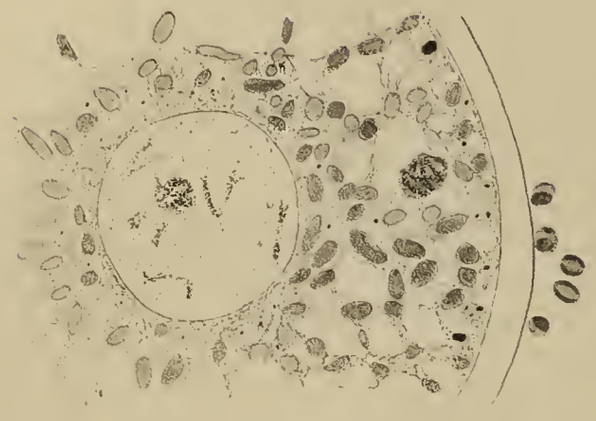


Fig.20.

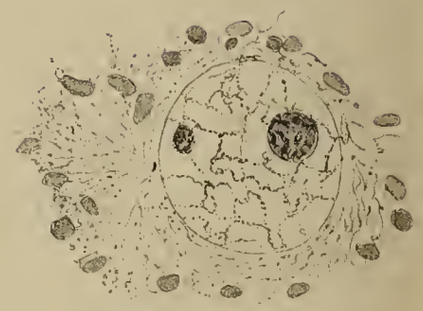
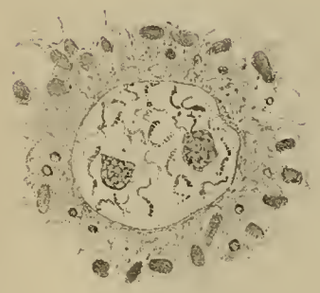


Fig.21.



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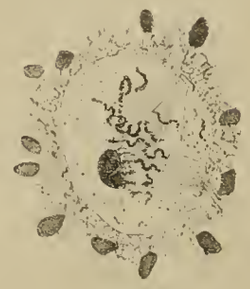

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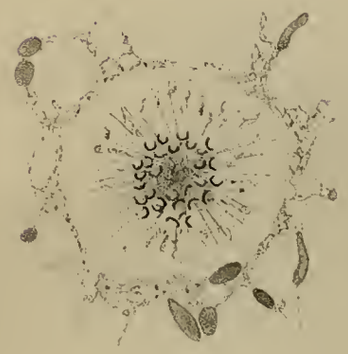

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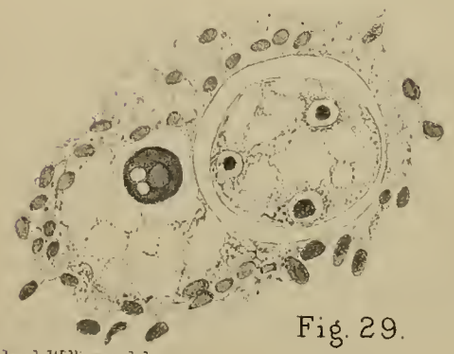

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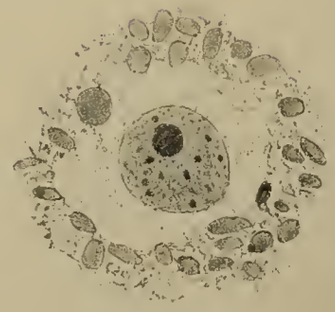


Fig.30.

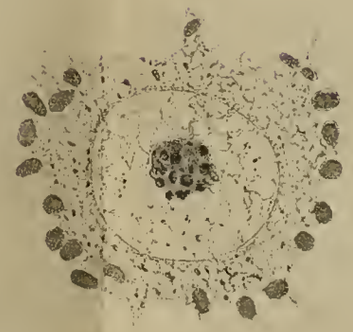
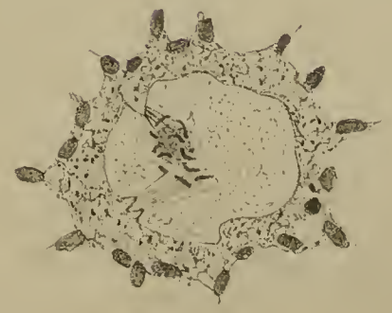


Fig.31.



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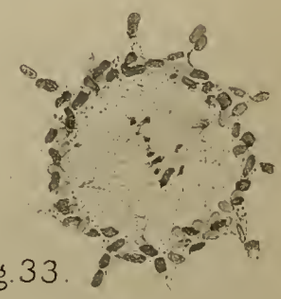

Fig.33.

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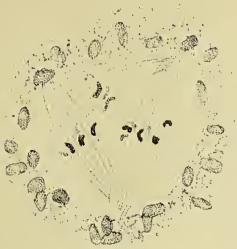
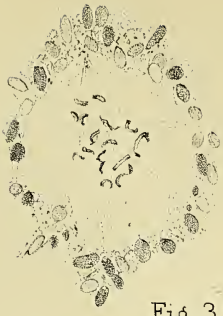


Fig. 34.



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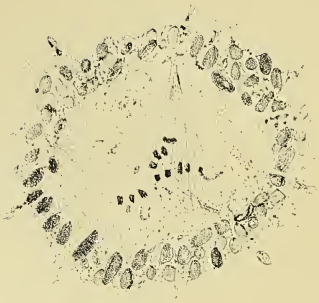

Fig. 36.

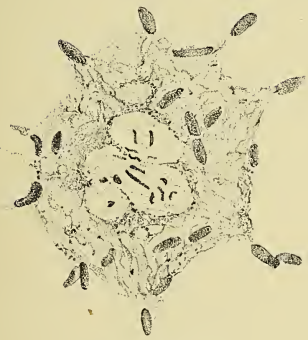

Fig. 37.

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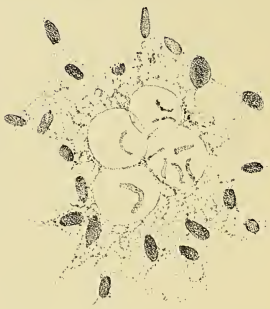

Fig. 39.

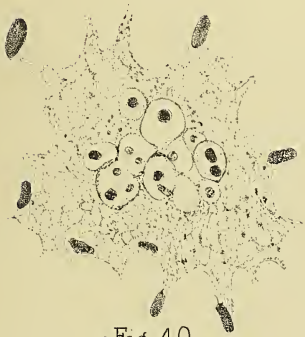

Fig. 40.

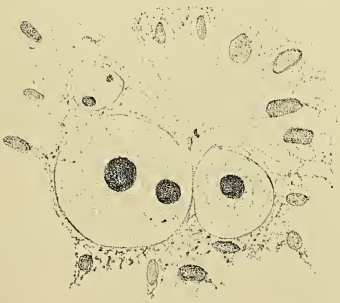

Fig. 41.

Fig 42.

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Ophioglossum simplex, Ridley.

BY

F. O. BOWER, F.R.S.,

Regius Professor of Botany in the University of Glasgow.

With Plate XV.

IN May, 1900, I received from Professor Groom, to whom my grateful thanks are due, a specimen of a plant which had been collected in Sumatra, in 1897, by Mr. Ridley; his description of it is as follows:—

'A NEW SPECIES OF *Ophioglossum*.

'The very remarkable little species of *Ophioglossum* I am about to describe was found by me in a dense wet forest on the banks of the Kelantan River, near Siak, in Sumatra, in December, 1897. I was only able to find three plants, for it is very inconspicuous, and as I was still far from camp, and it was very late when I perceived the plants, there was not sufficient time to search thoroughly the locality, which is one by no means easy of access. I would describe it as follows:—

'*Ophioglossum simplex*, n. sp.[1] Terrestrial, rhizome short, and tuberous, with few roots. Fertile fronds solitary, or two together, slender flattened, with a blunt apex, 4 to 6 inches long, $\frac{1}{8}$th inch wide, dark green, sterile division represented by a very small lateral process, or quite absent. Fertile portion about an inch long.

'*Hab.*—Dense wet forests on the Kelantan River, Siak, Eastern Sumatra.

'The affinity of this curious plant is with *O. Bergiana*, Schlecht, of South Africa, and with *O. pendulum*, L., an epiphytic plant common in Eastern Asia. The almost complete suppression of any trace of a sterile portion of the frond, and the consequent reduction of the plant to the very simplest elements, is the most peculiar feature in this species. Its habitat is very peculiar for the genus, for though there are two indigenous species of terrestrial *Ophioglossum*, viz. *O. nudicaule* and *O. reticulatum*, to be found

[1] This name has already been used by Rumphius in 1750 (Herb. Amb. vi, p. 152, Tab. 68, Fig. 1), but the plant so described falls under *O. pedunculosum* (Prantl, Beiträge z. Syst. d. Ophioglosseen, p. 329).

[Annals of Botany, Vol. XVIII. No. LXX. April, 1904.]

here, besides the epiphytic *O. pendulum*, L., none of these are forest plants, occurring only in open grassy spots ; while even the epiphytic one inhabits open woods, or grows on trees where there is plenty of light.'

Professor Groom examined the plant himself, and found its characters, so far as observed, to be those of *Ophioglossum* ; he then kindly forwarded it to me for further analysis. The description now given is based on only the one specimen ; two others were collected by Mr. Ridley, but they are said to be incomplete, and are at Singapore. The whole description must therefore be considered as provisional, and open to amendment when fresh material is available.

The specimen sent by Mr. Ridley was figured by Professor Groom in its unaltered state ; his drawing is shown in Fig. 1. The plant consists of a short stock, bearing three appendages of different ages. The largest is almost mature, and shows a structure like a fertile spike of *Ophioglossum*, with the lateral rows of sporangia almost mature. The second is similar in outline, but the sporangia are so young as to be barely perceptible ; still there can be no doubt that it is also of the nature of a fertile spike. The third is a small conical body of oblique position, and forced out of shape by the pressing. A careful external examination of the two largest appendages discloses no part which could be compared with the sterile lobe, or lamina of a subtending sporophyll, of other Ophioglossaceae. The leaf-stalks were examined throughout their length for scars or other traces of the insertion of a sterile lobe, but none was found. It is true there were scars of a reddish and rough appearance at various points on the stalks, but they are very irregular in form and position, and are not to be taken as scars of insertion of a withered sterile lobe, notwithstanding that one of them is near the base of the oldest leaf.

This is clearly seen in Fig. 2, which shows the base of the plant on a larger scale ; it would seem probable from the small size of the stock, its form, and from the entire absence of roots (with the exception of the small conical body which will be shown below to be a root) that the underground parts had been partially broken away in removing the plant from the soil. This was shown in the sections subsequently cut from the stock ; the insertions of roots which had been broken away while still actively functional were found. After the specimen had been soaked out in water, the stalks became sufficiently transparent for the vascular strands to be visible ; and it is clear that the course of the strands does not countenance the idea that the large scar above noted is of the nature of the insertion of a sterile lamina with a vascular supply. Moreover, scars of similar apparent texture, but of different outline, are found most irregularly scattered on the leaf-stalks, and are probably due to some pathological state. The fact that the scars do not correspond in position on the several leaves shows also that they are not constant morphological features. Thus

the external observation of the mature parts of the specimen affords no evidence of any sterile lobe or lamina, as in the known Ophioglossaceae. Mr. Ridley speaks of the 'almost complete suppression of any trace of a sterile portion of the frond'; I find in the specimen sent to me no need for the qualifying word 'almost.'

The small appendage at the base of the plant was removed with care, soaked out, and examined. It appears to have been compressed in drying, and after soaking the form is not recovered. As far as form is concerned there is nothing distinctive, while its oblique position on the specimen as received would allow of its being either a root or a young leaf displaced in the pressing. To decide the point, the whole appendage was removed and embedded; sections then showed that it is a root, and the following structural points were observed.

The stele appears to be of the usual type; it is diarch, and the xylems may remain separated by parenchyma-cells, which occupy the centre. The phloem forms an arc on either side, while even in these sections, which only partially recover from the pressing and drying, an endodermis can sometimes be traced. Outside this is a broad zone of cortical parenchyma, rather thin-walled; this merges into a peripheral band which contains the 'grumous' masses characteristic of mycorhiza: notwithstanding the only partial recovery of the section from drying, there is no room for doubt that the root has been mycorhizal, evidences of the presence of the fungus being seen in some four or five layers of the outer cortex. The periphery of the root is occupied by a layer of cells with their outer walls much thickened; the thickening sometimes extends to the inner walls as well: this layer appears to be of the nature of an exodermis, for at some points remains of an outer layer are still to be seen.

Comparison with *O. pendulum* shows that the details thus described correspond in the main to those there seen: the stele may, it is true, have more than two protoxylem groups in *O. pendulum*, but that character is known to be variable in that species. The characters of the cortex are very closely matched, including the mycorhizal band and the layer (probably exodermis) with the thickened outer wall. These details serve to strengthen the comparison of our plant with *O. pendulum* [1].

Since the external form of the mature appendages gives no indication of the existence of a sterile lamina, it will be well at this point to consider what views are open to us as to their nature in this remarkable plant. Two alternatives are possible, (1) that the appendages are spikes pure and simple, without any structural evidence of the subtending lamina

[1] Compare Atkinson, Bull. Iowa Bot. Club, xx, 1893, p. 356, on Symbiosis in the roots of the Ophioglossaceae: also Janse, Ann. Jard. Bot. de Buitenzorg, xiv, p. 65, Pl. IX, Figs. 11, 12. I have not however in either species noted the infection as local; it appeared to me to extend all round the cortex.

having figured in their past history, or (2) that they are leaves of the ordinary Ophioglossaceous type, in which the sterile lamina has become entirely abortive; in the latter case the whole appendage would be of a composite nature, the upper part being the spike, the lower part being of the nature of a leaf-stalk. A decision can best be approached on an anatomical basis, for there is between the sterile leaf and the fertile spike the general difference of orientation of the vascular strands: the xylem in the former being on the adaxial side, in the latter it is inverted, and facing the abaxial side.

The following notes on the distribution of the vascular strands in the leaves of certain species of *Ophioglossum* may be of use, as a basis for comparison.

In *O. Bergianum*, Schlecht, the leaf-trace originates, as in all the Ophioglossaceae hitherto examined on that point, as a single strand; in most species this single strand branches early, but in *O. Bergianum* it remains at first unbranched. The vascular supply for the fertile spike comes off as two lateral bundles from the margins of the leaf-trace; these fuse together to form the single bundle of the base of the fertile spike. This single strand may branch as it passes upwards, so that the transverse section of the fertile spike may show two, three, or even four strands, but they are always orientated with the xylem directed to the abaxial side. In the upper region there is only one strand, which passes through the rows of sporangia to the extreme tip[1]. The branchings in the sterile leaf are few and irregular, but the number of strands commonly seen is three, with the xylems on the adaxial side. This is the simplest species of the genus structurally, but the leading features are the same in the more complex. A slightly increased complexity is seen in *O. lusitanicum*, where the vascular supply of the sterile leaf branches into three, and the supply from the spike comes off from the lateral strands (Prantl, loc. cit. Pl. VII, Fig. 1).

In *O. vulgatum* the origin of the vascular supply of the spike is here again as two lateral strands, one from either marginal bundle of the sterile leaf[2]. These strands branch again as they pass upwards, and five strands usually appear in the transverse section, an arrangement which may be very nearly matched by some sections near the base of the leaf-stalk; but the distinction is always easily drawn by their inverted position: the xylem of the fertile spike being on the abaxial side. In *O. reticulatum*, L., the arrangement is similar to that in *O. vulgatum*, as regards the fertile spike, and it is probably the type general for the ordinary ground-growing species.

The similarity of *O. simplex* to *O. pendulum* in certain external points,

[1] See Prantl, Beitr. z. Syst. d. Ophioglosseen, Jahr. d. k. bot. Gart. Berlin, iii, p. 297. Also Bower, Studies in the Morphology of Spore-producing Members. II. Ophioglossaceae, p. 68.

[2] See Prantl, l. c., Taf. vii, Fig. 2.

and in the structure of the root, suggested a fresh examination of the vascular supply to the leaf and spike in that species. Prantl[1] in his diagnosis of the three sections of the genus gives for § *Euophioglossum*, 'petioli fasciculi basi tres': this section includes the bulk of the species of the genus. For § *Ophioderma* (including *O. pendulum*), and § *Cheiroglossa* (including *O. palmatum*), 'petioli fasciculi numerosi.' It is true that there are three bundles at the base of the leaf-stalk in *Euophioglossum*, but on entering the axis they fuse to one, before insertion on the main system[2]. Hitherto this insertion as a solitary strand has been found uniform in the Ophioglossaceae examined: but in *O. pendulum* it is not so, as the following description of a definite example will show; but it is possible that in a species which varies so greatly in size, the vascular complexity may vary also. The leaves in this species differ in vascular supply according as they bear spikes or are sterile. In the latter case the transverse section of the leaf-base in the specimen examined shows an open arc of some seven bundles (Fig. 3), which are reduced by fusion to five; these remain as distinct strands, till they insert themselves individually upon the system of the axis (Figs. 6 to 13, leaf to the left). In the case of leaves which bear spikes, the transverse section of the leaf-base shows a complete ring of bundles with their xylems facing inwards (Fig. 4); fusions on the adaxial side show that the distinction of the two margins near the base is not always maintained. As the base of the leaf is approached this foliar ring opens on the adaxial side—with or without some previous fusions on that side (compare Figs. 6 to 13). The strands which are reduced in number by further irregular fusions are then inserted *individually* upon the system of the axis. This, which is at times in form of an almost complete ring (Figs. 6, 7, 8), opens to receive the foliar strands upon the margin of the gap.

This vascular system is in itself not very uniform in detail, and differs from that of other Ophioglossaceae in the leaf-trace not being united at the base to a single strand.

In the sterile leaf the strands pass upwards from the system of the axis as a single curved series (Figs. 2–17, leaf *a*), showing occasional reticulations: the series is open on the adaxial face. As the strands enter the flattened region of the lamina the curve is flattened out also, with the xylems directed upwards. In the leaves which bear spikes the strands on passing from the system of the axis form a circular series, closed on the adaxial face (Fig. 4): irregular branchings and fusions are often seen between the bundles at the opposite margins of the curve, so that those strands which occupy the adaxial side are connected indifferently with both

[1] Beitr. z. Syst. d. Ophioglosseen, p. 299.

[2] Holle, Vegetationsorgane der Ophioglosseen, Bot. Zeit., 1875, p. 269; Rostowzew, Recherches sur *l'Ophioglossum vulgatum*, p. 21, Pl. I, Fig. 5.

margins. Proceeding up the leaf the ring flattens, and as the margins of the lamina become defined strands pass right and left from the ring; the circular series thus becomes broken up into the supply for the sterile lamina on the one hand, and the supply for the spike on the other. The latter consists of five or more strands, with their xylems directed abaxially, while the strands of the former are more numerous, and have their xylems directed adaxially (Fig. 5). Occasional connexions are found higher up, between the strands of the two systems, after their separation. The vascular supply of the spike may thus be held to be mainly, though not always exclusively, a product of marginal branching from the original vascular supply of the leaf-base, as in *Euophioglossum*.

Prantl's section *Cheiroglossa*, including only *O. palmatum*, is described as having 'petioli fasciculi numerosi.' The following observations on their relations to the spikes were made on a specimen sent to me by Mr. Fawcett from Jamaica. Transverse sections about the middle of the stalk show the vascular strands arranged, as in *O. pendulum*, in a ring, with their xylems directed centrally: they number about fifteen, and are of very variable size. Material was wanting for tracing the system downwards into the axis. Following it upwards no marked change took place at first, there being no obvious distinction of those strands which will enter the spikes till immediately below their insertion. Where the spikes are large, as in my leaf from Jamaica, and apparently also in that investigated by Professor Bertrand [1], a number of strands enter each spike. Immediately below the insertion of the lowest spike, though even the outline of the section shows where the stalk of the spike will be inserted, the ring of strands in the leaf-stalk remained undisturbed (Fig. 14); further up the ring opened, and with sundry branchings, four strands—subsequently reduced by fusion to three—passed out into the spike. Here as in other Ophioglossaceae the vascular supply appears to originate from both margins of the parent leaf, and not from one margin only (Figs. 14, 15, 16). The ring of strands in the leaf-stalk having thus opened, it did not again close (Fig. 17), but flattened out with the flattening expansion of the lamina into a wide arc; and in the leaf in question the vascular supply for the higher spikes came off from the margins of the arc, as shown in Figs. 18 to 23 (compare Bertrand's Fig. 97, l. c.).

In those specimens of *O. palmatum* where the spikes are numerous and small, their vascular supply appears to be only a single strand; this originates as a branch from one of the vascular strands, which may subsequently take a course distinctly intra-marginal in the lamina [2].

The characteristics of the vascular arrangements in the appendages

[1] Travaux et Mémoires de l'Univ. de Lille, Tome x, p. 189, Fig. 97.

[2] Compare Studies in the Morphology of Spore-producing Members. II. Ophioglossaceae, Pl. VIII, Figs. 120, 121; Pl. IX, Figs. 126, 127.

of the species of *Ophioglossum* investigated may then be summed up thus:—

(1) *The xylem in the strands of the leaf-stalk at first faces directly or obliquely to the adaxial surface:* in sterile leaves they constitute a more or less extended arc, open on the adaxial side; but in the fertile leaves [1], and especially clearly in *O. pendulum* and *palmatum*, as the strands pass upwards from the base *the arc closes in, and the strands together constitute a ring*, the margins of the arc being indistinguishable, and they may be related to one another by fusions. In the lamina the ring again opens out into a flattened arc, the opening taking place at the insertion of the spike, or of the lowest spike where there are several.

(2) *In the spike the strands are always arranged with their xylems directed abaxially, in a flattened arc, never in a closed circle.*

Thus diagnostic characters, though of a somewhat imperfect sort, exist, marking off the spike from the leaf-stalk. It remains to attempt the application of this diagnosis in the case of *O. simplex*, with a view to deciding the question of the morphological nature of the appendages which it bears. The following considerations as regards probable affinity may help towards the concentration of the problem down to a definite issue. Mr. Ridley suggests that the affinity of our plant is with *O. Bergianum* and with *O. pendulum*: the former affinity I should regard as doubtful, on the grounds of form as well as of anatomical character; the latter affinity seems for similar reasons more natural. But the nearest similarity in form is with the ground-growing plant designated *O. intermedium*, Hook., found by Lobb, near Sarawak, Borneo [2]. This plant was doubtfully included by Prantl in *O. pendulum* [3]. I think that these three plants constitute a natural group, or section of the genus, which may be designated with Prantl, § *Ophioderma*, and be held to consist of three species, viz. *O. pendulum*, L., *O. intermedium*, Hook. [4], and *O. simplex*, Ridley.

If the probable affinity of our plant be, as suggested, with *O. pendulum*, the anatomical issue assumes some degree of definiteness; for we know that in *O. pendulum* the vascular supply in the spike is in the form of a flattened arc, with the xylems directed abaxially, while that of the leaf-stalk shows the strands arranged in a complete ring, with their xylems directed centrally. The question may therefore be put thus: if we find in *O. simplex* that the strands are in a flattened arc and the xylems

[1] Compare Rostowzew, l. c., Text fig. 2, p. 7.

[2] Hooker, Century of Ferns, Tab. xcv.

[3] Prantl, l. c., pp. 331, 332.

[4] I see no sufficient reason for sinking this species, as Prantl does doubtfully. The discovery of *O. simplex* seems to me an additional reason for its retention as a valid species. I have compared the type specimen of *O. intermedium*, at Kew: the habit of the lower part of the plant is very similar to that of *O. simplex*; the difference chiefly lies in the presence of the small sterile lamina, and winged stalk below its insertion in *O. intermedium*, and in a narrower tract of tissue between the rows of sporangia of the spike than in *O. simplex*, but the latter character is variable in *O. pendulum*.

abaxial, the part in question will be of the nature of a spike; if we find that the strands are in a complete ring with the xylems central, the part where that occurs will probably be of the nature of a leaf-stalk. Further, if the leaf-trace continues downwards as separate strands, till the insertion of those strands separately upon the vascular system of the axis, then not only will the affinity with *O. pendulum* be confirmed, as apart from the rest of the genus, but also there will be a strong presumption that the appendages of *O. simplex* are the true correlatives of those of *O. pendulum*, notwithstanding the absence of the sterile lamina, as above noted for our plant. It may be said at once that the question of orientation is difficult in the upper region of any elongated succulent part, having approximately cylindrical form; and especially will this be the case when, as here, the only specimen has been pressed and dried. Certain results as to orientation can, in the present case, be obtained only at the base, and especially at the point of insertion of the appendage upon the axis. With this caution the facts available may be stated as follows.

A transverse section of the stalk at point *A*, Fig. 1, shows the vascular strands five in number, forming an arc open on the flattened side, which will presumably correspond to the abaxial side in a normal *Ophioglossum* (Fig. 24); it is however impossible in this dried specimen to be certain that its direction is actually abaxial. The xylems are directed towards this flattened side, and the structural arrangements are such as to indicate that here we have a part corresponding to that of a normal spike—a conclusion which the external form with its rows of sporangia amply bears out. It seemed useless in the absence of precise knowledge of orientation to pursue the details continuously downwards throughout the stalk, so the next section was taken at level *B*, in Fig. 1. Here the transverse section was more rounded, and the number of strands was found to be as high as seven, arranged in a regular circle, with their xylems directed centrally (Fig. 25); this result coincides fairly with the observations on the stalks soaked out (Fig. 2): the number seven is slightly in excess of the number of strands there shown—possibly in the crushed stalk one of the strands may have overlain another. Comparing this result with the transverse sections near the base of the stalks of fertile leaves of *O. pendulum* (Fig. 4) and of *O. palmatum* (Fig. 14), it appears that the form of the section and the vascular arrangement are similar, though of a rather simpler type, as shown by the smaller number of the strands; and thus the structural evidence points to the lower part being of the nature of a leaf-stalk. It remains to trace these strands downwards to their insertion on the system of the axis. This is shown in the successive sections (Figs. 27, 28, 29) for *O. simplex*, and it is clear from these that though there may have been reduction in number by fusions, the strands do not unite as in most Ophioglossaceae into a single strand, but remain as some four or five

separate strands; these insert themselves individually upon the rather irregular ring of bundles of the stock, which opens by a lateral gap to receive them. A comparison with *O. pendulum* (Figs. 6–13) shows that the arrangement in *O. simplex* is virtually the same, though somewhat smaller and simpler—a fact which still further accentuates the affinity with that species.

We are now in a position to discuss the morphology of this curious plant, and the bearing which its existence may have upon the general theory of the Ophioglossaceous form. It seems clear that the lamina, commonly present, is practically absent here: not even any vestigial trace of it was seen, though if young specimens were available it is possible that such might be found. So far as observation has been possible the appendicular organ appears simple, and to be terminated by a normal fertile spike. Either of two possible views may be based on these facts: (1) that the appendages are simple spikes, which have not, and never had, a subtending sporophyll; (2) that they are leaves of the ordinary Ophioglossaceous type, in which the sterile lamina is entirely abortive.

If the former be their real nature, then we see in *O. simplex* some support for the view put forward some years ago by Campbell [1]. He compared the Ophioglossaceous spike with the sporogonium of *Anthoceros*, and remarked: 'If we could imagine such a sporogonium to develop a root fastening it to the ground, and thus rendering it entirely independent of the oophyte, we should have the simplest possible form of a Pteridophyte.' The plant of *O. simplex* seems to consist of little more than two such 'sporogonia,' with root, or roots; in fact it would almost realize Campbell's forecast, and suggest more strongly than before that the Ophioglossaceae are, as he held, the most primitive Pteridophytes. If it were thus primitive it would point to the spike being the prior existent part, and the lamina a mere subsequent appendage upon it.

But in Celakovsky's view, which I have found reason to support elsewhere [2], the Ophioglossaceae are regarded as derivative forms from a Lycopodinous type of construction, in which a constant relation of the spore-bearing organ to the lamina existed throughout the descent. With such a type the condition of *O. simplex* could only be brought into conformity on a theory of abortion of the lamina, which subtends the spike in the usual Ophioglossaceous type: this is the alternative theory above suggested. Against this theory there can be no *a priori* objection, for in the Ophioglossaceae we see various degrees of abortion of the fertile spike, which lead to the condition of its complete absence; and there seems no reason to hold that what may happen to the spike may not equally

[1] On the affinities of the Filicineae, Bot. Gaz. xv, No. 1, Jan. 1890; also Mosses and Ferns, pp. 296, 297.

[2] Studies in Morphology of Spore-producing Members. II. Ophioglossaceae. Dulau and Co., 1896.

happen to the subtending leaf. As supporting this theory there is the fact of mycorhiza in the root. We have at present no means of measuring from mere structural evidence the nutritive powers of mycorhizal roots, or how far in any given case they may supplement or supersede the chlorophyll-nutrition. In proportion as the mycorhiza is more effective a reduction of the assimilatory system may be anticipated, such as our theory demands, while the spore-producing parts would retain their dimensions, provided that the efficiency of nutrition be not diminished [1]. A second point is the habitat, which Mr. Ridley specially describes as 'dense wet forests,' and pointedly compares it with the 'open grassy spots' where other ground-growing *Ophioglossums* are found. This seems to throw the onus of nutrition upon the mycorhizal roots. Applying these considerations to our present case, it is physiologically possible to contemplate a reduction, or even a complete abortion, of the sterile lamina to such a condition as that shown in *O. simplex*.

And here the anatomical evidence detailed above will come in. It has been above pointed out that the upper part of the appendage in *O. simplex* shows structure as well as form characteristic of the Ophioglossaceous spike; also that towards the base the structure is comparable with that found at the leaf-base in *O. pendulum*, while the insertion of the vascular supply upon that of the axis is characteristic of the leaf of that same species: thus the structural evidence falls in with a theory of abortion of the sterile lamina. This, however, presumes that there is a transition from spike to leaf-stalk as the length of this apparently undifferentiated stalk is traversed. Any theoretical difficulty which such a presumption may occasion will be relieved by comparison of the case of the staminal flowers in the genus *Euphorbia*: there the transition from floral axis to filament (a transition which is not less essential than that from spike to leaf-stalk) is only marked in the slightest way by the well-known articulation. In both cases, on the view above put forward, the simple condition has been arrived at by a process of abortion.

Of the two explanations of *O. simplex* thus put forward, I am disposed to prefer the second, but in all the circumstances of this peculiar case it is not possible to come to any certain conclusion, one way or the other. This can only be done when more material shall be available. Meanwhile the case cannot be held to invalidate the view of Celakovsky, so far as to dictate its rejection.

The systematic position of *O. simplex* will be, according to the characters above described, in Prantl's § *Ophioderma*. But while accepting Prantl's division of the genus (l. c., p. 299), it would I think be well to add to the diagnosis the fact that the insertion of the leaf-trace differs in § *Ophioderma* from that in § *Euophioglossum*. In all species of the latter

[1] Phil. Trans. 1903, vol. cxcvi, pp. 228 and 233–4.

which have been examined anatomically the leaf-trace unites at the base into a single strand before insertion on the system of the axis—a condition which would appear to be the more primitive, especially if the general view of the family be that they represent an ascending series from a small-leaved, polyphyllous ancestry [1]. But in § *Ophioderma*, of which *O. pendulum* and *O. simplex* have been examined (it was impossible to investigate the unique specimen of *O. intermedium* anatomically), the leaf-trace does not unite into a single strand at the base, but the individual strands are separately inserted on the system of the axis: this would appear to be the derivative condition, on any phyletic theory of the family as an ascending series of leaf complexity. I should propose, therefore, to add to Prantl's diagnoses as follows:—for § *Euophioglossum*, 'petioli fasciculi basi tres, deinde in unum conjuncti, in rhizomae fasciculos insertum,' and for § *Ophioderma* 'petioli fasciculi numerosi, separatim in rhizomae fasciculos inserti.' The case is still open for § *Cheiroglossa*, in which I am not aware that the stock has yet been examined anatomically. The anatomical difference thus brought forward, though not one which can be readily applied in ordinary systematic work, is more distinctive and trustworthy than the number of bundles at the base of the petiole, and for this reason it is to be preferred, if such a character is to figure at all in the diagnosis of the sections of the genus.

The provisional conclusion which may be drawn from the study of this new species, together with the two others which are grouped with it, in the § *Ophioderma*, is this: that they form a natural group, anatomically distinct, which illustrates three phases of proportion of the spike to the subtending leaf-lamina: in *O. pendulum* the sterile lamina is large, and sometimes irregularly branched; in *O. intermedium* it is small and simple, while the spike is still of considerable dimensions; in *O. simplex* it is absent, at least in the mature state, while the spike is still large. These three species may illustrate either a descending or an ascending series; the more probable view seems to be that they illustrate a decrease of the sterile leaf, and the extreme condition of *O. simplex* is to be attributed to the presence of mycorhiza, which makes nutrition of the large spike still possible in the dense, wet forest in which it grows, notwithstanding that the usual assimilating organ is functionally non-existent. Reduction is, however, not apparent in the spike itself, for, provided nutrition be kept up from whatever source, it would still maintain its character, being essentially a spore-producing, and not a nutritive member.

The specimen—at least what remains of it after the anatomical investigation above described—together with the original drawing by Professor Groom, will be deposited, according to the wish of Professor Groom, in the botanical department of the Natural History Museum, South Kensington.

[1] Compare Studies in Morphology of spore-producing members, Phil. Trans. 1903, B. vol. cxcvi, pp. 233–7.

DESCRIPTION OF THE FIGURES IN PLATE XV.

Illustrating Professor Bower's paper on *Ophioglossum simplex*.

Fig. 1. The only specimen available of *O. simplex*, from a drawing by Professor Groom, showing the two spike-like appendages, the short stock below, and small root. A. A. and B. B. indicate the levels at which sections were taken. Natural size.

Fig. 2. Base of the specimen on a larger scale after soaking out in water: the course of the vascular bundles in the appendages has been traced. × 4.

Figs. 3–5. Transverse sections of leaf-stalks of *O. pendulum*: Fig. 3, from the base of a sterile leaf; Fig. 4, from the base of a fertile leaf; Fig. 5, higher up on a fertile leaf. × 8.

Figs. 6–13. Successive transverse sections through the stock of *O. pendulum*, illustrating the mode of insertion of the leaf-trace upon the system of the axis. × 4. Whether the leaf be a sterile one (as that on the left side of each of these figures) or a fertile one (as were the other two), the strands do not coalesce to a single strand before insertion, but remain distinct till they join those of the axis.

Figs. 14–16. Transverse sections of the leaf of *O. palmatum*, showing the origin of the vascular supply to the lowest of its four spikes. × 4.

Fig. 17. Transverse section of the stalk of that spike.

Figs. 18–23. Successive sections higher up on the same leaf, showing the origin of the vascular supply into the second and third spikes. × 4.

Fig. 24. Transverse section of the larger appendage of *O. simplex*, at the level A. A. shown in Fig. 1. × 8.

Fig. 25. Transverse section at the level B. B. × 8.

Fig. 26. Transverse section just above the insertion on the axis. × 8.

Figs. 27–29. Successive transverse sections of the stock of *O. simplex* showing the insertion of the foliar strands upon the system of the axis. It will be seen that the foliar strands do not unite into one before insertion on those of the axis. × 8.

The Extra-floral Nectaries of Hevea brasiliensis, Müll.-Arg. (the Para Rubber Tree), an Example of Bud-Scales[1] serving as Nectaries.

BY

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With Plate XVI.

WHILE engaged in economic work on india-rubber in Ceylon during 1898–9, the Para Rubber Tree (*Hevea brasiliensis*)[2] was constantly under my observation, and peculiar nectaries occupying the position of bud-scales on its young shoots attracted my attention. Less conspicuous nectaries also occur on the foliage leaves proper. Though these latter are incidentally mentioned by systematists, the bud-scales generally, as well as their nectariferous nature, appear to have escaped their notice. This is not surprising, for the adult tree only puts forth fresh foliage annually, and the bud-scales being caducous, are merely evident while the shoots are in the immature condition; thus, unless the tree be examined during the short period of leaf-renewal, no bud-scales would be seen.

In the account of the genus *Hevea* in Martius' Flora of Brazil[3], the nectaries of the foliage leaves are mentioned, but no reference is made to the bud-scales. Delpino[4] in his elaborate work on extra-floral nectaries dismisses this genus in a few words, referring apparently only to the nectaries of the foliage leaves.

In a recent paper by Huber[5] on the periodicity in growth of *Hevea*

[1] The term 'bud-scale' is here used in the sense of a reduced leaf-structure situated on the shoot below the true foliage leaves. The author does not necessarily wish to imply that such structures in *Hevea* serve or have ever served as protective coverings to the bud.

[2] Introduced into Ceylon in 1876.

[3] Martius, Flora brasiliensis, vol. xi, pars. II, 1873. On p. 298 the genus (nine species including *H. brasiliensis*) is described as having 'petioli communes apice supra glanduligeri.'

[4] Delpino, Mem. Accad. Bologna, viii, 1887, p. 635. In giving examples of extra-floral nectaries in the Euphorbiaceae he refers to *Hevea* as follows: '8 specie di Hevea (petioli ima basi patellari-glanduligeri).'

[5] Huber, Bot. Centralb. lxxvi, 1898, pp. 259–64.

[Annals of Botany, Vol. XVIII. No. LXX. April, 1904.]

brasiliensis the bud-scales are just mentioned, but their nectariferous nature is not pointed out.

The description to follow is the result partly of observations made while resident at the Royal Botanic Gardens, Peradeniya, Ceylon, and partly of the examination of some spirit-preserved young shoots from adult trees brought back to England. The following account does not claim to be at all exhaustive. The object of this paper is chiefly to bring to notice a somewhat peculiar type of extra-floral nectary.

Morphology of the shoot. The adult trees at Peradeniya shed their leaves early in the year and remain bare for some days before the new foliage appears. On February 16, 1899, the new shoots had almost gained their full length, but their foliage leaves were still very immature. At this stage of growth the bud-scales are fully developed and their nectaries active. On the leaves attaining maturity these structures shrivel and drop off. According to Huber [1] the adult trees in their natural habitat, the Amazon valley, produce likewise only one crop of leaves in the year, but the time they are bare is about June ; hence the trees in Ceylon appear to have changed the time of the annual renewal of their foliage. This may be due to climate. The early months of the year constitute a moderately marked dry season in that part of Ceylon where Peradeniya is situated, and dryness is considered to have a direct bearing on leaf-fall. Yet on this idea the *Hevea* trees at Peradeniya ought not to burst into fresh leaf till about April, when the rains of the little monsoon commence: as it is they renew their foliage about the driest time of the year, while they cast off the old in January, a wetter and cooler month than either February or March.

Though mature trees produce only one set of leaves during the year, young trees—saplings—put forth several, showing a periodicity, which has been described by Huber [2]. Such saplings may produce fresh shoots about every month.

My attention was first called to the nectariferous bud-scales by noticing one day insects busy on the young shoots of some saplings growing in a plot. At a short distance away they looked as if they were devouring the immature foliage, leaving behind the stumps of the petioles, but on closer inspection I saw that they were a hairy kind of ant (?) imbibing the honey secreted by special foliar organs situated on the lower part of the shoot. Owing to the internodes between these structures having lengthened considerably, the general impression conveyed a little distance away was that of short petioles with the foliaceous part nibbled off. The true foliage leaves, however, were quite intact on the upper part of the shoot with their laminas as yet feebly developed. My first observations were made on these saplings, as I had to wait till the

[1] Huber, loc. cit.

[2] Huber, loc. cit.

proper time of the year to see if the young shoots of mature trees likewise possessed these nectariferous scales. Such was found to be the case.

The foliage leaves of *Hevea brasiliensis* are not evenly distributed along the whole length of the shoot, but are crowded together on the upper portion. The stretch of stem, which in the mature shoot appears to be a long internode below the foliage leaves, is really composed of several, the nodes of which were occupied in the young state by the nectariferous scales. That is to say, the internodes between the upper nectariferous scales have increased considerably in length—this is especially well seen in saplings. The leaf is trifoliate with a long petiole. The leaflets are large and lanceolate in shape, and are joined to the apex of the petiole by very short stalks (Plate XVI. Fig. 3). As a rule the length of the petiole and the size of the leaflets of a shoot decrease from the base upwards. For example, the petiole and leaflet of the lowest leaf may have a length of 30 cm. and 23 cm. respectively; whereas these measurements for the uppermost leaf may be only 3·5 cm. and 4·5 cm. respectively. The direction of the petioles is such that their laminas tend to be in one plane, and thus do not overshadow one another. The number of foliage leaves to a shoot varies, but is commonly twelve. They as well as the scales have a three-eighth arrangement on the stem-axis.

The nectary of the foliage leaf is situated on the upper surface just at the point of union of the leaflets with the petiole (Fig. 3 *n*). It may consist of either three contiguous saucer-shaped glands, one corresponding to each leaflet, or of only two of these, as in the figure, or it may assume the form of an irregular depression due to their fusion. In any case, they are not prominent structures, and do not differ as a rule in colour from the surrounding surface.

The bud-scales permit of division into two categories, viz. (1) the basal scales which are very small, non-nectariferous, and usually few in number; (2) the upper scales which are conspicuous, nectariferous, and numerous. Both kinds of scales as well as the foliage leaves possess each a pair of insignificant stipules (Figs. 1 and 2 *st*).

The basal non-nectariferous bud-scales. In the spirit material brought home for examination two types of young shoots could be distinguished, viz. those which had very few—one to three—non-nectariferous scales, and those possessing a great number, twenty or so. A drawing of each kind of shoot is shown in Figs. 1 and 2 respectively. A few shoots were intermediate in this respect, having several non-nectariferous scales, but not such an imbrication of them as represented in Fig. 2 *s*. No mention is made in my Ceylon notes of any large number of these basal scales having been noticed, but the shoots are referred to as possessing not

more than two or three each. Whether or not the possession of a large number of these scales be a common feature of young *Hevea* shoots cannot well be decided from the few examined, but the supposition is that such a shoot as the one shown in Fig. 2 is exceptional, and that as a rule only two or three non-nectariferous scales occur.

The nectariferous bud-scales. These vary considerably in number. The average for twenty-two shoots of adult trees examined was seven, ranging from five to twelve. The shoot from which Fig. 1 was drawn possessed eight, while that of Fig. 2 was exceptional in having twelve. Naturally, only part can be represented in the drawings. The lower nectariferous scales are small and short. The middle ones are usually the largest and possess the best developed nectaries, while the upper ones, though quite as long, are not so thick, and have the honey-secreting part reduced in extent; in fact in the uppermost one of all this part may be restricted to the apex, or perhaps even absent. The internodes increase in length as a rule from the base upwards; thus the lower nectariferous scales are near together, while the upper ones are some distance apart.

The inflorescences are borne in the axils of the nectariferous scales as well as in those of the lower foliage leaves (Figs. 1 and 2 *fl*).

Sapling. The young shoots of saplings resemble in most respects those of the adult trees, but being longer the internodes between the middle and upper nectariferous scales are more marked. From an examination of thirty-eight young sapling shoots the following numbers were obtained:—

non-nectariferous basal scales ranged in number from 0–3, aver. 1.
nectariferous scales ,, ,, 4–7, ,, 5.
foliage leaves—average 10.

Nine of these thirty-eight young shoots possessed each an arrested leaf between the nectariferous scales and the foliage leaves proper. This bore three leaflets well defined but quite small, while the nectariferous scales have mere points to indicate the remains of the leaflets. The nectary appeared to be absent[1]. This vestigial leaf did not persist, but withered and fell off with the scales.

Seedling. In germination the two cotyledons remain in the testa in the soil, so that what looks like a hypocotyl is really the epicotyl; it is quite long, 25 cm. or so in length. The first two foliage leaves formed quit the stem about the same level, and are similar in shape to those of older plants. Then comes an internode of about 3 cm., followed by two more foliage leaves situated at nearly the same level on the stem and alternating with the first pair; sometimes there may be only one

[1] Not microscopically examined—might possibly possess a trace of glandular tissue invisible to the naked eye.

leaf, or even three at this point. Occasionally the first pair of leaves may be vestigial, or only one of them fully developed.

If the plumule be fatally injured then the bud in the axil of one of the cotyledons develops into a shoot, bearing first three to four reduced leaves apparently without nectaries, before the true foliage leaves appear; sometimes the buds in both axils so sprout. The shoot arising from the axillary bud of the cotyledon simulates that derived from the plumule, but in the one case the length of stem produced before the foliage leaves are emitted is really composed of several internodes, the nodes being occupied by inconspicuous scale-leaves; while in the other it consists of one internode only, the epicotyl.

Unfortunately my notes do not connect the seedling with the sapling-stage, so as to see when the nectariferous scales first arise. This is probably at the second period of foliation. They apparently do not appear in the seedling, but rather later in the development of the plant.

Structure of the individual bud-scales. The structure of the non-nectariferous scales requires little description. A glance at Fig. 4 *s*, shows their size and shape. They are each accompanied by a pair of lateral bodies—stipules. In the mature or sprouting bud they are brown dead objects.

The nectariferous scales are fairly long, often bent structures and somewhat circular in transverse section; they project from the stem at right angles or with a downward inclination. Each bears at its apex three minute points, the sole remains of the leaflets. Their upper convex surface is covered with yellow honey-secreting tissue, and has often a median longitudinal groove. In the lower and middle scales the whole length of the upper surface is glandular. In the upper scales the glandular portion tends to recede from the proximal part, and in the uppermost one it is confined to the apex (Fig. 6 *ne*).

From a structural point of view the nectar-secreting tissue of plants can be divided into two classes[1], viz. (1) that consisting of small epidermal cells of the usual shape with thin hardly cuticularized outer walls, overlying a mass of closely packed cells full of contents, and secretory in function, and (2) that in which the epidermis itself assumes the form of a secretory epithelium with greatly thickened cuticle. In the first class the nectar reaches the surface by passing through the thin walls, while in the second class it escapes by bursting the cuticle.

The extra-floral nectaries of *Hevea brasiliensis* present a modification of the second type of structure, in that many of the original epithelial cells become divided in the mature nectary by tangential walls into two or three daughter-cells. That is, in the immature state the epidermis

[1] Bonnier, Les nectaires, Étude critique, anatomique et physiologique, Ann. d. Sci. Nat., 6e sér., T. viii, 1879, p. 96.

is a simple epithelium, but on approaching maturity it becomes in places two or three layered (Fig. 7). Conspicuous nuclei and much cytoplasm without prominent vacuoles are present in the epithelial cells, as well as in the small cortical cells below. The cuticularized part of the outer wall is quite thick, as is shown in the drawing (Fig. 7 *ct*).

Examples of extra-floral nectaries with an epithelium divided in places are to be met with in *Homalanthus populifera* and *Clerodendron Bungei*[1]; also a regularly two-layered epithelium exists in *Prunus avium*[2].

The diagram (Fig. 5) shows the position of the nectar-secreting epithelium in a transverse section of a typical median bud-scale; while that of Fig. 6 represents the epithelium as restricted to the apex in the uppermost scale.

The minute structure of the nectaries of the foliage leaves is similar to that of the scale ones.

General Remarks. This case of *Hevea brasiliensis* is about the first example cited of bud-scales—cataphyllary leaves—serving as nectaries. The only other instance I have found at all comparable is that mentioned by Reinke[3]. He points out that the bud-scales, as well as the foliage leaves of *Prunus avium*, have glandular teeth which are honey-secreting. But here the transformation is very partial. The scales are not so modified as to be merely nectaries. Their primary function is still that of bud-protection.

The Euphorbiaceae are rich in examples of plants with extra-floral nectaries. Baillon[4], in his work on this natural order, enumerates the various types, showing that their situation may be various, such as on the stem, petiole or lamina; and that different organs may be wholly transformed into them, such as stipules and leaflets. *Hevea brasiliensis* affords a still further type, viz. that of bud-scales serving as nectaries.

Two or three questions suggest themselves as to the origin of these cataphyllary nectaries of *Hevea*. Are they connected by descent with the petiolar glands, or are they a fresh production of glandular tissue in the evolution of the plant? What is the relationship between the non-nectariferous and nectariferous scales? Have they been derived independently at different periods from foliage leaves, or have the former arisen by further retrogression from the latter? From an identity in structure between the petiolar and scale nectaries and from the situation of the glandular tissue in the uppermost scale it looks as if the two classes of nectaries were directly connected. The petiolar glands have perhaps become much more developed in the scales, so that the function of these latter is now wholly that of secreting honey.

[1] Morini, Contributo all' anatomia ed alla fisiologia dei Nettarii Estranuziali, Mem. Accad. Bologna, 1886, vii.

[2] Reinke, Secretionsorgane, Prings. Jahrb., 1876, p. 125.

[3] Reinke, loc. cit. [4] Baillon, Étude générale des Euphorbiées, p. 230.

The view of the evolution of the shoot of *Hevea* that suggests itself to the author is as follows. Originally the base of the shoot had one, two, or three non-nectariferous bud-scales such as occur now; the rest of the foliar organs were true foliage leaves arranged equidistantly along the axis. Assuming that their laminas gradually increased in size towards the middle of the shoots and then decreased, the lowest and highest leaves would in consequence be the smallest and the middle ones the largest—a condition often occurring in shoots. That of the Beech (*Fagus sylvatica*) is a case in point. Providing that the *Hevea* shoot had an upward tendency, as it has at the present day, the large median leaves would tend to overshadow the lower smaller ones, and thus render these latter to a great degree functionless as assimilating organs, and through disuse a gradual reduction in their laminas might follow. The nectaries on the petiolar apices still remaining would be the first to secrete. Viewing their service as one of attracting ants to keep off leaf-destroying insects, it would be an advantage to the plant to retain the nectaries on these retrograde leaf-structures, and further to increase their size and consequently their secretion, in order to protect the expanding foliage leaves, till their nectaries became functional. Thus gradually a condition which now occurs would be brought about.

The Beech shoot has a few scales at its base without any lamina, which may be comparable, though not homologous as they are stipules, to the non-nectariferous scales of *Hevea*; then come the foliage leaves increasing in size as far as the middle of the axis, and then diminishing towards the apex. There is a tendency in some of its shoots for the small lower leaves to wither and fall early. This may be partly due to their being shaded by the higher leaves, though this overshadowing is largely guarded against by the shoot as a rule having a horizontal direction, and as a consequence the leaf-blades are in one and the same plane. If the shoot, on the other hand, were inclined considerably to the vertical as in *Hevea*, then the middle leaves would shade the lower ones much more effectually. Such a shoot as that of the *Rhododendron* demonstrates this. It is obliquely erect and has the foliage leaves crowded together on its upper part, thus resembling the shoot of *Hevea*. The length of stem below the rosette of foliage leaves is not a single internode, but composed of several, the nodes of which in the young state were occupied by small leaves which have shrivelled and disappeared. These, being perhaps originally smaller than the middle leaves and thus subject to shade, now no longer persist as functional foliage leaves; they have most likely decreased still further in size, and now apparently serve as protective scales to the bud. Consequently as a rule in horizontal shoots the lowest foliage leaves are the smallest, or at any rate smaller than the middle ones; while in shoots inclined to the vertical the lowest leaves are the largest, because

they represent probably the middle leaves of the primitive shoot, the lowest having ceased to act as foliage leaves.

The reason why in an ordinary shoot such as that of the Beech the middle leaves should generally be the largest is perhaps owing to the intensity of growth during development, first rising gradually to a maximum, then falling again till growth ceases. This would result in the first and last formed leaf-blades being the smallest.

The only other species of *Hevea* I have been able to examine is *H. spruceana*, Müll.-Arg., a very closely allied one. It possesses similar nectariferous scales.

Summary.

1. *Hevea brasiliensis* possesses two kinds of extra-floral nectaries:—

(*a*) Small inconspicuous glands situated on the upper surface of the foliage leaves, where the three leaflets join the petiole (Fig. 3 *n*).

(*b*) Large conspicuous glands borne on vestigial foliar structures—'bud-scales'—which are situated on the shoot below the foliage leaves proper (Figs. 1 and 2 *ns*).

2. The 'bud-scale' nectaries are a prominent feature of the young expanding shoot, and are functional till the foliage leaves are mature, when they wither and drop off. They are present in saplings, as well as in adult trees, but were not observed in seedlings.

3. Besides these nectariferous structures, one or more insignificant bud-scales without nectaries may be present at the base of the shoot (Figs. 1 and 2 *s*).

4. The minute structure of the foliar and 'bud-scale' nectaries is the same. Each consists of a well-defined secretory epithelium with a thick cuticle. The original cells of this epithelium may be divided here and there by one or two tangential walls to form in places a two- or three-layered epidermis (Fig. 7). The nectar escapes by the bursting of the cuticle.

5. The two kinds of extra-floral nectaries are considered as homologous; that is to say, the 'bud-scale' one may be regarded as a further development of what was at one time a petiolar nectary.

6. These nectariferous structures, occupying relatively the same position on the shoot as ordinary bud-scales, probably never had a protective function, but have been derived directly from what were once foliage leaves by the disappearance of the lamina and an increase in size of the nectary.

7. According to the usual view taken of the function of extra-floral nectaries, the 'bud-scale' glands may be looked upon as attracting ants to keep off insects injurious to the developing foliage. As soon as the foliage

leaves mature, their own nectaries become functional, and the scale ones being no longer required wither and drop off.

8. This case of *Hevea brasiliensis* is the first striking instance recorded, as far as the author is aware, of bud-scales—cataphyllary leaves—serving solely as nectaries.

Postscript.

Just on the completion of this paper a communication on the extra-floral nectaries of *Hevea*, read before the Academy of Sciences, Paris, on November 9, 1903, came to my notice. The article[1] resulting from it in the corresponding number of the 'Comptes Rendus' deals wholly with the structure of the petiolar nectaries, and makes no reference whatsoever to the nectariferous bud-scales; hence the chief subject-matter of my paper is not in the least affected. The authors state that the number of individual glands composing the petiolar nectary of *Hevea brasiliensis* may vary between two and five, but is usually three. They point out that the secretory epidermis of the nectary is two-layered in places, and lay stress on the two following structural features: (1) the presence of a ring of lignified parenchyma in the interior of the raised border which surrounds the secretory surface of each gland; (2) the laticiferous tubes, occurring in fair abundance in the specialized parenchyma of the gland, either end just below the secretory epidermis, or even pass between the epidermal cells to the exterior. J. P.

Cambridge, *December*, 1903.

[1] Daguillon et Coupin, Sur les nectaires extra-floraux des *Hevea*, Comp. Rend. cxxxvii, No. 19, 1903, pp. 767–9.

EXPLANATION OF FIGURES IN PLATE XVI.

Illustrating Mr. Parkin's paper on the Extra-floral Nectaries of *Hevea brasiliensis*.

Fig. 1. Young shoot from adult tree bearing the immature foliage leaves on its upper part. Natural size. *s*, single non-nectariferous basal bud-scale; ns_3, ns_5, ns_6, ns_8, represent respectively the 3rd, 5th, 6th, and 8th nectariferous bud-scales; this shoot possessed eight of these structures; the other four are not depicted in the drawing; the shaded areas show the position of the nectar-secreting parts of the scales; in ns_8, the uppermost scale, this part is restricted to the apex; *l*, lowest foliage leaf; *pn*, position of the petiolar nectary; *st*, stipules; *fl*, young inflorescences in the axils of the upper scales and lower foliage leaves; *ls*, leaf-scars of previous year's shoot.

Fig. 2. Young shoot from adult tree with many basal bud-scales. Natural size. *s*, imbrication of non-nectariferous basal scales; *ns*, length of stem bearing nine nectariferous bud-scales (the shoot possessed twelve altogether); *fl*, inflorescences in the axils of the nectariferous scales; *l*, lowest foliage leaf; *st*, stipules; *ls*, leaf-scars of previous year's shoot.

Fig. 3. Part of the upper surface of a foliage leaf. Natural size. *n*, twin-nectary; *p*, upper part of petiole, *l*, lower part of one of the three leaflets; *s*, short stalk of leaflet.

Fig. 4. Individual bud-scales from shoot represented in Fig. 1. Natural size. *s*, single non-nectariferous basal scale, *a*, dorsal view showing pair of stipules, *b*, side view; ns_1—ns_7 (inclusive), side views of seven nectariferous bud-scales in order of succession from base upwards, the position of the honey-secreting tissue is indicated by shading; ns_8, uppermost nectariferous bud-scale, *a*, side view, *b*, ventral view showing three points, the vestiges of the three leaflets—the nectar-secreting part is restricted to the apex and is shown by the small shaded area in this region.

Fig. 5. Diagram of a median transverse section of a nectariferous bud-scale. × 30. *e*, ordinary epidermis; *ne*, honey-secreting epithelium on the upper surface with groove, *g*; *fv*, ring of fibro-vascular bundles.

Fig. 6. Diagram of a longitudinal section of the apical part of the uppermost nectariferous bud-scale (ns_8 in Figs. 1 and 4) showing the restricted distribution of the glandular tissue. × 30. *e*, ordinary epidermis; *ne*, honey-secreting epithelium in a position corresponding to that occupied by the nectary of the foliage leaf; *p*, one of the three apical points—vestige of a leaflet; *h*, hairs; *v*, vascular strands.

Fig. 7. Section of the honey-secreting epithelium of a bud-scale. × 400. *e*, undivided epithelial cell; e_1, epithelial cell divided into two daughter-cells; e_2, epithelial cell divided into three daughter-cells; *ct*, the thick cuticle; *n*, nuclei; *c*, small cortical cells full of contents and without intercellular spaces. The empty areas in the figure represent cells in the section from which the contents had accidentally disappeared during preparation.

fl

n

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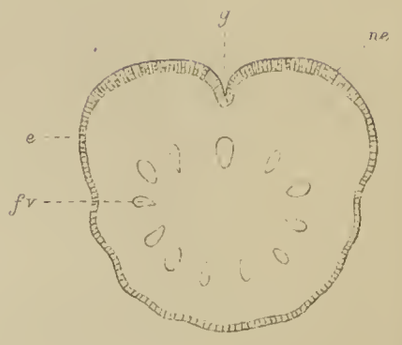

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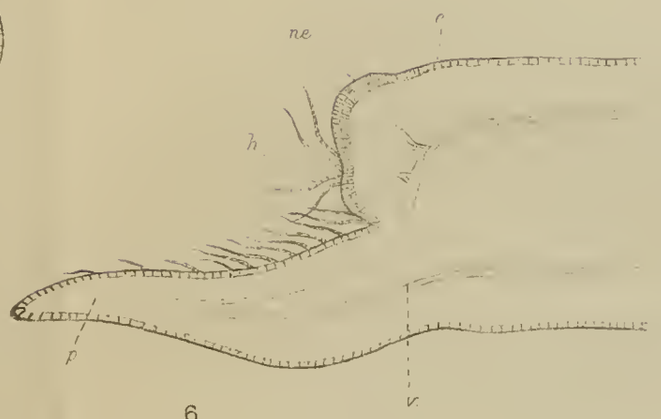

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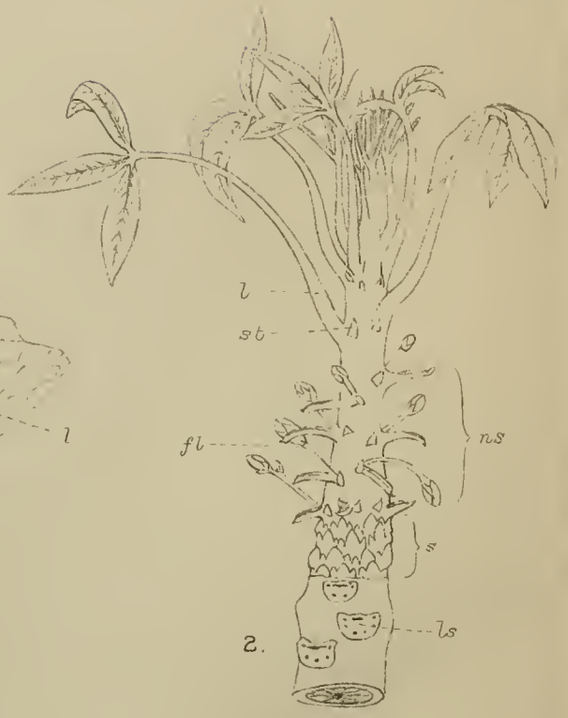

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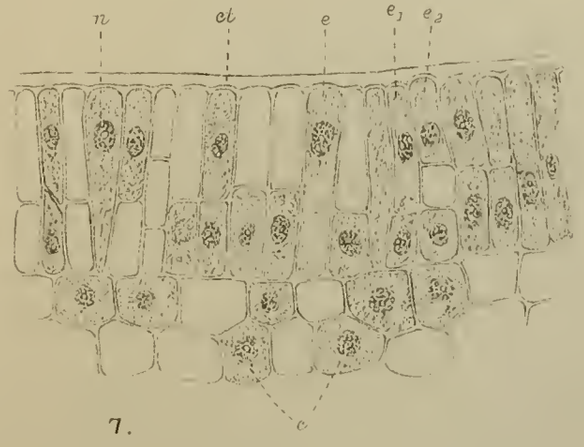

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University Press, Oxford

PARKIN.—HEVEA BRASILIENSIS.

The Principles of Phyllotaxis.

BY

ARTHUR H. CHURCH, M.A., D.Sc.,

Lecturer in Natural Science, Jesus College, Oxford.

With seven Figures in the Text.

IN a preliminary note published some time ago[1], exception was taken to the conventional methods adopted for the description and even interpretation of phyllotaxis phenomena, and a suggestion was made that appeared to be not only more in accord with modern conceptions of the phenomena of energy distribution, but it was further indicated that such a theory when carried to its mathematical limits threw a strong light both on the mechanism of shoot production and the inherent mathematical properties of the lateral appendage usually described as a 'leaf-member,' as opposed to any secondary and subsidiary biological adaptations.

As publication of the entire paper has been delayed, and the new standpoint has not received any special support from botanists to whom the mathematical setting proved possibly a deterrent, the object of the present note is to place the entire argument of the original paper in as concise a form as possible[2]. The preliminary discussion is sufficiently familiar[3].

The conventional account of phyllotaxis phenomena involves a system of 'fractional expressions' which become interpreted into *angular divergences*; and in practice the appearance of 'orthostichies' has been taken as a guide to the determination of the proper 'fractional expression.' This method, elaborated by Schimper (1830–5), has more or less held the field to the present time; and, for want of something better, has received the assent, though often unwilling, of such great investigators as Hofmeister and Sachs, to say nothing of lesser lights. Although elaborated into a system by Schimper and Braun, who added the peculiar mathematical properties of the Fibonacci series to the academical account

[1] Note on Phyllotaxis, Annals of Botany, xv, p. 481, 1901.

[2] On the Relation of Phyllotaxis to Mechanical Laws. Part I, Construction by Orthogonal Trajectories, 1901. Part II, Asymmetry and Symmetry, 1902.

[3] Descriptive Morphology-Phyllotaxis. New Phytologist, i, p. 49.

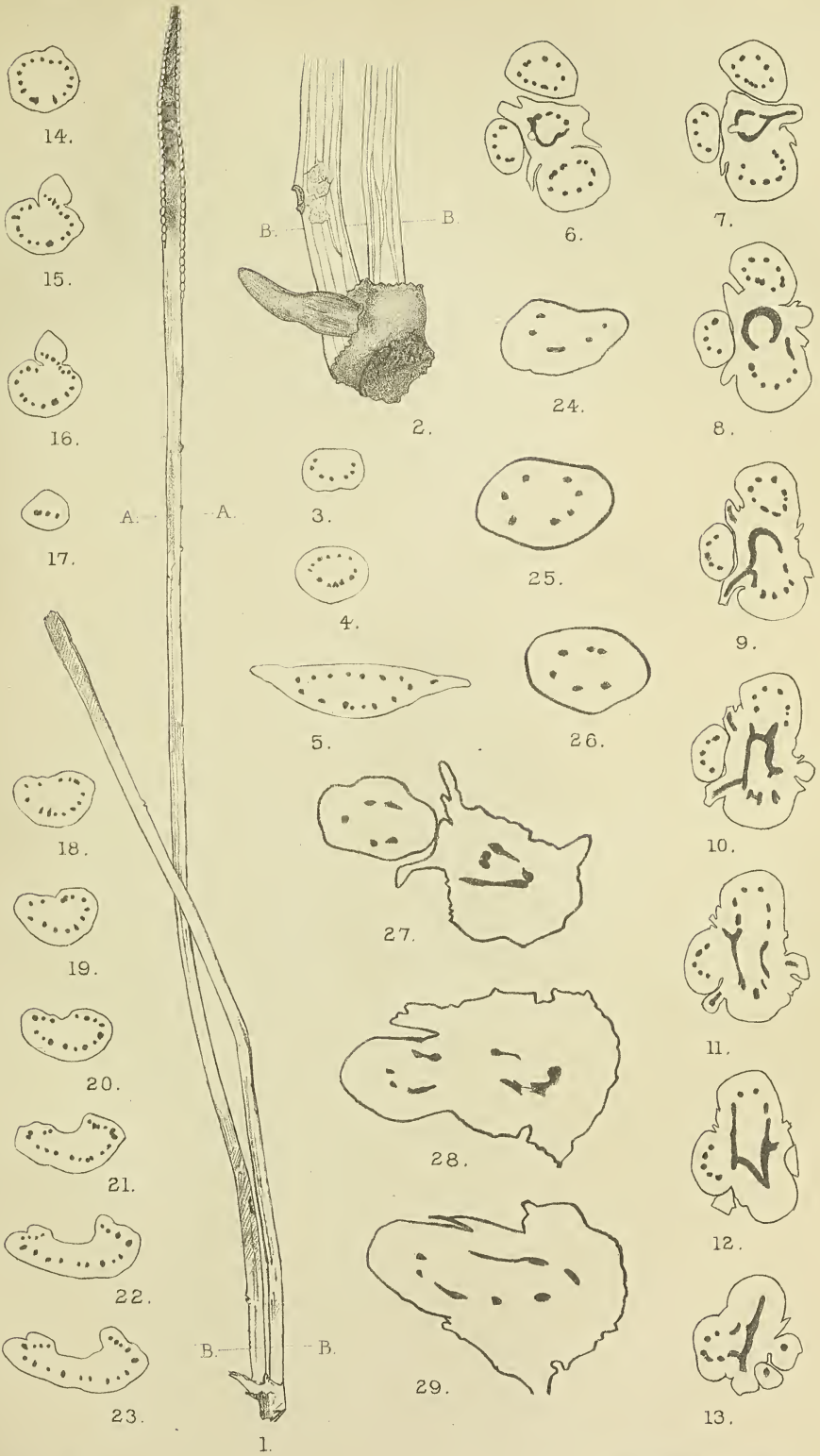
[Annals of Botany, Vol. XVIII. No. LXX. April, 1904.]

of the subject, the geometry of the system is based solely on a *mathematical conception* put forward by Bonnet and Calandrini in 1754; and this mathematical conception applied only to adult shoots and adult members of equal volume arranged in spiral sequence, and thus involved a system of *intersecting helices* of equal screw-thread, or, reduced to a plane expression, of spirals of Archimedes, also with equal screw-thread. A system of helical mathematics was thus interpolated into botanical science, and these helical systems were correctly tabulated by 'orthostichies' and 'divergence angles' obtained from simple fractional expressions themselves deduced from the observation of orthostichies.

But in transferring the study of phyllotaxis to the ontogenetic sequence of successively younger, and therefore *gradated*, primordia at the apex of a growing plant-shoot which was not cylindrical, these mathematical expressions were retained, although the helices originally postulated have absolutely vanished; and it is somewhat to the discredit of botanical science that this simple error should have remained so long undetected and unexpressed. As soon as one has to deal with spirals which have not an equal screw-thread, the postulated orthostichies vanish as straight lines; the fractional expressions therefore no longer present an accurate statement of the facts; and the divergence angles, calculated to minutes and seconds, are hopelessly out of the question altogether; while any contribution to the study of phyllotaxis phenomena which continues the use of such expressions must only serve to obscure rather than elucidate the interpretation of the phenomena observed. That the required orthostichies were really non-existent at the growing point, a feature well known to Bonnet himself, has thus formed the starting-point for new theories of *displacement* of hypothetically perfect helical systems, as, for example, in the contact-pressure theory of Schwendener. But once it is grasped that the practice of applying helical mathematics to spiral curves which, whatever they are, cannot be helices, is entirely beside the mark, it is clear that the sooner all these views and expressions are eliminated the better, and the subject requires to be approached without prejudice from an entirely new standpoint.

The first thing to settle therefore is what this new standpoint is to be; and how can such a remarkable series of phenomena be approached on any general physical or mathematical principles?

Now in a transverse section of a leaf-producing shoot, at the level of the growing point, the lateral appendages termed *leaves* are observed to arrange themselves in a gradated sequence as the expression of a *rhythmic production of similar protuberances*, which takes the form of a pattern in which the main construction lines appear as a grouping of intersecting curves winding to the centre of the field, which is occupied by the growing point of the shoot itself. As the mathematical properties



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BOWER.—OPHIOGLOSSUM SIMPLEX.

of such intersecting curve systems are not specially studied in an ordinary school curriculum, a preliminary sketch of some of their interesting features may be excused, since geometrical relationships have clearly no inherent connexion with the protoplasmic growth of the plant-shoot, but are merely properties of lines and numbers.

Thus, by taking first, for example, a system in which spiral curves of any nature radiate from a central point in such a manner that 5 are

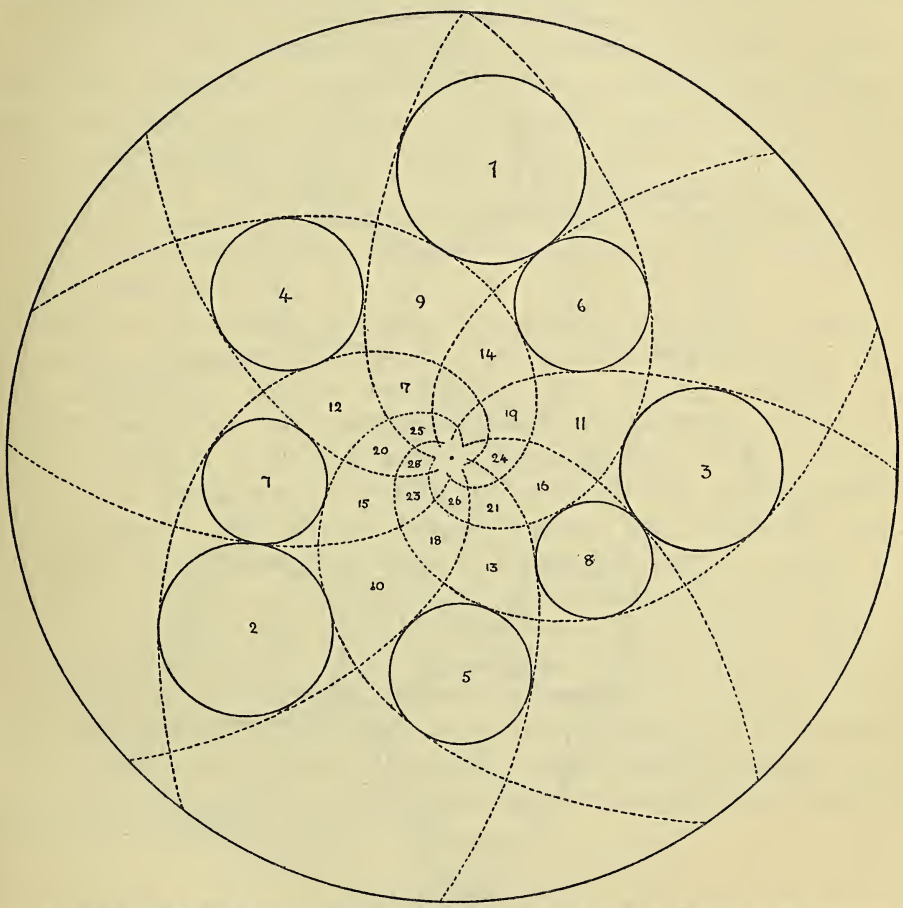

FIG. 35. Curve-system (5+8): Fibonacci series. A full contact-cycle of eight members is represented by circular primordia.

turning in one direction and 8 in the other, giving points of intersection in a uniform sequence, a system of *meshes* and *points of intersection* is obtained, and to either of these units a numerical value may be attached. That is to say, if any member along the '5' curves be called 1, the next inmost member along the same series will be 6, since the whole system is made of 5 rows, and this series will be numbered by differences of 5.

In the same way differences of 8 along the '8' curves will give a numerical value to these members; and by starting from 1, all the meshes, or points, if these are taken, may be numbered up as has been done in the figure (Fig. 35, (5+8)).

Observation of the figure now shows what is really a very remarkable property: all the numerals have been used, and 1, 2, 3, 4, &c., taken in order, give also a spiral sequence winding to the centre. This is merely

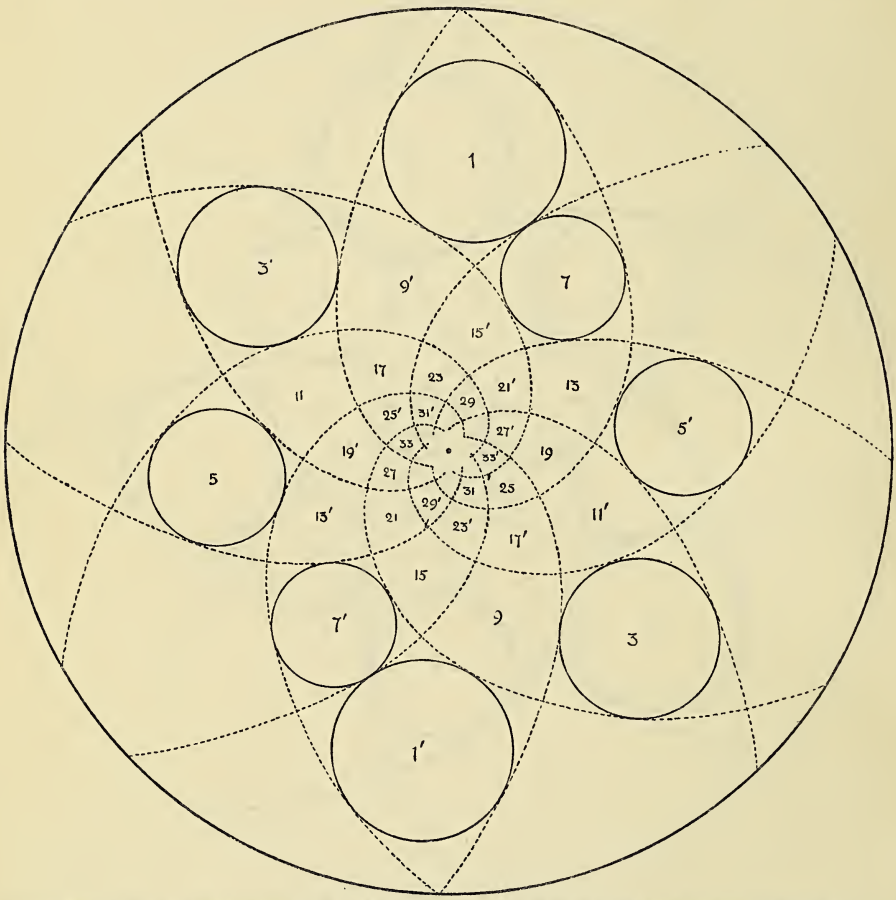

FIG. 36. Curve-system (6+8): Bijugate type. Contact-cycle as in previous figure.

a mathematical property of the system (5+8), in that these numbers are only divisible by unity as a common factor; but the single spiral thus obtained becomes in a botanical system the *genetic-spiral* which has been persistently regarded as the controlling factor in the whole system, since if such a construction be elongated sufficiently far, as on a plant-shoot, this spiral will alone be left visible.

The first point to be ascertained in phyllotaxis is the decision as to

which is to be the prime determining factor; that is to say, does the possession by the plant of a 'genetic-spiral' work out the subsidiary pattern of the parastichies, or are the parastichies the primary feature, and the genetic-spiral a secondary and unimportant consequence of the construction?

Now, other systems may quite as easily be drawn; thus take next a system of 6 curves crossing 8. On numbering these up by differences of 6 and 8 respectively in either series, it will be found that this time all the numerals are *not* employed, but that there are two sets of 1, 3, 5, &c., and 1′, 3′, 5′, &c., showing that pairs of members on exactly opposite sides of the system are of equal value. There is thus no single genetic spiral now present, but two equal and opposite systems—a fact which follows mathematically from the presence of a common factor (2) to the numbers 6 and 8. The existence of such factorial systems in plants has created much confusion, and the term *bijugate* applied to such a construction by the brothers Bravais may be legitimately retained as its designation (Fig. 36, system (6 + 8)).

Again, on constructing a system of 7 curves crossing 8, and numbering by respective differences, this time of 7 and 8; as in the first case, since these numbers have 1 only as common factor, all the numerals are utilized in numbering the system; the genetic-spiral may be traced even more readily than in the first example, the adjacent members along it being now in lateral contact, so that the resulting spiral obviously winds round the apex. This effect is common among Cacti, and is the result of a general property of these curve systems which may be summed up as follows:—Given a set of intersecting curves, the same points of intersection (with others) will also be plotted by another system of curves representing the diagonals of the first meshes, and the number of these curves, and also of course the difference in numerical value of the units along their path, will be given by the *sum* and *difference* of the numbers which determine the system, for example, 5 and 8 have as complementary system 3 and 13; and also other systems may be deduced by following the addition and subtraction series, e. g.:—

5 — 8
3 — 13
2 — 21
1 — 34.

Whereas the (7 + 8) system gives only 1 and 15; the single so-called 'genetic-spiral,' which includes all the points, being reached at the first process. Thus a Cactus built on these principles would show an obvious 'genetic-spiral' winding on the apex and 15 ridges, which in the adult state become vertical as a true helical construction is secondarily produced as the internodes attain a uniform bulk (Fig. 37 (7 + 8)).

Finally, take the case of 8 curves crossing 8, and number in the same way by differences of 8 along both series. It immediately becomes clear that there are 8 similar series: all other spirals have been eliminated; there is no 'genetic-spiral' at all, but only a system of alternating circles of members of absolutely identical value in each circle. We have now, that is to say, systems of true *whorls*, and also learn in what a true whorl consists—the members must be exactly and

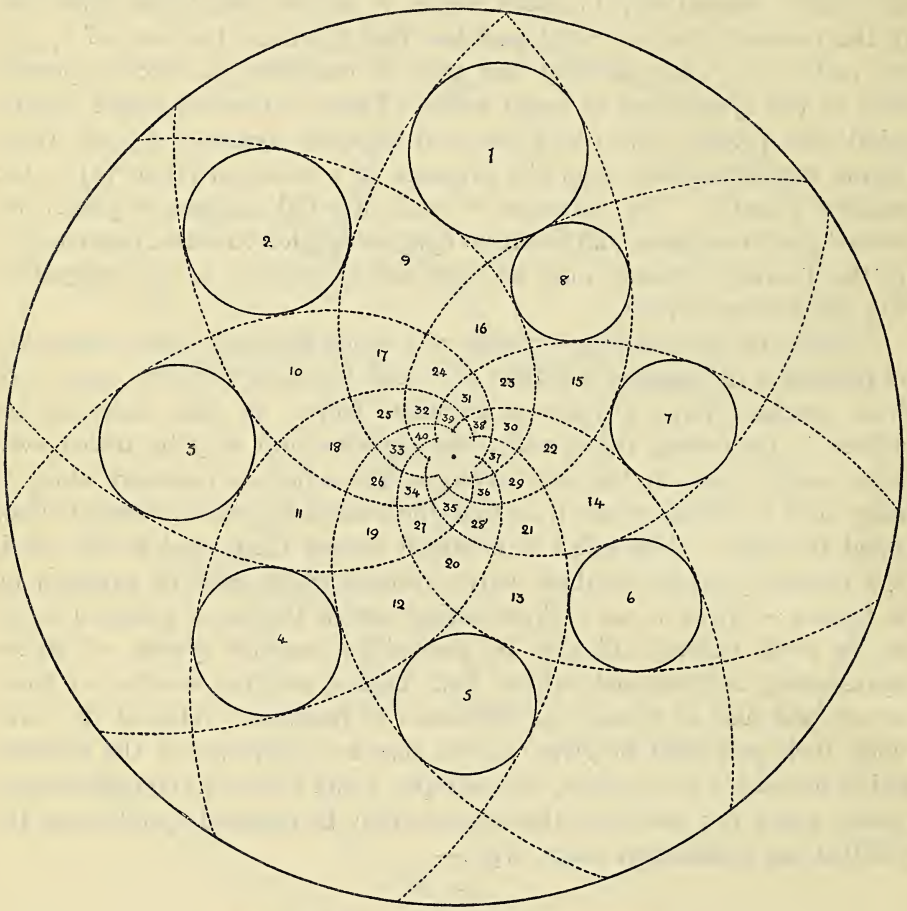

FIG. 37. Curve-system (7 + 8): anomalous type.

mathematically equal in origin—while the expression a *successive whorl* is a contradiction in terms.

From such simple and purely geometrical considerations it thus follows that the so-called 'genetic-spiral' is a property solely of intersecting curve-systems which only possess 1 as a common factor, and is therefore only existent in one case out of three possible mathematical forms (Figs. 35, 36, 38). While if these four systems were subjected to

a secondary *Zone of Elongation*, No. 1 would pull out as a complex of spirals in which four distinct sets might be traced; No. 2 as two spiral series leaving paired and opposite members at each 'node'; No. 3 as a spiral series with two complementary sets only; while No. 4 would give the familiar case of *alternating whorls* with 8 members at each 'node.' Further these cases are not merely arbitrary: they may all occur in the plant-kingdom, though the first is admittedly

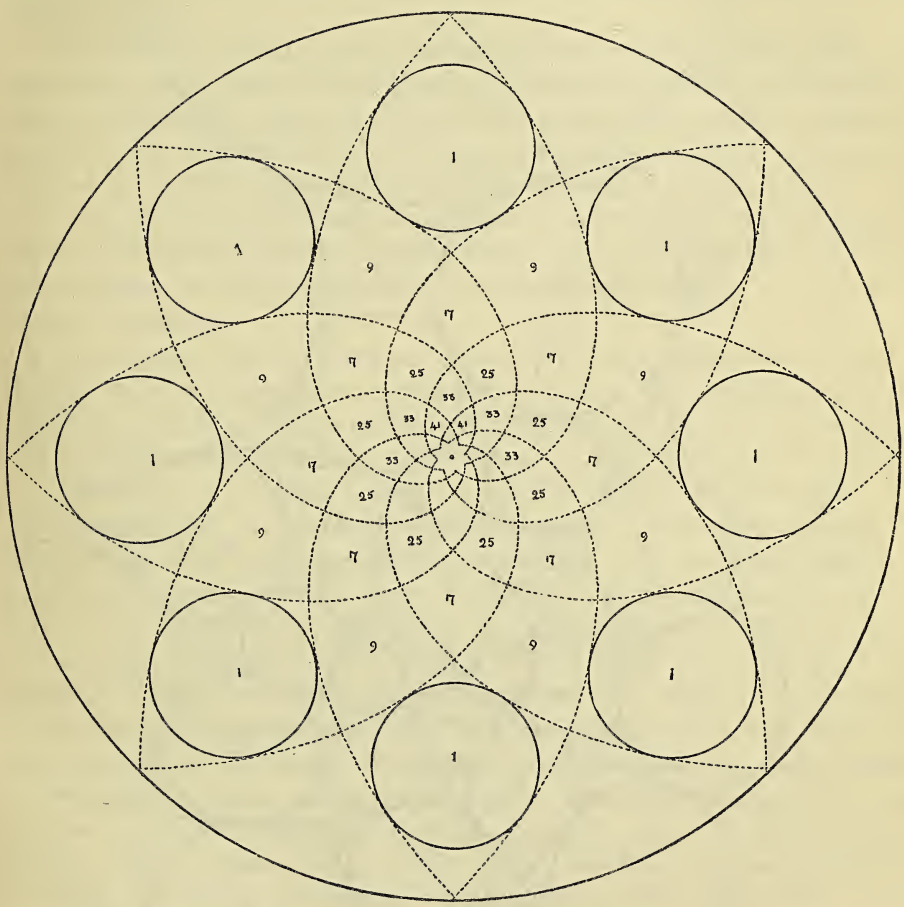

FIG. 38. Curve-system (8 × 8): symmetrical type.

the most frequent; but any theory which interprets one should equally well interpret the others. Similarly all changes of system may be discussed with equal readiness from the standpoint of the addition or loss of certain curves, *and only from such a standpoint*; since it is evident that once it is granted that new curves may be added to or lost from the system, the numerical relations of the members may be completely altered by

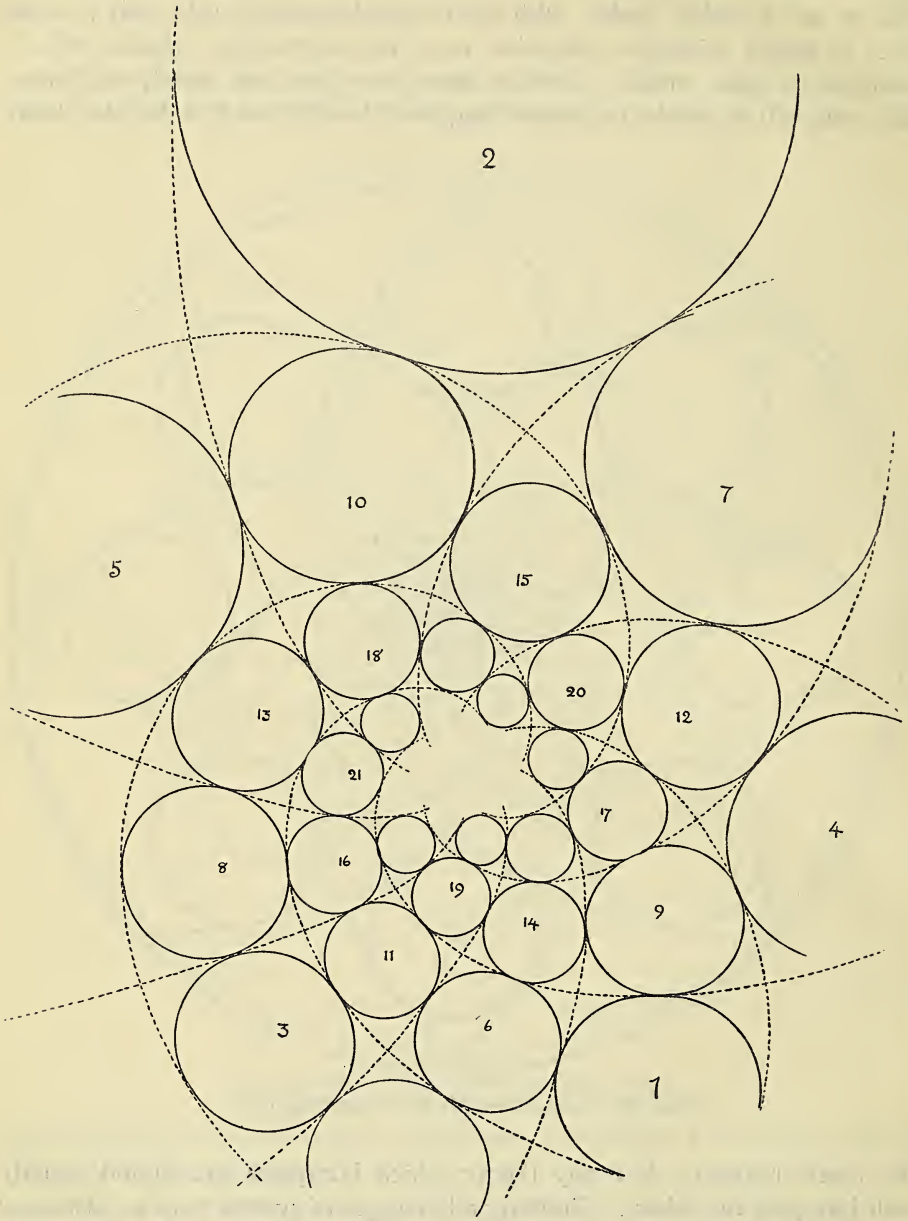

FIG. 39. System (5 + 8): eccentric construction in the plane of No. 2.

the addition of one curve only, as in the difference between the systems (7 + 8), (8 + 8), &c. (Figs. 35–38)[1].

Thus the hypothesis of a *genetic-spiral*, since it entirely fails to account for the arrangement of the members of all phyllotaxis systems in a single spiral, may be conveniently wholly eliminated from future discussions of these systems. It remains as a mere geometrical accident of certain intersecting curve-systems, and the fact that such systems may be very common in plant construction does not affect the main principle at all.

On the other hand, it may be urged that in these special cases one cannot get away from the fact that it does actually represent the building-path as seen in the *visible ontogeny* of the component members, and must therefore ever remain the most important feature of these systems as checked by actual observation apart from theoretical considerations. But even this view is not absolute; and such a case in which the ontogenetic sequence of development is not the single spiral obtained by numbering the members in theoretical series would naturally confuse the observer of direct ontogeny.

For example, in the previous cases figured the proposition of *centric* growth systems was alone considered, as being the simplest to begin with; it is obvious that even a small amount of structural *eccentricity* will produce a very different result. Thus in Fig. 39 the (5 + 8) system is redrawn in an eccentric condition, the so-called 'dorsiventrality' of the morphologist; on numbering the members in the same manner as before it is clear that the series obtained is very different from any empirical ontogenetic value which would be founded on the observation of the relative bulk of the members at any given moment. The occurrence of such systems in plant-shoots—and it may be stated that this figure was originally devised to illustrate certain phenomena of floral construction in the case of *Tropaeolum*—gives in fact the final proof, if such were any longer needed, of the simple geometrical generalization that such systems of intersecting curves are always readily interpreted in terms of the number of curves radiating in either direction, and not in any other manner. The presence of a circular zone (*whorl*) or a *genetic-spiral* is a wholly secondary geometrical consequence of the properties of the numerals concerned in constructing the system. The preference of any individual botanist, either in the past or at present, for any particular method

[1] Cf. Relation of Phyllotaxis to Mechanical Laws. Part II, p. 109, Rising and Falling Phyllotaxis. Part IV, Cactaceae.

Though the figures (35–38) have, as a matter of fact, been drawn by means of suitable orthogonally intersecting logarithmic spirals, because these curves are easily obtained and the schemes are subsequently held to be the representation of the true construction system of the plant-apex, the nature of the spirals does not affect the general laws of intersection so long as this takes place uniformly.

of interpreting any of these systems has little bearing on the case: the subject is purely a mathematical one; and the only view which can be acceptable is that which applies equally well to all cases, in that the question is solely one of the geometrical properties of lines and numbers, and must therefore be settled without reference to the occurrence of such constructions in the plant.

If all phyllotaxis systems are thus to be regarded solely as cases of intersecting curves, which are selected in varying numbers in the shoots of different plants, and often in different shoots of the same plant, with a tendency to a specific constancy which is one of the marvellous features of the plant-kingdom, it remains now to discuss the possibility of attaching a more direct significance to these curves, which in phyllotaxis construction follow the lines of what have been termed the *contact-parastichies*; that is to say, to consider

I. What is the mathematical nature of the spirals thus traced?

II. What is the nature of the intersection? and

III. Is it possible to find any analogous construction in the domain of purely physical science?

The suggestion of the logarithmic spiral theory is so obvious that it would occur naturally to any physicist: the spirals are primarily of the nature of *logarithmic spirals*; the intersections are *orthogonal*; and the construction is directly analogous to the representation of lines of equipotential in a simple plane case of electrical conduction. In opposition to this most fruitful suggestion, it must be pointed out however that the curves traced on a section are obviously never logarithmic spirals, and the intersections cannot be measured as orthogonal. But then it is again possible that in the very elaborate growth-phenomena of a plant-shoot secondary factors come into play which tend to obliterate the primary construction; in fact, in dealing with the great variety of secondary factors, which it only becomes possible to isolate when the primary construction is known, the marvel is rather that certain plants should yield such wonderfully approximately accurate systems. To begin with, logarithmic spiral constructions are *infinite*, the curves pass out to infinity, and would wind an infinite number of times before reaching the pole. Plant constructions on the other hand are *finite*, the shoot attains a certain size only, and the pole is relatively large. The fact that similar difficulties lie in the application of strict mathematical construction to a vortex in water, for example, which must always possess an axial tube of flow for a by no means perfect fluid, or to the distribution of potential around a wire of appreciable size, does not affect the essential value of the mathematical conception to physicists. And, though the growth of the plant is finite, and therefore necessarily subject to retarding influences of some kind, there is no reason why a region may not be postulated,

however small, at which such a mathematical distribution of 'growth-potential' may be considered as accurate; and such a region is here termed a '*Growth-Centre*.' Since the interpretation of all complex phenomena must be first attacked from the standpoint of simple postulates, it now remains to consider the construction and properties of as simple a centre of growth as possible.

Thus in the simplest terms the growth may be taken as *uniform*

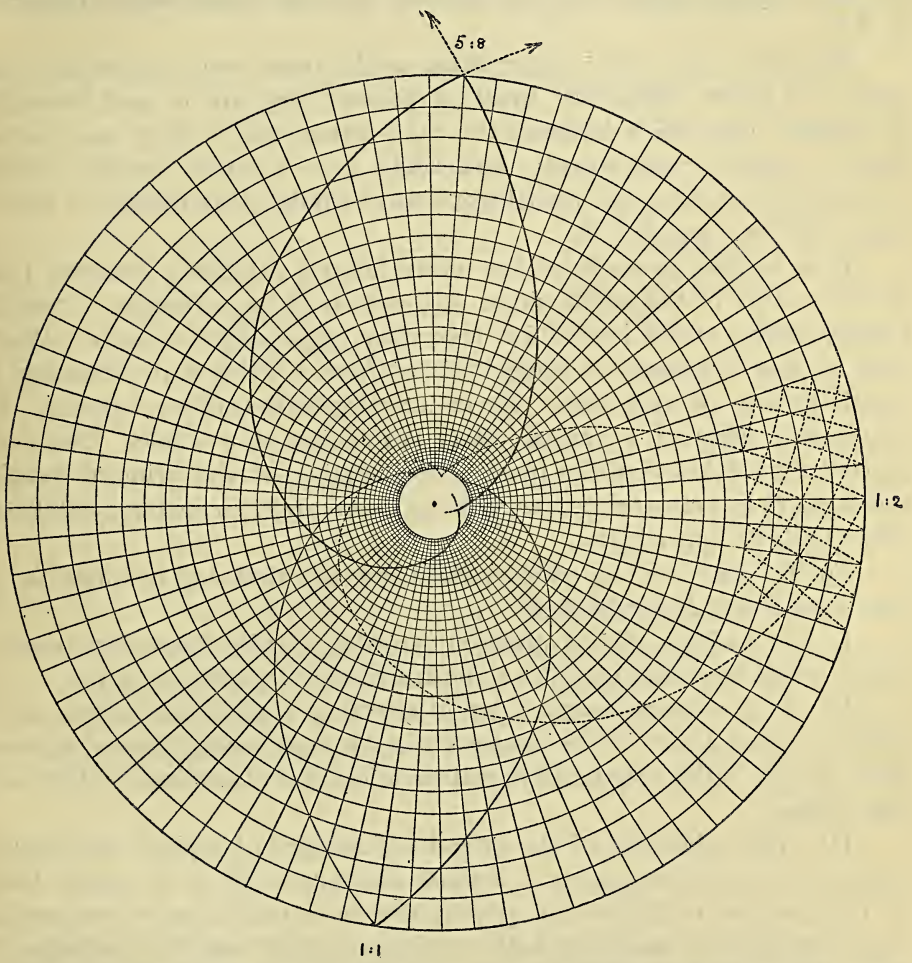

FIG. 40. Scheme for Uniform Growth Expansion: a circular meshwork of quasi-squares. Symmetrical construction from which asymmetrical homologues are obtained by the use of logarithmic spirals.

and *centric*: the fact that all plant growth is subject to a *retardation* effect or may be frequently *eccentric*, may at present be placed wholly on one side, since the simplest cases evidently underlie these. The case of uniform centric growth is that of a uniformly expanding sphere; or,

since it is more convenient to trace a solid in separate planes, it will be illustrated by a diagram in which a system of concentric circles encloses a series of similar figures, which represent a uniform growth increment in equal intervals of time. Such a circular figure, in which the expanding system is subdivided into an indefinite number of small squares representing equal time-units, is shown in Fig. 40, and presents the general theory of mathematical growth, in that in equal times the area represented by one 'square' grows to the size of the one immediately external to it[1].

Now it is clear that while these small areas would approach true squares if taken sufficiently small, at present they are in part bounded by circular lines which intersect the radii orthogonally; they may therefore be termed *quasi-squares*: and while a true square would contain a true inscribed circle, the homologous curve similarly inscribed in a quasi-square will be a *quasi-circle*.

It is to this quasi-circle that future interest attaches; because, just as the section of the whole shoot was conceived as containing a centric growth-centre, so the lateral, i. e. secondary, appendages of such a shoot may be also conceived as being initiated from a point and presenting a centric growth of their own. These lateral growth-centres, however, are component parts of a system which is growing as a whole. The conception thus holds that the plane representation of the primary centric shoot-centre is a *circular system enclosing quasi-circles* as the representatives of the initiated appendages.

To this may now be added certain mathematical and botanical facts which are definitely established.

I. Any such growth-construction involving *similar figures* (and quasi-circles would be similar) implies a construction by logarithmic spirals.

II. A growth-construction by intersecting logarithmic spirals, and only by curves drawn in the manner utilized in constructing these diagrams (Figs. 35–38), is the only possible mathematical case of *continued orthogonal intersection*[2].

III. The primordia of the lateral appendages of a plant only make contact with adjacent ones in a *definite manner*, which is so clearly that of the contacts exhibited by quasi-circles in a quasi-square meshwork, that Schwendener assumed both a circular form and the orthogonal arrangement as the basis of his Dachstuhl Theory: these two points being here just the factors for which a rigid proof is required, since given these the logarithmic spiral theory necessarily follows.

A construction in terms of quasi-circles would thus satisfy all theo-

[1] The same figure may also be used to illustrate a simple geometrical method of drawing any required pair of orthogonally intersecting logarithmic spirals.

[2] For the formal proof of this statement I am indebted to Mr. H. Hilton.

retical generalizations of the mathematical conception of uniform growth, and would be at the same time in closest agreement with the facts of observation; while no other mathematical scheme could be drawn which would include primordia arranged in such contact relations and at the same time give an orthogonal construction. If, that is to say, the *quasi-circle* can be established as the mathematical representative of the primordium of a lateral appendage, the orthogonal construction, which is the one point most desired to be proved, will necessarily follow.

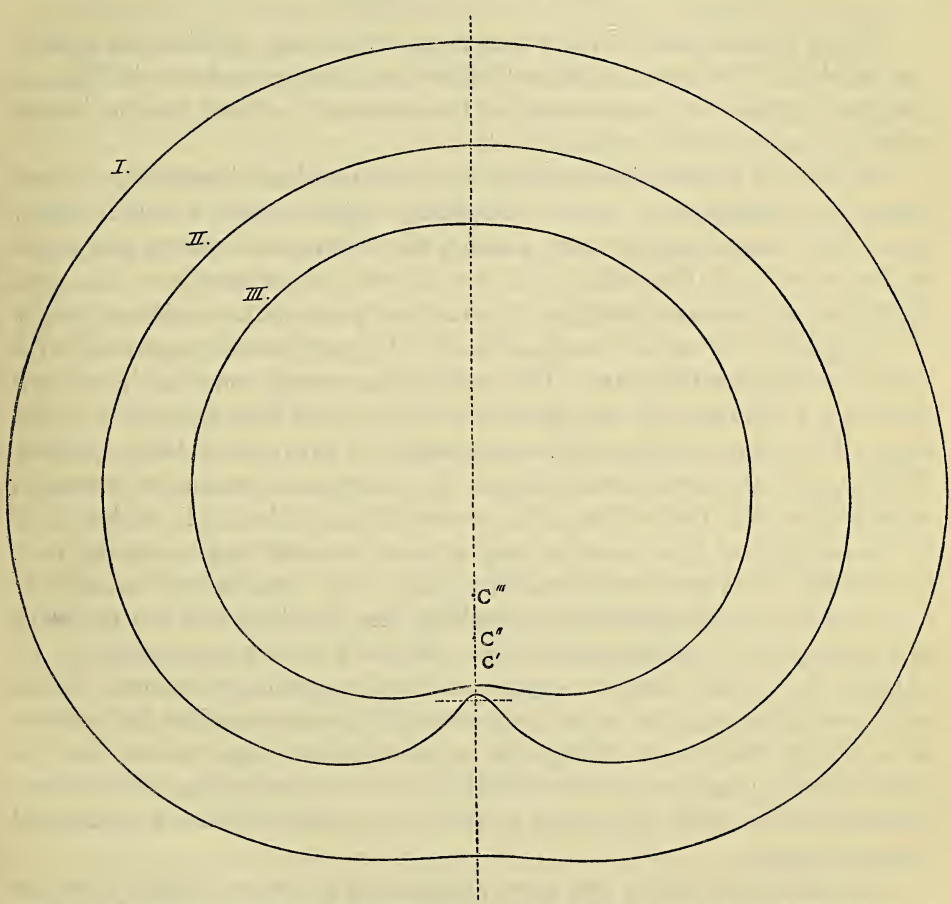

FIG. 41. Quasi-circles of the systems (2 + 2), (1 + 1) and (1 + 2) arranged for illustration in the plane of median symmetry. *C'*, *C''*, *C'''*, the centres of construction of the respective curves. (After E. H. Hayes.)

It remains therefore now to discuss the nature of the curves denoted by the term *quasi-circles*; their equations may be deduced mathematically, and the curves plotted on paper from the equations. These determinations have been made by Mr. E. H. Hayes. Thus a general equation for the quasi-circular curve inscribed in a mesh made by the orthogonal inter-

section of m spirals crossing n, in the manner required, is given in such a form as,

$$\log r = \log c \pm 1{\cdot}36438 \sqrt{\frac{1}{m^2+n^2} - {\cdot}000030864\,\theta^2},$$

where the logarithm is the tabular logarithm, and θ is measured in degrees; or where the logarithm is the natural logarithm and θ in circular measure:

$$\left(\log \frac{r}{c}\right)^2 + \theta^2 = \frac{\pi^2}{m^2+n^2}.$$

From these equations the curve required for any phyllotaxis system can be plotted out; and a series of three such curves is shown in Fig. 41, grouped together for convenience of illustration, i. e. those for the lowest systems (2 + 2), (1 + 2) and (1 + 1).

It will be noticed immediately that the peculiar characters of these curves are exaggerated as the containing spiral curves become fewer: thus with a larger number than 3 and 5, the difference between the shape of the curve and that of a circle would not be noticeable to the eye. While in the kidney-shaped (1 + 1) curve the quasi-circle would no longer be recognized as at all comparable in its geometrical properties with a true centric growth-centre. But even these curves, remarkable as they are, are *not* the shape of the primordia as they first become visible at the apex of a shoot constructing appendages in any one of these systems. The shape of the first formed leaves of a decussate system, for example, is never precisely that of the (2+2) curve (Fig. 41), but it is evidently of the same general type; and it may at once be said that curves as near as possible to those drawn from the plant may be obtained from these quasi-circles of uniform growth by taking into consideration the necessity of allowing for a growth-retardation. Growth in fact has ceased to be uniform even when the first sign of a lateral appendage becomes visible at a growing point; but, as already stated, this does not affect the correctness of the theory in taking this mathematical construction for the starting-point; and, as has been insisted upon, the conception of the actual existence of a state of uniform growth only applies to the hypothetical 'growth-centre.'

On the other hand, the mere *resemblance* of curves copied from the plant to others plotted geometrically according to a definite plan which is however modified to fit the facts of observation, will afford no strict proof of the validity of the hypothesis, although it may add to its general probability, since there is obviously no criterion possible as to the actual nature of the growth-retardation; that is to say, whether it may be taken as uniform, or whether, as may be argued from analogy, it may exhibit daily or even hourly variations. Something more than this is necessary before the correctness of the assumption of quasi-circular leaf-homologues can

be taken as established; and attention may now be drawn to another feature of the mathematical proposition.

It follows from the form of the equation ascribed to the quasi-circle that whatever value be given to m and n, the curve itself is *bilaterally symmetrical* about a radius of the whole system drawn through its centre of construction. That it should be so when $m=n$, i. e. in a symmetrical (*whorled*) leaf-arrangement, would excite no surprise; but that the primordium should be bilaterally symmetrical about a radius drawn through its centre of construction, even when the system is wholly asymmetrical and spiral, is little short of marvellous, since it implies that identity of leaf-structure in both spiral and whorled systems, which is not only their distinguishing feature, but one so usually taken for granted that it is not considered to present any difficulty whatever. Thus, in any system of spiral phyllotaxis, the orientation of the rhomboidal leaf-base is obviously *oblique*, and as the members come into lateral contact they necessarily become not only oblique but asymmetrical, since they must under mutual pressure take the form of the full space available to each primordium, the quasi-square area which appears in a spiral system as an oblique unequal-sided rhomb (Fig. 35). Now the base of a leaf (in a spiral system) is always such an oblique, *anisophyllous* structure, although the free appendage is *isophyllous*, bilaterally symmetrical, and flattened in a horizontal plane [1]. The quasi-circle hypothesis thus not only explains the inherent *bilaterality* of a lateral appendage, but also that peculiar additional attribute which was called by Sachs its '*dorsiventrality*,' or the possession of different upper and lower sides, and what is more remarkable, since it cannot be accounted for by any other mathematical construction, the *isophylly* of the leaves produced in a spiral phyllotaxis system [2].

It has been the custom so frequently to assume that a leaf-primordium takes on these fundamental characters as a consequence of biological adaptation to the action of such external agencies as light and gravity, that it is even now not immaterial to point out that *adaptation* is not *creation*, and that these fundamental features of leaf-structure must be present in the original primordium, however much or little the action of environment may

[1] These relations are beautifully exhibited in the massive insertions of the huge succulent leaves of large forms of *Agave*: the modelling of the oblique leaf-bases with tendency to rhomboid section, as opposed to that of the horizontal symmetrical portion of the upper free region of the appendage, may be followed by the hand, yet only differs in bulk from the case of the leaves of *Sempervivum* or the still smaller case of the bud of *Pinus*.

[2] *Anisophylly* is equally a mathematical necessity of all *eccentric* shoot systems.

It will also be noted that the *adjustment* required in the growing bud, as the free portions of such spirally placed primordia tend to orientate their bilaterally symmetrical lamina in a radial and not spiral plane, gives the clue to those peculiar movements in the case of spiral growth systems, which, in that they could be with difficulty accounted for, although as facts of observation perfectly obvious, has resulted in the partial acceptance of Schwendener's Dachstuhl Theory. This theory was in fact mainly based on the necessity for explaining this 'slipping' of the members, but in the logarithmic spiral theory it follows as a mathematical property of the construction.

result in their becoming obvious to the eye. The fact that the quasi-circle hypothesis satisfies all the demands of centric growth systems, whether symmetrical or asymmetrical, as exhibited in the fundamental character of foliar appendages, and that these characters may be deduced as the mathematical consequences of the simple and straightforward hypothesis of placing centres of lateral growth in a centric system which is also growing, may be taken as a satisfactory proof of the correctness of the original standpoint. And it is difficult to see what further proof of the relation between a leaf-primordium as it is first initiated, and the geometrical properties of a quasi-circle growth system is required; but it still remains to connect this conception with that of orthogonal construction.

This however naturally follows when it is borne in mind, firstly that no other asymmetrical mathematical growth-construction is possible, except the special quasi-square system which will include such quasi-circles; and secondly, that the contact-relations of the quasi-circles in these figures are identical with those presented by the primordia in the plant, and could only be so in orthogonal constructions. It thus follows that with the proving of the quasi-circle hypothesis, the proof is further obtained that the intersection of the spiral paths must be mutually orthogonal; and it becomes finally established that in the construction of a centric phyllotaxis system, along logarithmic spiral lines, the segmentation of the growth system at the hypothetical growth-centre does follow the course of paths intersecting at right angles; and the principle of construction by orthogonal trajectories, originally suggested by Sachs for the lines of cell-structure and details of thickened walls, but never more fully proved, is now definitely established for another special case of plant-segmentation, which involves the production of lateral appendages without any reference to the segmentation of the body into 'cell' units.

But even this is not all; the point still remains,—What does such construction imply in physical terms? Nor can it be maintained that the present position of physical science affords any special clue to the still deeper meaning of the phenomena. The fact that the symmetrical construction in terms of logarithmic spirals agrees with the diagram for distribution of lines of equipotential and paths of current flow in a special case of electric conduction, while the asymmetrical systems are similarly homologous with lines of equal pressure and paths of flow in a vortex in a perfect fluid, the former a static proposition, the latter a kinetic one, may be only an 'accident.' On the other hand it must always strike an unprejudiced observer that there may be underlying all these cases the working of some still more fundamental law which finds expression in a similar mathematical form.

In conclusion, it may be noted that if the proof here given of the principle of plant construction by orthogonal trajectories is considered satis-

factory, it adds considerably to the completeness of the principles of protoplasmic segmentation, and may be extended in several directions with further interesting results. It is only necessary to point out that the case of centric-growth is after all only a first step; and the most elaborate growth forms of the plant-kingdom, as exhibited for instance in the segmentation of the leaf-lamina, may be approached along similar lines, and by means of geometrical constructions which are consequent on the more or less perfect substitution of *eccentric* and ultimately wholly *unilateral* growth-extension, which again must ever be of a retarded type. The subject thus rapidly gains in complexity; but that the study of growth-form, which after all is the basis of all morphology, must be primarily founded on such simple conceptions as that of the 'growth-centre' which has here been put forward, should I think receive general assent, and in the case of the quasi-circle, there can be little doubt as to the extreme beauty of the results of the mathematical consideration.

The Development of the Spermatozoid in Chara.

BY

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With Plate XVII.

WHILE the discovery of the fact that the cilia-bearing part of the spermatozoid in *Chara* and in certain Archegoniates originates from a cytoplasmic structure resembling a centrosome has aroused a keen interest in the study of spermatogenesis among plants, yet the diversity of opinion and the controversies that have arisen concerning the probable relation existing between the centrosome and the cilia-bearer, or blepharoplast, have become an equal stimulus to research. Because of the fact that in certain Gymnosperms and Pteridophytes the cilia-bearing structure is derived from a centrosome-like body, the investigator naturally expects to find a similar origin for this structure in the spermatogenesis of other plants possessing male gametes that may be called spermatozoids. In this respect the expectations of the writer were not realized in a study of the development of the spermatozoid of *Chara fragilis*, and as certain important details observed differed from the accounts given by Belajeff ('94) and others, the publication of the following seems not superfluous.

The mature spermatozoid of *Chara*, as is well known, consists of a thread-like body, making two or more spiral turns, and bearing two long cilia inserted a short distance behind the anterior end. This body is composed of a nucleus occupying the middle portion and a cytoplasmic band or thread, the blepharoplast, which bears the two cilia. The blepharoplast, therefore, extends some distance posteriorly as well as anteriorly beyond the nuclear portion.

Up to the present time Belajeff ('94) has given the most complete account of the development of the spermatozoid in the Characeae, using chiefly *Chara foetida* as well as species of *Nitella*. As the earlier part of the development described by Belajeff differs somewhat in detail from the results of my own observations, a brief outline of the process as described by this author will be given first for the sake of greater clearness.

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The first indication of the development of the spermatozoid is the movement of the nucleus from near the centre to one side of the cell and a slight contraction of the entire protoplast. The side towards which the nucleus moves is designated by Belajeff as the 'dorsal,' and that directly opposite as the 'ventral' side of the cell. By the side or lateral wall is meant that which is parallel with the longitudinal axis of the spermogenous filament. The contraction of the protoplasm is most pronounced at the sides, while it is withdrawn very little or not at all from the transverse walls. About this plasma-cylinder there now appears a ring-shaped groove (loc. cit., p. 33) which, it is stated, is to play an important rôle in the further development of the spermatozoid, since in this groove the cilia are to be developed. There now appears a small plasmic protuberance at the boundary between the nucleus and the cytoplasm, i. e. at the dorsal side of the cell, or that which will become the convex side of the developing sperm, from which the cilia arise. 'Den Beginn der Entwickelung des Spermatozoidenkörpers kennzeichnet das Auftreten eines kleinen Plasmahöckers an der Grenze zwischen Kern und Cytoplasma. Dieser Höcker begiebt sich in die ringförmige Rinne und ist der Seitenwand der Zelle zugewandt. Am deutlichsten ist das Hervortreten dieses Höckers von einer der flachen Seiten der spermatogenen Zelle aus zu verfolgen (loc. cit., Fig. 15 *a*). Dieser Höcker entspricht augenscheinlich den "glänzenden Pünktchen" des Mettenius und den von Goebel beschriebenen "Köpfen." Aus dem Höcker wachsen zwei kurze, elastische Fäden hervor, die beide parallel der Seitenwand, aber in entgegengesetzter Richtung verlaufen.'

With the further development of the spermatozoid the protuberance or nodule (Höcker) undergoes a change in position, whereby it gradually moves to the side of the cell opposite the nucleus. From the protuberance to the nucleus extends a delicate thread staining intensely red with fuchsin. With the further growth of this thread the protuberance is pushed farther away from the nucleus (loc. cit., Fig. 16). This thread lies in the cytoplasm, from which only the protuberance, bearing the cilia, protrudes into the groove. The thread with its cilia-bearing protuberance forms the anterior end of the spermatozoid. Simultaneously with the development of the anterior end, that of the posterior extremity is to be observed. At the side of the nucleus opposite the point of origin of the plasmic protuberance, there appears in the cytoplasm a homogeneous thread or band, which grows parallel with the side-wall of the cell and in the opposite direction to that of the anterior end. This thread is considerably thicker than that which bears the cilia. Its free or posterior extremity protrudes out of the cytoplasm into the groove, and forms a beak-like outgrowth (loc. cit., Figs. 16, 17, and 19). The cilia-bearing filament continues its growth within the cytoplasm and until it has reached a middle point on the

ventral side of the cell. The cilia are fastened to its anterior end. From this time onwards the point of insertion of the cilia is unchanged, so that the further growth of the thread leads to the formation of that part of the sperm anterior to the point of insertion of the cilia. The transformation taking place in the nucleus during the development, as described by Belajeff for *Chara foetida*, differs in no important detail from my own observations on *Chara fragilis*, a detailed account of which is to follow.

From the foregoing it will be seen that, according to Belajeff, the cilia arise from a cytoplasmic protuberance or knob (Höcker), lying near the nucleus at the side which is to become the convex side of the mature spermatozoid. The formation of a cytoplasmic thread now carries the cilia-bearing protuberance some distance from the nucleus, and constitutes the anterior cytoplasmic part of the sperm. At the same time a similar, though somewhat broader, thread or band develops from the opposite side of the nucleus. This constitutes the posterior end of the spermatozoid. Judging from Belajeff's figures these cytoplasmic extremities of the sperm seem to be direct transformations of the plasma-membrane (Hautschicht), although he does not make a direct statement to that effect. He leaves the impression also that the two ends of the sperm are separate pieces, being fastened to the respective ends of the nuclear portion.

Belajeff's study was made on material stained and observed *in toto* after mounting in glycerine—a method that cannot be regarded at present as altogether reliable, yet more improved methods show that many of the finer details were brought out with remarkable accuracy. In my own study *Chara fragilis* was used exclusively. The material was fixed in chrom-osmic-acetic acid, embedded in paraffin, sectioned and stained on the slide with aniline-safranin, gentian-violet and orange G. For the most part the sections were cut from three to five microns in thickness, but in order to bring whole cells into observation thicker sections were prepared.

Of all the plants below the Ferns, the Characeae are probably the most suitable objects for the study of spermatogenesis. The mature spermatozoids are relatively large, and the protoplasmic structures of their mother-cells can be fairly well differentiated by present cytological methods. Yet even here, as in the most favourable cases, there are phases in the process which present extreme difficulties, and consequently there are points concerning which there is always more or less doubt. This I have found true in some of the earlier stages in which cytoplasmic differentiation begins.

As is well known, the spermogenous cells of *Chara* are arranged in long filaments coiled up in the globular antheridium. The cells are in the form of flat, cylindrical disks, two or three times as broad as long

(Figs. 2, 3). Observed from the end they are, therefore, circular in outline, and in longitudinal section somewhat rectangular. Before differentiation begins, the nucleus is spherical or elliptical, and lies near the middle of the cell. The diameter of the nucleus is almost as great as the length of the cell, so that there appears to be room enough only between nuclear membrane and either transverse wall for the plasma-membrane. The chromatin is arranged in the form of a somewhat regular but interrupted spirem, i. e. there are thin places in the nuclear thread in which little or no chromatin is present (Pl. XVII, Figs. 1–3). There is no definitely recognizable differentiation in the cytoplasm at first. This appears to be a uniformly granular network or alveolar structure. The first indication of the development of the spermatozoid, as has been correctly observed by Belajeff, is the withdrawal of the nucleus towards one side of the cell, and a slight contraction of the entire protoplast. That part of the lateral wall towards which the nucleus moves will be next to the convex side of the developing sperm. This is spoken of as the dorsal side by Belajeff. The first indication of a cytoplasmic differentiation observed by me appears in the form of a very delicate thread or band extending partly around the cell and embracing the nucleus in its arc (Fig. 1). This thread seems to be merely a differentiation of the plasma-membrane. The end of this thread, which will become the anterior end of the sperm, is thinner than the opposite or posterior end. In Fig. 1 the anterior end is below the nucleus, and the posterior above. The row of sharply defined granules included in the concave side of the posterior part of the spermatozoid, and which can be so readily observed in later stages, has just begun to appear. This line of granules marks the line of separation between the posterior end of the sperm and the general cytoplasmic body of the cell. I do not agree with Belajeff that these ends are separate, but that they are the ends of a continuous thread, the blepharoplast, which in the majority of cases is obscured where it lies next to the nucleus. That this interpretation is correct is seen in later stages, where the cell has been shrunken by the reagents (Fig. 5). I was not able to observe any cytoplasmic knob or protuberance (Höcker) from which the cilia arise as described by Belajeff, nor does the band or thread extend around the middle of the cell in a groove. In this respect my observations agree with those of Strasburger ('92), who did not observe any groove. About the same developmental stage shown in Fig. 1, or perhaps a little later, is represented in Fig. 2, as seen from the side or in longitudinal section. The band is seen to lie along one end of the cell. In this figure it will be further noticed that, on the side near the band, the cytoplasm has become denser, while on the opposite side there are a very few granules in the very delicate cytoplasmic network. This portion of the cell, which is comparatively free from granules, might easily be taken

for a vacuole. Fig. 2 seems to be the same stage as represented by Belajeff's Fig. 12. Fig. 3 is a median longitudinal section of a cell in the same stage as Fig. 1, but passing through the region between the ends of the thread, or on a line in the plane of *ab*, Fig. 1. The cytoplasm on the side opposite the nucleus, or that which will become the concave side of the mature sperm, is somewhat concave at this time, becoming much more so in a later stage. I observed nothing to correspond with Belajeff's Fig. 15 *a*. In the stage of Fig. 1 no trace of cilia was seen. At this stage the chromatin is disposed in a hollow spirem with numerous interruptions, i. e. places free from chromatin but continuous as to linin. The chromatin is in the form of larger or smaller lumps or pieces. A little later in the development, the blepharoplast is more distinctly differentiated (Figs. 4, 5). It has become longer, and extends almost entirely around the cell. The anterior end is thinner and narrower than the posterior. In Fig. 5 it seems that the posterior end broadens rather abruptly. In this instance it seems that the thread, by shrinkage, has become separated from the nucleus at its point of contact. As a rule, the condition of things is as shown in Figs. 4 and 6, in which case the thread lies in such close contact with the nuclear membrane that it is not definitely distinguished there. This gives the impression that the thread consists of two separate pieces growing in opposite directions from a point or points on the nucleus as described by Belajeff. The two ends of the blepharoplast rarely lie in the same plane at this stage. In the stage of Fig. 6, the cilia are present and of considerable length. In all cases in which their point of origin could be made out with certainty, that was always some distance from the anterior end. In no case were they found attached at the extremity, as figured by Belajeff in his Fig. 11.

At this stage the entire protoplast has become more contracted. The diameter of the nucleus is also shorter, but the difference between that in Fig. 1 and in Fig 4 is due in some measure to a difference in the size of the cells. The surface of the cytoplasm at the point between the ends of the blepharoplast now begins to become concave (Figs. 6, 8), although this substance assumes, at a later stage, the form of a rounded vesicle (Figs. 9–13). A marked change is also manifested by the nucleus. It loses its spherical or elliptical shape, becoming denser and flattened on one side (Fig. 8), and assuming the form of a half moon or crescent. In structure it is dense and uniform. Both posterior and anterior ends of the blepharoplast have become longer and thicker, projecting much beyond the cytoplasmic mass of the cell. At a later stage (Figs. 11 and 12) the nucleus increases in length, becoming sausage-shaped, and makes about one complete turn in a spiral. In Fig. 13, the body of the sperm makes about three turns, the anterior end describing sharper curves than the posterior end. The cytoplasm of the cell now consists of a finely granular mass

embraced largely by the nuclear portion of the spermatozoid (Figs. 9, 11, 13). The blepharoplast is of a uniformly homogeneous structure, staining well with gentian violet of the triple stain. Along the concave side of the posterior end is the conspicuous row of granules mentioned in a preceding paragraph. These granules stain densely, appearing almost black in preparations otherwise well stained to bring out the remaining parts distinctly. Fig. 10 *a* and *b* represent two spermogenous cells as seen from the side (in lateral view) at the stage of development shown in Fig. 9: *a* represents the sperm as seen from the surface, while *b* is an optical section. The dots on the right and left of the body of the sperm represent cross-sections of the cilia. In *b*, it is seen that the nucleus is cylindrical, being circular in section. The cytoplasmic mass is strongly concave on the side opposite the nucleus. In this concavity are shown two crescents which are sections of the ends of the blepharoplast. The larger crescent, above in the figure, is a section of the posterior end, and the lower that of the anterior end. From optical sections it is clearly seen that the blepharoplast is a band with the outer surface convex and the inner surface somewhat concave. The section of the blepharoplast at the left of the nucleus shows that the cilia-bearing band is closely applied to the nuclear membrane at the place of contact. The cilia are relatively very long, and lie rather loosely coiled in the spermogenous cell. Sometimes they seem to lie in contact with each other for certain distances (Fig. 14). In the final stages of the development of the spermatozoid the general cytoplasmic portion seems to be reduced to a mere vestige.

Unfortunately material was not available at the time of my study to enable me to fix the living spermatozoids upon the slide, and to stain them in that condition. This I hope to be able to accomplish sometime in the near future.

In discussing the probable relation of the blepharoplast in *Chara* and in some of the lower Archegoniates, the observations recently published by Ikeno ('03) upon spermatogenesis in *Marchantia polymorpha* are of special interest. His excellent paper is the most thorough and complete account thus far given for any Liverwort. Only those who have attempted cytological work in these plants can fully realize the difficulties encountered, because of the small size of the spermogenous cells, and the difficulty with which cytoplasmic structures, especially, are differentiated.

Ikeno begins his study with the last two or three mitoses in the spermogenous tissue. In these mitoses he finds that centrosomes are present during certain stages, and that this structure functions finally as a cilia-bearer.

In 1898 the writer (Mottier, '98) called attention to the presence of centrosomes in the vegetative cells of the gametophyte of *Marchantia polymorpha*, and further study was made by Van Hook (1900) with similar

results. Although we found such a body present during certain stages of mitosis, yet we could not trace it from one cell-generation to the next, and our conclusion was that it was only a temporary organ. There is no question but that Ikeno has observed a similar phenomenon in the spermogenous cells, for in all known cases wherever centrosomes are present in vegetative cells, they are to be observed also in reproductive cells of the same organism. The behaviour of these structures in the spermogenous tissue, as described by Ikeno, seems to be similar to that which I have mentioned for the vegetative cells; for Ikeno ('03, pp. 70, 71) states explicitly that, in the 'aster' and 'diaster' stages of mitosis, the centrosomes are only occasionally to be observed, and that they disappear at the end of each mitosis and reappear again at the beginning of the next successive karyokinesis.

In the last division of the spermogenous tissue the cubical cells divide obliquely, and the two resulting cells, which are triangular in shape, are not separated by a cell-wall. This division differs from the preceding division in that the centrosomes are always present (loc. cit., pp. 74, 75), so that each triangular protoplast, which is to become transformed into a spermatozoid, has a centrosome that eventually passes into an angle of the cell (loc. cit., Figs. 22–26). This centrosome now elongates somewhat and places itself in close contact with the inner contour of the cell, so that it appears to have arisen as a local thickening of the plasma-membrane (Hautschicht). Out of this elongated centrosome the two cilia now grow. As soon as the cilia have begun their development, a thread or band-like cytoplasmic differentiation appears, which grows in the direction of the anterior end of the future sperm, and finally connects the nucleus with the centrosome (loc. cit., Fig. 31). According to this statement, therefore, the centrosome-like body gives rise to the cilia only, whereas the specially differentiated cytoplasmic thread, or band, is of another origin. 'Bald nachdem die Cilien sich zu entwickeln begonnen haben, rundet sich die Spermatide ab (loc. cit., Figs. 29, 30), . . . der cytoplasmatische Fortsatz beginnt sich auszubilden und wächst nach der Richtung des vorderen Endes des jetzt sich bildenden Spermatozoids hin, um schliesslich das Zentrosom zu erreichen, so dass dieser Fortsatz den letzteren mit dem Zellkern verbindet (loc. cit., Fig. 31).'

This statement does not harmonize with Ikeno's figures. It would seem from his Figs. 30 and 31 that the 'Fortsatz' mentioned, connecting nucleus with the elongated, cilia-bearing band is derived from the cilia-bearer itself, as represented in his (loc. cit.) Figs. 29 and 30, and not as a separate formation.

If Ikeno's statement be correct, then the development of the specialized cytoplasmic band, which is known as the blepharoplast, differs in *Marchantia* from that known in all other Archegoniates, for, in the Ferns and zooidogamous Gymnosperms, the centrosome-like body gives rise

directly to this band. Ikeno figures in colours a mature spermatozoid, in which the central, or nuclear portion, is coloured green, while the anterior part with the cilia and the posterior end are coloured red. I do not find in his text any statement concerning the development of the posterior extremity which is undoubtedly of cytoplasmic origin.

Ikeno discusses at some length the question of the probable homology of the cilia-bearer, or blepharoplast, and the centrosome, concluding that these structures are homologous in the phylogenetic sense. In the elaboration of his argument, it seems to me that he ignores or leaves out of consideration the most fundamental principle, namely, that structures to be homologous must have, at least, morphological rank in the cell, i. e. they must be organs in the morphological sense. As a matter of fact the centrosome is not an organ of the cell with morphological significance. In *Chara*, certain Pteridophytes and spermogenous Gymnosperms, where the development of the blepharoplast is best known, there are no centrosomes with which to homologize these structures. Even in *Marchantia* where there seem to be both centrosome and blepharoplast, it has not been shown that the former is a permanent structure in the cell, since it cannot be followed from one cell-generation to the next. Ikeno expressly states that, in the aster and diaster stages of mitosis, the centrosome is only occasionally to be seen, and in this he is in accord with the observations of the writer and Van Hook. Ikeno has, of course, brought forth in *Marchantia* the strongest evidence that has, as yet, been advanced, pointing to a relationship between centrosome and blepharoplast, but this evidence cannot be accepted as final. The fact that two bodies look alike and stain alike in different cell-generations, is not conclusive proof that they are the same. Personally I cannot admit that all the bodies that Ikeno figures in cells of *Marchantia* are centrosomes, as similar bodies with exactly the same behaviour have been found in cells of plants in which it is known with absolute certainty that centrosomes, as understood among plants, do not exist. If, on the contrary, we attribute a morphological rank and phylogenetic relationship to the substance of which centrosomes and blepharoplasts are made, such as kinoplasm, or whatever we wish to call it, and if we admit that a morphological differentiation exists in the cytoplasm—a view that has much in its favour—then our theory will bring all known facts into line. Otherwise it seems that we shall be obliged to be contented with facts as they are known, and patiently await other facts and unquestionable evidence.

Summary.

The spermatozoid of *Chara* is a spirally coiled body consisting of a nucleus and a specially differentiated part of the cytoplasm which exists in the form of a thread or band, the blepharoplast, and bears two long

cilia. The nucleus occupies the middle part of the spermatozoid, while the blepharoplast extends its entire length. The anterior end of the blepharoplast is thinner than the posterior. The two cilia are borne some distance back of the anterior end. The posterior portion is thicker, and usually ends bluntly. In cross section the blepharoplast is crescentic, being convex on the outside and concave within. It is of a homogeneous structure, excepting a strip of granular substance along the concave side of the posterior end. The entire spermatozoid, as far as I was able to observe, makes two and one-half or three turns in a spiral.

The blepharoplast arises as a delicate thread-like differentiation of the cytoplasm at the surface of the cell, extending some distance along the cell from the nucleus and on opposite sides of the latter. Whether the terminal portions of this thread extending from the nucleus originate as a single piece, or as two pieces, could not be determined with certainty in the earliest stages, but, later, the blepharoplast is clearly seen to be one piece, extending the entire length of the sperm. The blepharoplast seems to be a modification of the plasma-membrane, i. e. it did not seem to be formed within or without this membrane, but as a direct transformation of it that increased in thickness inwardly. No centrosome-like body, or 'Plasmahöcker,' was observed from which the cilia develop, as described by Belajeff, Strasburger and others. The nucleus is transformed from an elliptical or oval body, with a hollow chromatin spirem, to a dense, homogeneous, sausage-shaped structure making one or more spiral turns. The cilia were always found attached some distance back of the anterior extremity of the blepharoplast. They did not seem to grow at the expense of a protuberance or centrosome-like body.

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Strasburger, E., 1892: Histologische Beiträge, iv, 107, 1892.

EXPLANATION OF FIGURES IN PLATE XVII.

Illustrating Professor Mottier's paper on the Spermatozoid of *Chara*.

All figures were drawn with the aid of the camera lucida and with Zeiss apochromatic homogeneous immersion 2 mm., apert. 1·40 with compensating ocular 8. All are magnified about 2,000 diameters.

Fig. 1. Sporogenous cell seen from the end at the beginning of the development of the spermatozoid. The blepharoplast appears as a heavier line at the surface of the protoplast above and below the nucleus; the anterior end being on the side below the nucleus.

Fig. 2. Two spermogenous cells in about the same stage of development as Fig. 1, seen from the side, or in longitudinal section. The blepharoplast is seen along the lower transverse wall in each cell. The cytoplasm has accumulated into a denser mass along the side next the blepharoplast.

Fig. 3. A median longitudinal section of a cell in the same stage as Fig. 2, passing through the cell in the plane *a–b* of Fig. 1. The blepharoplast is, therefore, not visible.

Figs. 4, 5. Later stages in the development as seen from the end. The blepharoplast is well differentiated and extends almost or quite around the nucleus. The posterior end, which is on the upper side in the figure, is seen to be much broader than the anterior end; its extremity projects as a beak beyond the surface of the protoplast. The anterior end is a very delicate thread curved or hooked at its extremity. In Fig. 5, the blepharoplast is separated from the nucleus, probably on account of shrinkage, and it is seen to be a continuous thread. The cilia are present at this stage.

Figs. 6, 7. Similar to the preceding. Fig. 6 shows the mode of attachment of the cilia; in Fig. 7 only a part of one cilium is shown.

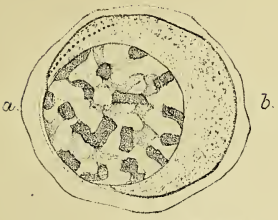
Fig. 8. A later stage. The spermatozoid has begun to assume its characteristic spiral form. The ends of the blepharoplast do not lie in the same plane, and were drawn by changing the focus. The nucleus is now in the form of a half moon or crescent, and the cytoplasm is correspondingly concave. The cilia lie in contact for a part of their length.

Fig. 9. A later stage than Fig. 8. The nucleus has become sausage-shaped. The granular cytoplasm is embraced by the middle portion of the sperm.

Fig. 10 *a* and *b*. Optical longitudinal sections of two cells in about the stage of Fig. 9. *a* is a surface view, and *b* an optical section. The dark dots in the cells on the right and left of the sperm represent sections of the cilia. In *b* the cytoplasm is concave at the right to correspond to the concave side of the sperm. The two crescents seen in this concavity are the transverse sections of the ends of the blepharoplast, that of the posterior end being above. These sections show that the blepharoplast is a convexo-concave band. At the left of the nucleus is seen a section of the blepharoplast closely applied to the nuclear membrane.

Figs. 11, 12, 13. Successively older stages. The sperm has now become spirally coiled by the growth or extension in length of both nucleus and blepharoplast. The cytoplasmic vesicle adhering chiefly to the nuclear portion is being gradually diminished.

Fig. 14. A nearly ripe spermatozoid. The spermatozoids are usually more closely coiled up than this one. The granular cytoplasm is more reduced.



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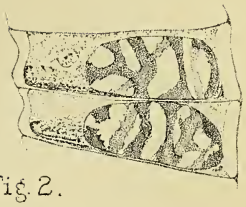

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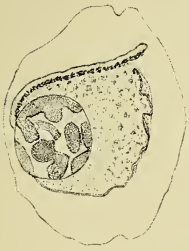

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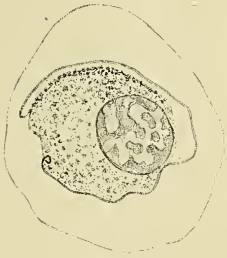

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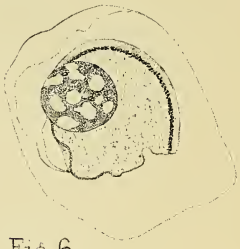

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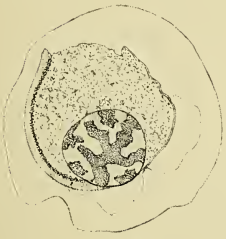

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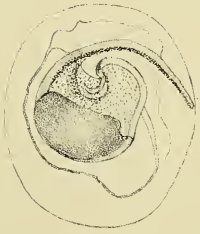

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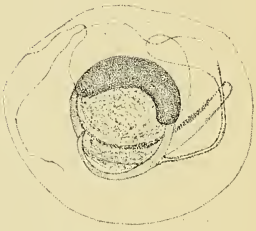


Fig. 9.

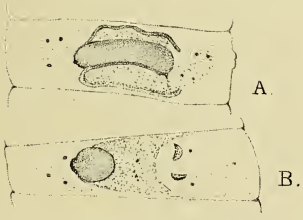
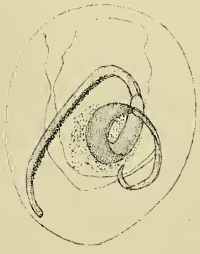


Fig. 10.

Fig. 11.

Fig. 12.



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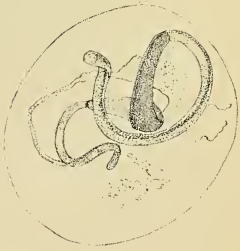

Fig. 14.

Mottier del.

University Press, Oxford.

MOTTIER—SPERMATOZOID OF CHARA.

A Mycorhiza from the Lower Coal-Measures.

BY

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With Plates XVIII and XIX and a Figure in the Text.

ALL who have investigated the microscopic structure of fossil plants are familiar with traces of fungal hyphae and occasional fungal sporangia in and around the plant-remains. An excellent critical account of our knowledge of such fossil Fungi will be found in Seward's 'Manual of Fossil Plants' ('98), in which he has not only recorded the Fungi described by Williamson, Renault, Conwentz, and other observers, but discusses their possible systematic position. In summing up our knowledge of this group of plants, he remarks that 'we have fairly good and conclusive evidence of the existence in Permo-Carboniferous times of Phycomycetous Fungi.'

Judging from the appearance of the tissues in which these Fungi are found, one is led to the conclusion that they were for the greater part of a saprophytic nature. This would seem more particularly so in the case of the fossil plants from the English Coal-Measures, the internal structure of which is so fully known from the remains found in the nodular concretions, the so-called 'coal-balls' of the Bullion Coal. In these coal-balls, which, according to Lomax ('02), were probably not formed *in situ,* the plant-remains are often of a very fragmentary character, and show traces of having undergone considerable decomposition. The tissues are often penetrated by Stigmarian rootlets, and show signs of having been bored by wood-eating animals. They also show not infrequently internal mycelia, while apparent fungal sporangia are found both within the fossil plants and in the débris lying between them. Indeed, the conditions under which these nodules were formed would seem to have been most favourable for the growth of saprophytic Fungi. Some of the fossil Fungi, however, from the silicified nodules at Grand Croix which have been described by Renault ('83) and Bertrand ('85), and more recently by Oliver ('03), seem to have been of a parasitic nature and to have belonged probably to the group of Chytridiaceae. One form, indeed, which appears to have been parasitic on the

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fronds of *Alethopteris aquilina*, Magnus ('03) considers sufficiently like the recent form *Urophlyctis* to warrant its inclusion in that genus.

From the observations so far made we are able to picture to a certain extent the modes of life of the Fungi in Palaeozoic times, and we come to the conclusion that they differed very little from those of recent Fungi.

But, besides leading saprophytic or parasitic existence, Fungi are at the present day also found living together with green plants in a state of symbiosis, in which they do not destroy the tissues of the green plant, but seem rather to be of some use to it, while at the same time they derive some benefit from the green plant. Living in such mutual relations with algal cells, the Fungi form the group of organisms known as Lichens, while when they inhabit the roots or root-stocks of many higher plants they form the so-called mycorhiza, the significance of which is still under discussion. Remains of Lichens are, according to Schimper and Schenk ('90), known from the Tertiary period, some of them being preserved in Amber; but none have so far been recorded from Secondary rocks. Mycorhizae, on the other hand, have to my knowledge not been described in a fossil condition. Yet, at the present time this peculiar association of Fungi with the roots of higher plants is a fairly widespread phenomenon. This is perhaps more particularly the case in tropical forests, where, according to Janse's investigations, sixty-nine plants out of seventy-five chosen from various divisions of the vegetable kingdom had their roots inhabited by apparently symbiotic Fungi. Whatever may ultimately turn out to be the significance of these endophytic Fungi, there can be no doubt that this mutual adaptation represents a considerable specialization of the two organisms forming the Mycorhiza. The latter might, therefore, be expected to have arisen at a comparatively recent period in the evolution of plants. But apparently this form of symbiosis is of considerable antiquity, for it seems to have existed as far back as the Lower Coal-Measures. I am conscious that this announcement will very naturally meet with some scepticism; nevertheless, I venture to think that the evidence which will be brought forward in the following pages warrants the conclusion that this highly specialized mutual adaptation of Fungus and cormophyte did actually exist in the Palaeozoic age.

The root or rhizome in question I found on two slides in the Cash collection of Coal Measure plants in the Manchester Museum, Owens College (slides No. Q 527 and Q 529), both from the Halifax Hard Bed, which, according to Binney ('62), must be correlated with the Bullion Mine of the Burnley district and the Gannister Mine of Dulesgate, Todmorden. These three seams of the Lower Coal-Measures are characterized by the possession of the nodular concretions (coal-balls) referred to above, and it is from one of these that the preparations were made. On communicating my view of the nature of these specimens to Dr. Scott, he very generously placed at my disposal

a similar specimen from the collection of the late Mr. James Spencer, of Halifax, which he had purchased some years ago. It turned out to be undoubtedly of the same nature as the specimens in the Cash Collection, and is in all probability from the same locality, as most of Mr. Spencer's material came from the Halifax Hard Bed.

The Host Plant.

The only remains that we have of the host plant are a few delicate root-like organs ranging from about 1 to 2 mm. in thickness. In all the specimens so far discovered the tissues are well preserved in comparison with the surrounding plant-remains, which have for the most part undergone considerable destructive changes. Among the débris are seen spore-cases, apparently of a fungal nature, and numerous opaque, rounded masses (see Pl. XVIII, Fig. 1), which are generally taken to be excrements of wood-boring animals. The whole has the aspect of a mass of humus in which Fungi and animal organisms were causing a gradual breaking-down of the vegetable remains. Only a few hard macrospores, some Stigmarian rootlets, and the mycorhiza in question seem unaltered—a fact which is suggestive of their having grown in the humus-like mass.

The internal structure of the mycorhiza at once suggests a root of the diarch type, but differs in several respects from other diarch roots found among Coal Measure plants. It should be added that these root-like remains are very constant in character, and therefore easily recognizable. As seen in Pl. XVIII, Fig. 1, the root has two groups of wood distinctly separated by well-marked ground-tissue cells. These are present in all the specimens, and as the larger ones are evidently mature we may consider that the xylem-rays did not meet in the adult organ, as is generally the case in the roots of Ferns. In a smaller specimen than that in Fig. 1 these medullary cells are filled with curious granules (Pl. XVIII, Fig. 2, and Text-fig. 42), the nature of which is uncertain. At first sight they closely resemble a number of starch-grains, but it is somewhat doubtful whether starch would be preserved with quite so definite an outer boundary. On the other hand, they do not seem in any way connected with the Fungus, which does not as a rule penetrate to such depths; nor does the Fungus in other parts of the plant, where it occurs, produce granules quite of this kind. It would, therefore, seem more probable that the granules are the normal cell-contents at a certain stage in the development of the organ or under certain conditions of nutrition.

With regard to the xylem-groups, it is not always easy, or indeed possible, to distinguish the protoxylem-elements. In Fig. 2 the smaller elements seem to be on the outside of the wood; but, as will be seen from Fig. 1, this is not always the case. In one group, indeed, the smaller elements appear to be on the inside. Their position is in fact irregular, and

in that respect, as well as in others to which reference will be made later on, the stele resembles somewhat that of the rhizome of *Psilotum*. It is true that in this latter plant the xylem-rays, whether two or more, are generally connected in the centre; but in certain parts of the plant, according to Bertrand's ('82) figures and description, the xylem-groups may remain separate. Owing to the uncertainty as to the position of the protoxylem, I prefer, therefore, to leave it an open question whether the organ under consideration was a root or a rhizome. If the latter, then it must have

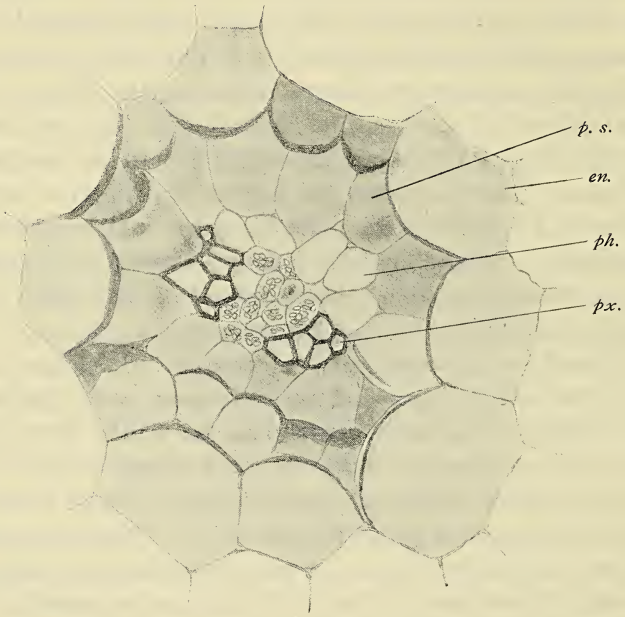

FIG. 42. Enlarged drawing of stele from specimen represented in Fig. 2, Pl. XVIII. The protoxylem elements (*px*) are on the outside of the groups of wood. The ground tissue is filled with granules (starch?). The stele is surrounded by a distinct endo-cortex (*en*) within which is a phloem-sheath (*p.s.*).

been a leafless rhizome of the type found in the Psilotaceae or in *Corysanthes*, *Corallorhiza*, and some other Saprophytes.

In longitudinal section, as seen in Plate XVIII, Fig. 3, which is taken from Dr. Scott's specimen, it will be seen that the xylem consists mainly of scalariform tracheids. I have in fact not been able to discover any other elements in the longitudinal section; but it is of course possible that the latter did not pass through the spiral elements if such were present in the plant. There are no very clearly-marked phloem elements, though certain cells on either side of the xylem-groups might be considered as bast cells (*ph*). In this respect we have another agreement with *Psilotum* and also with other Lycopodiaceous plants in which the phloem is not made up of well-defined sieve-tubes. Similarly in some cases, as in Plate XVIII, Fig. 2,

there is an indication of a phloem-sheath (*p. s.*) or pericycle. The characteristics of these tissues are not well pronounced, and except for the xylem-groups the structure of the stele is somewhat unsatisfactory.

The cortical tissues are well developed and consist of thin-walled cells. There is no such thickening of the walls as one frequently finds in the roots of Filicineae, nor any such specialized lacunar tissue as is typical of the stem and roots of most of the Lycopodiales, particularly of the Lepidodendraceae.

It will be seen from Fig. 1 and Fig. 3 that some of the inner layers of the cortex are slightly more elongated in the radial direction, while the outer layers appear in the transverse section somewhat flattened owing to the tangential extension of the cells. It will be convenient, therefore, to use the terms exo- and medio-cortex for these two portions of the cortex in the same sense in which they have been used by Groom ('95) in his descriptions of the roots of monocotyledonous saprophytes where they show a similar differentiation. The difference between these two layers of the cortex can most readily be seen in the longitudinal section (Fig. 3) in which it will be further seen that the innermost row of cortical cells (endo-cortex, possibly endodermis) is drawn out longitudinally, while the tangentially and radially elongated cells of the exo- and medio-cortex are short, more particularly the latter. The radially elongated cells, as can be seen both in the transverse and in the longitudinal sections (Figs. 1 and 3), are also characterized by their very dark contents. These will be described in detail later on, but it may be mentioned at present that they show indications of fungal hyphae and closely resemble in their appearance and in their position in the cortex the curious contracted masses (clumps) described by various authors in the aerial roots of Orchids and in the absorptive organs (roots or rhizomes) of saprophytic Monocotyledons and of *Psilotum*. The exo-cortex, though also containing hyphae, possesses none of these 'clumps,' and consequently looks at first sight devoid of the Fungus. This specialization of the hyphae in two different regions of the cortex is a very common phenomenon in mycorhizae and is constantly met with in the plants mentioned above. Fungal hyphae are very rarely met with in the endo-cortex. Only in one transverse section were a few hyphae discovered in this layer. This, again, is in conformity with the behaviour of the fungal mycelium in the mycorhizae of living plants, and supports the conclusion I have arrived at, that the Fungus is not of a destructive nature; or it would probably have penetrated into all the living tissues of the plant.

The cells of the epidermis are smaller in size than the cortical cells as can be seen in Plate XVIII, Figs. 1 and 3, and are often drawn out into long absorptive hairs (Plate XIX, Fig. 2). This piliferous layer may possibly be regarded as evidence of the root-nature of the organ, but it

must be remembered the rhizome of *Psilotum* and *Tmesipteris*, as well as some of the modified absorbing rhizomes of saprophytes, bear such hairs. It is indeed difficult, as Groom ('95, II) has shown, to distinguish between roots and rhizomes in these plants, as they may be very considerably altered both externally and internally. Not only may the leaves be considerably atrophied but, as Groom points out, 'the structure of the stele in absorbing rhizome-axes of hemi- and holo-saprophytes is frequently remarkably like that of a root (*Corysanthes, Burmannia, Corallorhiza*), so the root-like structure of the stele of the absorbing organ is no proof of its root-nature.' It would seem, therefore, best to suspend our judgement with regard to the nature of the organ in question, and similarly we shall have to remain in doubt as to the systematic position of the plant until other portions of it have been discovered.

The Fungus.

The general distribution of the mycelial threads within the host-plant has been mentioned above as well as the different appearance of the Fungus in the exo-cortex and medio-cortex respectively. In the epidermis too hyphae may be noticed, but their occurrence in a cell does not necessarily mean that this has been a centre of infection. Sometimes, as in the cell bearing a root-hair in Plate XIX, Fig. 2, hyphae may be running longitudinally through the cell. Sometimes, however, numerous hyphae are seen running radially across the epidermal cells (*h* in Fig. 2); but in no case have I been able to trace them beyond the outer wall. Even in such cases, however, we have no proof that it was through these cells that the Fungus had gained admission, as it is known that hyphae may grow out from the mycorhiza into the surrounding medium. Thus Groom ('95, II), in his description of *Thismia*, mentions (p. 354) that it is possible to observe 'that frequently free hyphae are deserting, not entering, the host-plant.' Whether the particular hyphae shown in Fig. 2 are entering or leaving the tissues must of course remain an open question in the case of a fossil plant.

In the outer cortical layers the course of the Fungus is somewhat irregular, both horizontally and vertically running hyphae being met with. On the whole, however, the mycelium seems to grow along the mycorhiza just as Janse ('97) observed in many of the roots which he investigated. Plate XIX, Fig. 1, which is a portion of the transverse section represented on Plate XVIII, shows the majority of the hyphae cut more or less transversely, and also exhibits them running, as they seem generally to do, close along the inner face of the cell-walls. This is often found to be the case in the living mycorhizae, where, however, the Fungus often forms more or less definite coils on the inside of the cell-walls. From these thicker coiled hyphae very thin haustorial filaments are sent into the

cell-contents in *Neottia*, *Psilotum* and other plants according to Werner Magnus ('00) and Shibata ('02). But as these are very delicate, and soon disappear, we should not expect to find them in the exo-cortex of the fossil plant. Otherwise the latter presents very much the same appearance as it does in recent plants. In one or two cases the numerous hyphae attached to the inner walls of these cortical cells remind one of the mycelial pegs described by Groom ('95) in the roots of *Galeola*.

For the most part the hyphae seem to be intra-cellular, but there are indications that a few of them run between the cells. In no case, however, is there any sign that the Fungus was in any way destroying the tissues of the host-plant. In some of the cells of the exo-cortex curious pear-shaped bodies are found at the ends, or apparently at the ends, of certain hyphae. These may be fairly numerous, as in Plate XIX, Fig. 3, or there may be only two or three in a cell. They are generally most numerous in the sub-epidermal cells, while in the deeper layers of the exo-cortex they are fewer in number, larger in size, and more rounded. They resemble somewhat the pear-shaped swellings described and figured by Williamson ('81) on the hyphae of *Peronosporites antiquarius*, a Fungus found in the bark of *Lepidodendron*. Such pear-shaped bodies are, however, of very common occurrence in the outer cortical layers of recent mycorhizae. Thus in *Psilotum*, Janse ('97) figures a dozen of them in a sub-epidermal cell, just as they occur in the fossil mycorhiza. The nature of these vesicles, formed by endophytic Fungi, is a much-disputed point. Some authors look upon them as possibly of the nature of reproductive organs. Thus Bruchmann ('85) considered that they might possibly be oosporangia, while Goebel ('87) thought they might be gonidia (Dauergonidien) in the case of the Fungus inhabiting *Lycopodium*, which they supposed to be related to *Pythium*. Groom ('95), on the other hand, who made a very careful examination of these bladders and their mode of formation in the mycorhiza of *Thismia*, found that they were not terminal but intercalary dilatations, though appearing terminal by hypertrophy. He is consequently inclined to attribute a nutritive function to them, and regards them as of importance in increasing the absorptive area of the Fungus which is supposed to feed upon the host-plant in the outer cortical layers. This view of the purely vegetative character of the vesicles had also been entertained by Mollberg, who was apparently the first observer of these intercalary hypertrophies of the Fungus (in *Platanthera* and *Epipactis*). Groom found that the vesicles accumulated a large amount of densely-staining cytoplasm, which afterwards became vacuolated and diminished in amount, ultimately degenerating and depositing 'a homogeneous yellow substance in which are rod-like bodies which remind one of the regular rod-like bacteroids in leguminous tubercles.'

In the fossil plant the vesicles, whether large or small, are generally

devoid of contents, but a few of the larger more rounded ones show homogeneous contents. In one or two instances vesicles were found among the cells containing the dark clumps and these contained what appear to be spores (Plate XIX, Fig. 6), but as they are really in the medio-cortex it is possible that they were formed in a different manner from the vesicles described above. In some cells lying near those with large vesicles (see Plate XIX, Fig. 5) there are found curious granules distributed very evenly through the cell and apparently attached to the cell-wall. The nature and formation of these I was not able to elucidate from the specimens at my disposal.

In the medio-cortex, as described above, we find the characteristic clump formation (see Plate XVIII, Figs. 1, 3, and Plate XIX, Fig. 4)—the clumps consisting no doubt partly of the cell-contents, partly of fungal filaments; but they are as a rule so dark in colour that no details of their structure can be made out. They are connected to the cell-walls by threads, which are sometimes very delicate and appear as if they were protoplasmic filaments, though they are probably contracted hyphae, as these can in some cases be seen very clearly as shown in Plate XIX, Fig. 4. These hyphae are, however, usually thinner and more delicate than those in the outer layers of the cortex. In this particular the fossil mycorhiza agrees with recent ones in which various observers have noted this difference. The fossil mycorhiza can of course give us no clue as to the formation and significance of these clumps, but their excellent preservation in a fossil condition may be considered to support the view of Werner Magnus ('00), that they consist of the non-digestible and unalterable remains of the Fungus after the host-plant has derived from it all possible nutriment. For the fact that these clumps are so well preserved would indicate that the Fungus had passed into an unalterable condition before fossilization.

Should the same degenerative changes have taken place in the medio-cortex of the fossil plant as take place in recent mycorhizae these would readily explain such appearances of degeneration as one meets with in some of the specimens. Thus in Fig. 8 will be seen curious vacuolated masses which, as indicated by the presence of delicate radiating filaments, are probably produced in a similar way to the mycelial clumps. They obviously correspond to the so-called 'traubenförmige Körper' described by Bernatzky ('99) in the rhizome of *Psilotum*. Ultimately they would seem to break up into separate particles not unlike bacteroids (Fig. 7), but which may also be compared to the curious 'Eiweisshyphen' described by Magnus ('00) in some of the cells of the medio-cortex (Verdauungszellen) in which the host-plant is digesting the Fungus.

The obvious resemblance between these clumps in the fossil plant and those of recent mycorhizae, together with the close agreement in the structure and behaviour of the Fungus in the outer layers of the cortex,

with those of the Fungus in recent mycorhizae will, I think, be regarded as sufficient evidence for the conclusion that we are dealing in the case of this fossil plant with a mycorhiza or mycorhizome. The Fungus differs materially in its manifestations from other cases of endophytic Fungi so far observed in fossil plants, and in no way suggests that it was living either saprophytically or parasitically upon the host-plant. The excellent preservation of both the Fungus and the host-plant and the specialization of the cortex into two layers comparable with the 'Pilzwirthzellen' and 'Verdauungszellen' of recent mycorhizae, would suggest that, as in the case of the latter, the host-plant is deriving some benefit from the presence of the Fungus.

We cannot of course expect from the investigation of a fossil mycorhiza to elucidate the difficulties that surround the mycorhiza-question, but it is of no little interest to find that already at the Coal-Measure period Fungi and cormophytes exhibited a mutual adaptation of such complexity as that involved by the formation of a mycorhiza.

Of the systematic position it is difficult to say much on the slender evidence before us. I have not been able, in any of the longitudinal sections, in which one sees occasionally considerable lengths of the Fungus, to detect any transverse walls, and this would incline me to the belief that the Fungus belonged to the group of Phycomycetes. This view would be supported by the fact stated by Seward ('98), that the Phycomycetes certainly existed in Permo-Carboniferous times. Among recent endophytic Fungi showing symbiotic adaptation some apparently also belong to this group, according to Bruchmann ('85) and Goebel ('87).

The systematic position of the host-plant is almost as difficult to determine as that of the Fungus. Leaving out of consideration the Gymnosperms and Cycadofilices with which it shows no affinities, we may confine ourselves to the consideration of the Vascular Cryptogams. Of the four divisions of these it seems, as mentioned above, to have most affinity to the Lycopodiales, though it differs considerably from most of these. It has not the specialization of the cortical tissues characteristic of the Lepidodendraceae and, if it is a root, does not possess the usual monarch arrangement found in that group. The absence of large intercellular passages such as are found in the roots of Lepidodendraceae and Calamarieae would lead us to infer that it was not rooted in marshy ground as were probably these larger fossils, and its association with a Fungus would rather point to a saprophytic existence in rich leaf-mould or to an epiphytic existence like that of *Tmesipteris* on the stems of Tree-ferns. In either case it would be likely to become infected with fungal hyphae, and might develop the special adaptation which its mycorhiza exhibits. That a mycorhiza has not been found in other Coal-Measure plants should not astonish us when we remember that

the greater number of Lycopodiales and Equisetales were probably rooted in marshy places in which the conditions would not be very favourable to the formation of mycorhizae. In our present state of uncertainty as to the nature of the host-plant, of which the root or rhizome only is known, it would not be advisable to give it more than a provisional name, merely to facilitate reference to the specimen. For such use it would be best to employ a non-committal designation, like that of *Rhizonium*—which was invented by Corda ('45) to describe certain roots which he took to be those of an Orchidaceous type, and which was afterwards used by Williamson ('89) for the roots of an unknown plant. In consideration of the peculiar character of the fossil described above, I would suggest that, until we know more about the plant to which it belonged, it should be referred to as *Mycorhizonium*.

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EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Professor Weiss's paper on a Mycorhiza from the Coal-Measures.

(Photographs by Mr. Abraham Flatters, Manchester.)

PLATE XVIII.

Fig. 1. Transverse section of a mycorhiza from the Cash Collection in the Manchester Museum (Q. 827), about 1 mm. diameter. Above the section are seen some dark oval masses, probably the excrements of a wood-eating animal. In the exo-cortex (*ex*) traces of the fungal hyphae can be seen projecting from the cell-walls into the cell-spaces. In the medio-cortex (*mc*) are seen dark masses (clumps) consisting largely of fungal remains.

Fig. 2. Vascular cylinder of another mycorhiza 2 mm. diameter from the same slide as Fig. 1 (Q. 827), showing a well-marked endo-cortex (*en*). Between the two groups of wood-elements are a few parenchymatous cells containing numerous granules. *ps* = phloem-sheath.

Fig. 3. Longitudinal section of a mycorhiza from Dr. Scott's preparation (No. 1527), showing the vascular cylinder, the endo-cortex (*en*), the radially-elongated medio-cortex (*m.c*) with dark mycelial clumps, the exo-cortex (*ex*) with scattered hyphae.

Fig. 4. Portion of the medio-cortex from a tangential longitudinal section on Dr. Scott's slide (No. 1527), showing the dark mycelial clumps (*cl*). An enlarged drawing of a portion of this photograph is shown in Fig. 4, Plate II.

PLATE XIX.

Fig. 1. A portion of the external tissues of the root figured in Plate I (Cash Collection, No. 527), showing the fungal hyphae (*h*) in the exo-cortex and one of the cells of the medio-cortex with clump formation (*c*).

Fig. 2. A portion of the epidermis from a transverse section of another root, from the same slide as Fig. 1, showing the radial course of the fungal hyphae (*h*) in an epidermal cell. *r. h* = root-hair.

Fig. 3. Formation of vesicles by the fungal hyphae in the sub-epidermal layer. Portion of the longitudinal section from Dr. Scott's collection shown in Plate XVIII, Fig. 3.

Fig. 4. Enlarged view of a portion of medio-cortex shown in photograph 4 on Plate XVIII, to show the hyphae connecting the mycelial clumps with the cell-walls.

Fig. 5. Portion of the cortex of transverse section on Slide No. 1527 (Dr. Scott's Collection), showing one of the large vesicles found near the medio-cortex, and also a cell containing curious granulations (see p. 262).

Fig. 6. Portion of the tangential section on Dr. Scott's preparation (No. 1527), showing sporangia (?) (*sp*) and spores (?) in a cell of the medio-cortex. The other cells contain mycelial clumps (*cl*).

Fig. 7. A cortical cell from a transverse section on Slide 529 of the Cash Collection, in which the cell-contents seem to have undergone degeneration.

Fig. 8. Two cortical cells from the same section as Fig. 7, showing earlier stages of degeneration of cell-contents with indication of previous formation of mycelial clumps.

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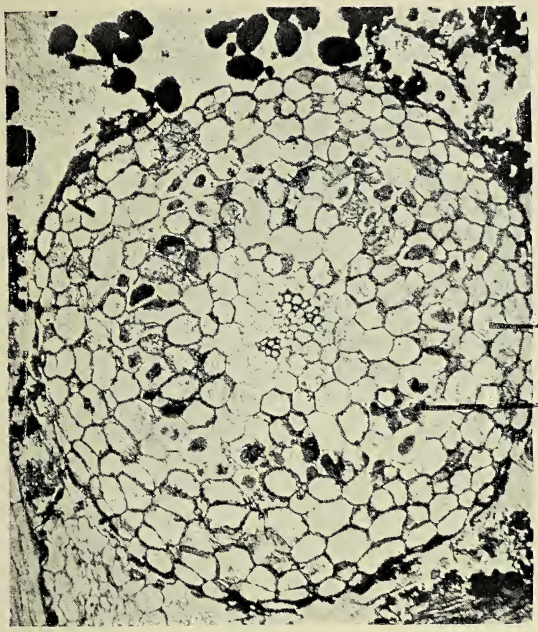

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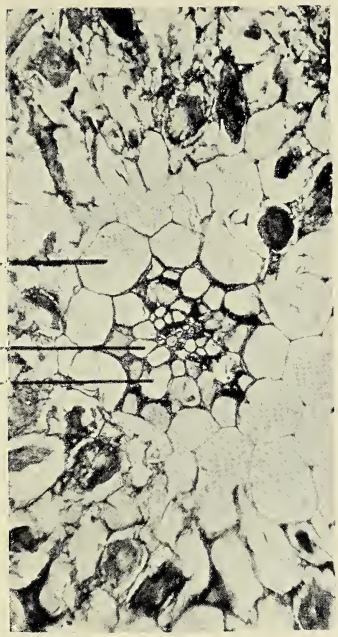

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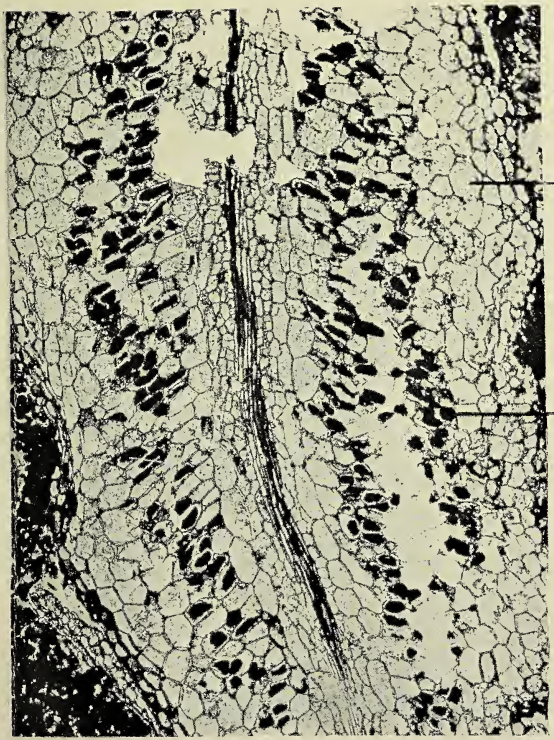

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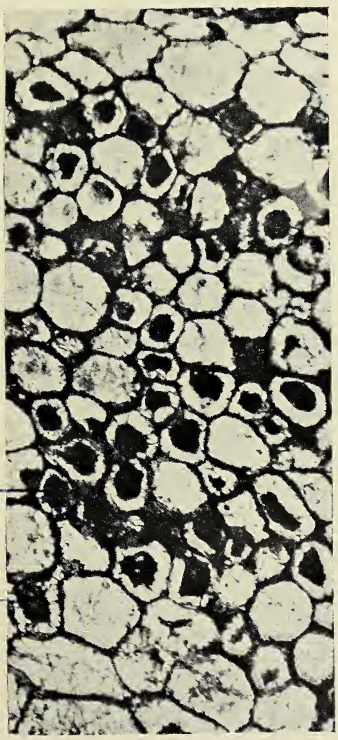
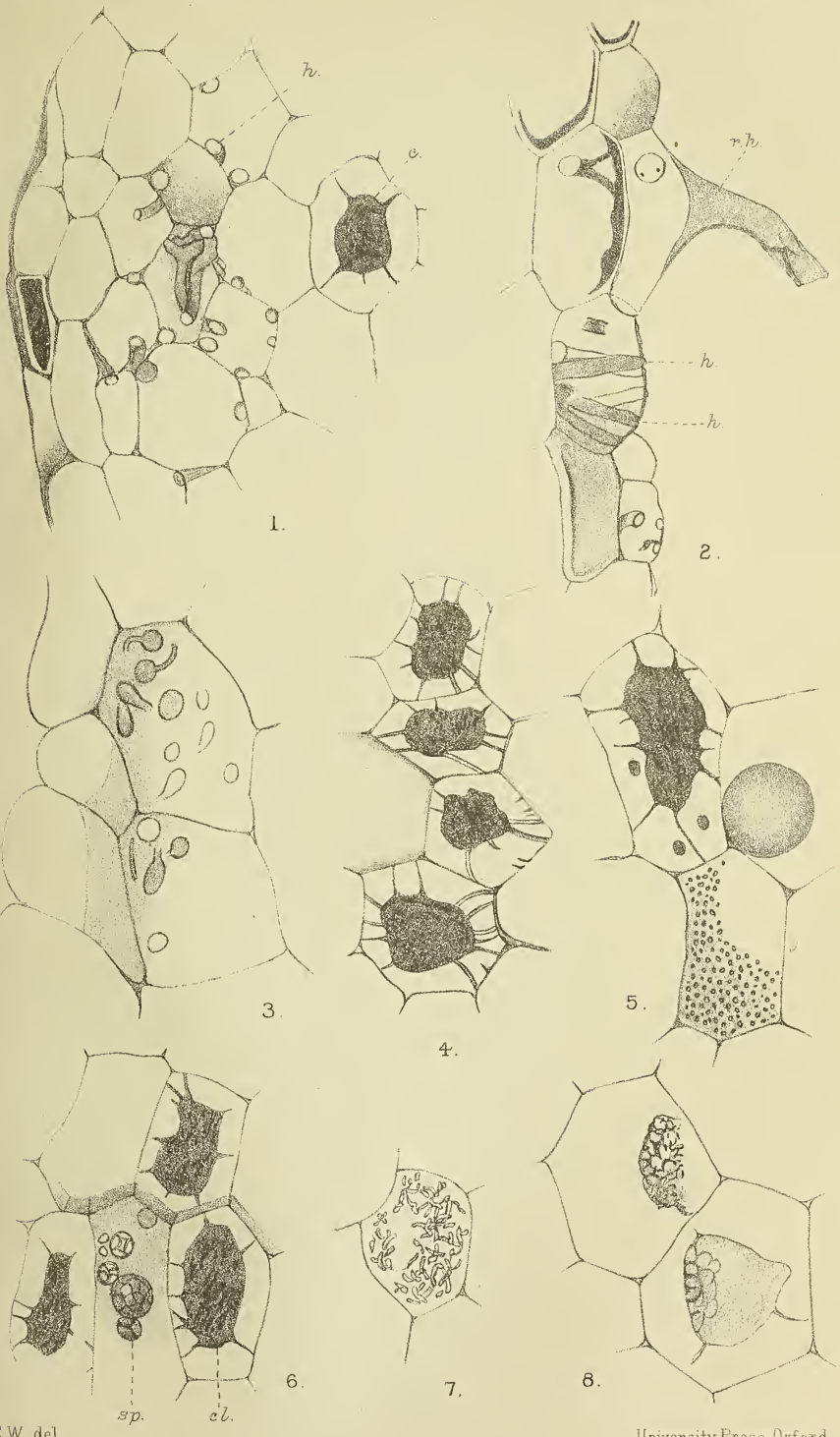


WEISS.—MYCORHIZA FROM THE COAL-MEASURES.



F.E.W. del.

University Press, Oxford.

WEISS.—MYCORHIZA FROM COAL-MEASURES.

A Study of the Enzyme-secreting Cells in the Seedlings of Zea Mais and Phoenix dactylifera[1].

BY

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With Plate XX.

I. Historical.

THE morphology and physiology of secreting-cells offer an inviting field for study. Their metabolism is more active and continues for a longer time than that of embryonic tissue. The constant removal of the products of synthetic activity affords opportunity for observing the changes which accompany their production. Some of the most instructive descriptions of these cells have been given by students of animal histology, the highly specialized glands of animals affording a favourable subject for investigation. A brief résumé of the most important contributions to our knowledge of the subject is here given.

Charles Darwin ('75) described the changes occurring in the stimulated gland-cells of *Drosera rotundifolia* during the process of digestion. His experiments were carried farther by his son, Francis Darwin ('76, '77, '78), using *Drosera rotundifolia* and *Dipsacus fullonum*. Following their work came that of Schimper ('82) on *Sarracenia*, *Drosera*, and *Utricularia*; Fromann ('84) on the glandular hairs of *Pelargonium*; and de Fries ('86) on *Drosera*. The first detailed cytological study of secreting-cells in plants was that of Gardiner ('85) on the gland-cells in the tentacles of *Drosera dichotoma*. He saw that the process of secretion was accompanied by vacuolization and destruction of the cytoplasm in the distal end of the cell, but the nucleus was always surrounded by a dense layer of protoplasm. His explanation of the process of secretion was that the cytoplasm contains a 'formed substance' derived from the protoplasm, and that the out-pouring of the secretion is caused by the repeated breaking down of the protoplasm into this 'formed substance,' which is of a mucous nature and, attracting water, escapes as the secretion to the external surface.

[1] LXX. Contribution from the Botanical Laboratory of the University of Michigan.

[Annals of Botany, Vol. XVIII. No. LXX. April, 1904.]

Korscheldt ('89) studied the gland-cells in the genital organs of *Branchipus* and in the glands of butterfly larvae. He found that the nuclei moved to the apex of the cells during active secretion and returned to the base during the resting period of the gland; and concluded that the position of the nucleus in a cell indicates a participation in the activities at that place. In some cases where he thought that solid granules pass from the nucleus into the cell, there was apparently a direct change from nuclear substance into secretion, but he was not sure of the identity of the substances thus produced from the nucleus. During secretion, amoeboid movements were frequently observed in the nuclei.

The results of Müller ('96), ('98) are very instructive, especially as to the extra-nuclear processes connected with the production of secretions.

The first detailed cytological study of secreting-cells in the botanical realm was on the effect of stimulation of the glandular-cells of *Drosera* with egg-albumen by Miss Huie ('97), and a later paper ('99) by the same author on the effect of stimulation with substances of various composition. She found that the cytoplasm, which in the resting stage is abundant and stains blue with Mann's Eosin-Toluidin blue, is exhausted by the process of secretion and shows strongest affinities for the red stain. During the period in which the secretion is being formed there is a constant increase in the amount of chromatin in the nucleus accompanied by a decrease in the size of the nucleolus. By the use of chemically different stimuli, very characteristic alterations are obtained in the morphology and colour-reactions of the cell. The cytoplasm is the cell-constituent most noticeably affected by external stimuli, but the nucleus is said to be the seat of metabolic activity. In all cases the process of recuperation begins in the nucleus. She concludes that the rate of plasmic changes depends on the rate of absorption, but that the rate of nuclear changes is commensurate only with the digestibility of the food.

Rosenberg ('99) has given a very accurate and valuable description of the cytological changes accompanying the stimulation of the gland-cells of *Drosera rotundifolia*, making comparisons between the secreting-cells and the reproductive-cells of that plant. He gives the following interesting observations on the behaviour of the nucleus. The volume of the nucleus constantly grows smaller during secretion; the chromatin shows a constant increase. At first the chromatin is in the form of granules on the nodes of the linin network throughout the nucleus. Gradually the granules collect at the nuclear membrane, where they fuse into longer or shorter rods; finally, when the effect of the stimulus is particularly energetic, these rods unite and form a single thread with richly anastomosing branches. When the digestive process is ended the thread gradually becomes thinner, and here and there segments, until finally the original condition of the nucleus is reached. In certain cases where the leaf is very strongly stimulated and the feeding

process lasts for a long time, the chromatin content increases farther and chromatin granules lie within the interior of the nucleus. Simultaneous with these changes the nuclear membrane becomes indistinct, and at times almost invisible. Miss Huie reported the same appearance and expressed the belief that the nuclear membrane was absorbed. Rosenberg believes that the membrane undergoes a change in composition and staining qualities which perhaps facilitates the communication between nucleus and cytoplasm. The nucleolus constantly grows smaller until at the close of secretion it is very small. Yet he thinks there is no close connexion between nucleolus and chromatin, for there are nuclei with abundant chromatin which nevertheless contain large nucleoli. Chromatin is apparently an active component of the nucleus, yet it remains an open question whether the increase of chromatin is the result of the formation of an enzyme, or of the abundant absorption of nutrition. He thought that the latter theory was improbable.

In his work on the secreting-cells of the pancreas, Mathews ('99) found that during secretion the nucleus moves nearer the centre of the cell and that the fibrillar zone increases in size, while the granular and reticulate cytoplasm disappears. The granules called zymogen-granules arise as products of the decomposition of the threads which in turn are formed by the chromatin. Neither the nucleoli nor chromatin showed any periodic alteration in amount or staining reactions. The synthetic processes in the nucleus were thought to resemble in many ways catalytic or fermentative actions.

Another interesting and careful study is that of Garnier ('00) on various animal glands, but his results are not different from those already given.

While my work was still in progress, Torrey ('02) published an account of the cytological changes occurring in the cells of the scutellum of *Zea Mais* during the secretion of enzyme. His observations and conclusions differ from mine in many important respects, as I shall show later.

He locates the origin of the diastase granules in the nucleus, where they exist as fine, deeply-staining granules. From the nucleus, fine rows of granules extend through the nuclear membrane and out into the cytoplasm. After germination has progressed for about eighteen hours the cells are much larger than in the resting stage, the nuclei are for the most part devoid of granules, which are now to be found in the cytoplasm. After twenty-four hours certain cells are to be found in which the granules are massed together in the ends nearest the endosperm, but otherwise the cytoplasm and nuclei are entirely destitute of them. At the beginning of the second day of germination the secreting-cells are in a resting condition ; there is no increase in size and the nuclei are clear.

The second period of secretion begins at the end of the second day. It is indicated by the presence of darkly staining granules in the nucleus

and their subsequent discharge. After seventy-two hours the periods of secretion are not well marked. At the end of eleven days there are signs of degeneration in some of the epithelial cells as indicated by an abnormal swelling and vacuolization of the cytoplasm. After twenty-two days the cytoplasm is very scanty.

II. Material and Methods.

In my work I have attempted to study the morphology of the enzyme-secreting cells in the scutellum of *Zea Mais* and in the 'absorbing organ' of the seedling of *Phoenix dactylifera*.

It has been proved by the work of Brown and Morris ('90), Hansteen ('94), and Grüss ('97) that the diastase produced by the scutellum of the Gramineae is formed and secreted by the columnar epidermal cells of that organ. In *Phoenix dactylifera* the production and secretion of enzymes occurs in the columnar epidermal cells of the absorbing organ (Grüss, '94, Puriewitsch, '98). It is to a consideration of the morphological changes that this paper is devoted; the physiological changes will be made the subject of a subsequent study. My work was carried out at the Botanical Laboratory of the University of Michigan during the years 1902 and 1903. It is with great pleasure that I take this opportunity of expressing my thanks to Professor F. C. Newcombe for his invaluable suggestions and criticisms.

I used the large variety of *Zea Mais* known in agriculture as 'White Dent,' and the seeds of *Phoenix dactylifera* obtained from the dates of commerce. The resting embryos were cut from the dry seeds and killed in strong alcoholic killing fluids. The embryos of different ages were obtained by germinating the seeds in moist sawdust or between layers of moist filter-paper. Light was always excluded in order to prevent any manufacture of food by photosynthesis. The embryos of *Phoenix* were grown in an incubator at a temperature of 30° C., those of *Zea* were grown at a temperature of 22° to 25° C.

The study of fixed and stained material was supplemented by that of living cells in both plants. Sections of the living material were cut with a razor and mounted in water, or in dilute sugar-solution if they were to be studied for any length of time. Methylene blue in aqueous solution was used for *intra vitam* staining. In many respects the use of living material was not as satisfactory as one would expect, owing to the difficulties in identifying the different substances in the cell. It was, nevertheless, valuable as a check on the artificial appearances produced by killing fluids and other reagents. It is well known that certain methods of fixing and staining give characteristic appearances to the tissues upon which they are used, especially upon cells containing large amounts of plastic material in a fluid state. Many of the discrepancies between the descriptions of different investigators are doubtless due to different processes of fixing and

embedding. An interpretation of the effects produced awaits an extension of our knowledge of the chemical and physical reactions between protoplasm and the various reagents used in micro-technique.

In order to obviate as far as possible particular effects due to chemical or physical action of the killing fluids, a number of different fluids were used and different stains employed after each method of fixing. The technique was rather difficult on account of the delicacy of the material. The following results were observed with the respective killing fluids.

Saturated Solution of Picric Acid in 50 per cent. Alcohol. The material prepared for study by this reagent was unsatisfactory. The protoplasmic structures were not fixed well enough to show with any definiteness. On the other hand, this is a good reagent for fixing the proteid granules. (Zimmermann, '93, p. 216.)

Aqueous Picro-corrosive Fluid. I used the following modification of Mann's method as given by Huie ('97). One volume of a saturated aqueous solution of mercuric bichloride was added to three volumes of a saturated aqueous solution of picric acid. The material was allowed to lie in this fluid for twelve to eighteen hours, then washed in water and dehydrated in the usual way with alcohol. This proved to be a very satisfactory killing fluid. The amount of picric acid present was sufficient to fix perfectly the granules, while the mercuric bichloride hardened the protoplasm and precipitated the soluble proteids. In a few cases there was a tendency to contract the cell-contents in the scutellum of *Zea*.

Kleinenberg's Picro-sulphuric Acid. The results obtained by the use of this reagent indicated that it was better than picric acid alone, but not so good as the picro-corrosive fluid. The material seems to be insufficiently hardened.

Chromo-Osmo-Acetic Acid. Mottier's formula, Pring. Jahrb. Bd. 30, p. 170. This fluid is a good fixing agent for the protoplasmic structures of the cell, but not for the granular structures. It produced no shrinking in any of the cases where it was used.

Iridium chloride in Acetic Acid. The formula used was—

Iridium chloride 1 per cent. aqueous solution . .	25 cc.
Glacial acetic acid	75 cc.

It leaves the tissue in better condition for the stain than the following killing fluid, but in other respects the two act similarly.

Worcester's Killing Fluid. This fluid gave uniformly good results whenever used. The formula according to which it is made is as follows:—

Mercuric bichloride, saturated aqueous solution .	96 parts.
Formalin (40 per cent. formaldehyde) . . .	4 „
Acetic acid, 10 per cent.	10 „
Formic acid, to each litre of solution . . .	5 drops.

The tissue is allowed to remain in the killing fluid from ten to twenty hours, then transferred for washing to 70 per cent. alcohol, which contains about 1 per cent. of potassium iodide. If the killing fluid is not completely removed, it interferes with the action of the stains, more particularly the basophil stains.

Probably the good results obtained with this reagent are due to the large amount of soluble chloride it contains, which precipitates the proteids in the cells.

Saturated Solution of Mercuric Bichloride in Absolute Alcohol. This reagent was used to kill resting embryos in the dry seeds, where the presence of air in the tissues hinders the penetration of heavy liquids. It produced good preparations, but did not leave the nuclei of the cells as susceptible of staining as Worcester's fluid.

It will be seen from the foregoing accounts that the killing fluids which gave the best results were those containing a large proportion of soluble chloride and a small proportion of acid. Those fluids which contained a large proportion of acids appeared to have a corrosive action upon the proteid granules in the cells. In small amounts the presence of acids appeared to facilitate the penetration of the reagent without corroding the granules.

Most of the material was embedded in paraffin by the ordinary method. In the case of dry seeds I used a method for which I am indebted to Mr. B. J. Howard ('03). By the use of this method I completely infiltrated the pieces of seeds (which were always small) with paraffin, and succeeded in obtaining uniformly good preparations.

The results obtained by the use of different stains were as variable as those of the different killing fluids. No one stain could be depended upon in all cases. The use of stains is twofold—to render the objects more opaque for study, and to give an indication of their acidity and alkalinity. While I did not find it possible to apply the terms 'cyanophil' (basophil) and 'erythrophil' (eosinophil) with precision, yet the absorption of different stains serves in a general way to indicate the nature of cell-contents. The use of the different stains gave the results described below.

Picro-Nigrosin. This was found to be a very satisfactory stain for general cytological purposes. It brings out the granules, nuclei, and cytoplasts very plainly, but does not give them a differential stain.

Kleinenberg's Haematoxylin. The results obtained by the use of this stain were much the same as the preceding. It is a very valuable stain for chromatin, and was used chiefly on that account.

Haidenhain's Iron Alum Haematoxylin. The method described by Torrey ('02), using Congo Red as a counter-stain, was employed. It is a very valuable stain when used in connexion with others, but cannot be

relied upon when used alone. It gives no indication of the acidity or alkalinity of the cell-contents, nor does it differentiate the granules in the cytoplasm from those in the nucleus.

Zimmermann's Fuchsin-Iodine-Green. Repeated attempts with this otherwise valuable stain were not successful in producing a single good preparation. The stain seems to have no affinity for the granules, and not even the protoplasmic structures were stained satisfactorily.

Anilin-Gentian-Violet-Iodine-Eosin. Gram's Method. The sections were not stained deeply enough when this stain was used to afford any satisfactory study.

Mann's Eosin-Toluidin Blue. This proved to be the most valuable stain used, and was employed more extensively than any other in my work. The method described by Huie ('97) was employed in staining the sections. It not only differentiates cell-walls and cell-contents, but differentiates one kind of granules from another in the same cell. The cell-walls were stained blue; starch-grains were stained bluish-green; cytoplasm, blue (red in cells where secretion had progressed for some time); zymogen granules, blue (or purple); chromatin, reddish-purple. The best results were obtained in material which had been fixed by Mann's aqueous picro-corrosive killing fluid.

Eosin and Anilin Blue. This stain was used to good advantage with sections of *Zea*, where a differentiation of starch- and proteid-grains was desired.

Eosin and Gentian Violet. The same methods were employed with this stain as for the two preceding, but it did not give as good results. The cell-contents were not plainly differentiated.

Flemming's Triple Stain. Good preparations were obtained by the use of this stain, but it did not differentiate the different granules in the cell sufficiently to make it a valuable stain. It works best after the use of Chromo-osmo-acetic acid, but does not give good results after the use of fluids containing mercuric bichloride.

III. Observations on the Scutellum of *Zea Mais*.

A cross-section of the scutellum, when examined under the microscope, is seen to consist of large, nearly isodiametric cells, which are bounded on the side next the endosperm by a single layer of columnar epidermal cells. There is not only a noticeable difference in the size and shape of the two kinds of cells, but also in their contents. The granules found in the epidermal cells are always small, and are composed of proteid; the granules in the large cells of the scutellum may be either starch or proteid; and the proteid may be in the form of large or small granules.

A. Studies on Living Material.

Twenty-four hours old. The protoplasmic body of the epidermal cells of the scutellum still shows the characteristics of the resting condition; it does not entirely fill the cell-cavity. The cells contain a large amount of granular substance which is coloured yellowish-brown by iodine. The underlying cells of the scutellum contain two kinds of granules—proteid and starch.

Two days old. The cells of the epidermal layer are still closely packed with fine granules, but the larger proteid granules have disappeared from the underlying layers of cells. The nuclei of the epidermal cells, where observable, present an uneven contour. The scutellar cells in the vicinity of the plumule contain a small amount of starch.

Three days old. This lot of seeds had made rapid growth; when the embryos were removed for study the radicles were 1·5 to 2·5 cm. long, but the condition of the secreting-cells was only slightly more advanced than that described for the second day. The epidermal cells were full of small proteid granules, but the large proteid granules had disappeared from the first three or four layers of hypodermal cells. There was again an evident accumulation of starch about the plumule.

Four days old. The radicles of this lot of seeds only average 1 cm. in length, but the cytological changes in the scutellum are farther advanced than in the last set. The epidermal cells contain proteid granules only in the distal[1] half or third of the cell. A few cells are entirely free from granular material.

Seven days old. Cotyledons 1 cm. long, radicles 2 cm. long. The epidermal cells are nearly free from granules. In those cells where granules are found they are small, and occur only in the distal end of the cell. The scutellar cells have lost all of the large proteid granules, but still contain large numbers of the small granules. In favourable cases the vacuolate protoplasm may be seen. When the small granules of the epidermal cells disappear, the small granules of the scutellar cells, which in their turn have probably resulted from the disintegration of the large granules, also begin to disappear.

The cells of the scutellum show an increasing amount of starch in the region of the plumule. After the application of iodine to the sections the consequent darkening can be seen plainly by the naked eye. The presence of this starch has two possible explanations—it may arise indirectly from the breaking down of proteid matter in the scutellum (cf. Timberlake, '01), or it may be formed by the anhydration of some soluble carbohydrate derived from the endosperm.

[1] In the subsequent descriptions of epidermal cells, the term 'distal' refers to the end of the cell in contact with the endosperm, 'proximal' refers to the opposite end.

Nine days old. Nearly all the epidermal cells are devoid of granular material; the remainder contain granules evenly distributed throughout the cytoplasm. The starch in the vicinity of the plumule is beginning to disappear. The substance of the endosperm is almost completely dissolved and absorbed at this time. Apparently the epidermal cells of the scutellum have ceased to be actively secreting cells and are now principally vegetative in function. A little later the contents of these cells disappear; possibly they are absorbed by the growing plant.

B. Studies of Microtome Sections.

Cells in the Resting Condition. The protoplasm of the epidermal cells is contracted away from the lateral walls and often from the distal wall of the cell. With a low magnification the protoplasm appears as a fine granular substance, homogeneous throughout. In a number of the cells there are irregular vacuoles in the proximal end of the cell. With higher magnification one can distinguish the granules arranged on the protoplasmic network.

There is no regularity as to the position of the nucleus in the cell at this time. It has a slightly irregular, elliptical outline. The nucleus contains fine granular material which renders it darker than the cytoplasm. There is only a small amount of chromatin present, and it occurs in the form of small spheres at the surface of the nucleus. These can be distinguished easily from the other granular material, because the latter appears to be evenly distributed throughout the interior of the nucleus, while the chromatin is at the surface. The faint outline of a nucleolus may be distinguished in favourable sections. (Plate XX, Fig. 1.)

The other cells of the scutellum are so densely filled with granules of proteid matter that none of the protoplasmic structures are visible except the nucleus.

Cells after imbibition with water for three hours. The contents of many of the epidermal cells do not yet completely fill the cell-cavity (Fig. 2). The cytoplasm is full of small, flocculent granules which stain blue with Mann's Eosin-Toluidin Blue. The nucleus contains two kinds of nucleo-proteid matter in the form of granules. Undoubtedly the larger granules are chromatin and the smaller ones may be a reserve product stored in the nucleus; because as the cell-metabolism proceeds these fine granules rapidly disappear from the nucleus. The nucleolus stains red, the chromatin and fine granules, dark purple.

The large isodiametric cells of the scutellum are completely filled with two kinds of granules at this stage, a number of large and small proteid grains which nearly fill the cell and stain red with Mann's Eosin-Toluidin Blue, and larger spherical starch grains which stain blue.

Condition of the scutellum after thirty hours of activity (Fig. 3). The cells of the epidermal layer are noticeably larger than those in the stage last studied, and are completely filled with granular protoplasm, which is slightly denser at the distal end of the cell and stains purple with Mann's Eosin-Toluidin Blue. The granules show some conformity in their arrangement to the strands of the cytoplasmic network. The nuclei are of the sort one finds in cells where active metabolism is going on. Nearly every nucleus is surrounded by a vacuole and lies in the proximal end of the cell. The karyolymph, or nuclear plasm, contains, as in the last stage, two kinds of material. However, there is much less of the fine granular material than in any preceding stage, and the larger bodies of nucleo-proteid, chromatin, have increased in size. It can also be seen that the chromatin is arranged on the linin network. The nucleolus, although distinct, is not prominent. The staining properties of the granules in the cytoplasm indicate that they are of a different character from those in the nucleus; the former stain blue with Mann's Eosin-Toluidin Blue, the latter stain red. Part of the proteid granules have disappeared from the more deeply lying cells of the scutellum. There is a slight increase in the amount of starch in the scutellum, the greatest increase being near the plumule.

Morphology of the cells at the end of two days of activity. Radicles 1 cm. long. There is a further decrease in the amount of the granular substance in the cytoplasm and an increase in the amount of chromatin in the nucleus (Fig. 4). The nucleolus is hardly to be distinguished because it has become smaller and lost most of its ability to absorb stain. The nucleus is usually located in the proximal half of the cell. The contents of the subepidermal cells of the scutellum are beginning to diminish. They are probably being used for the nourishment of the plant or to form enzymes.

Morphology of the cells at the end of the third day of activity. Temperature 25°. On the third day the scutellum appears to be secreting diastase very actively. The epidermal cells contain a fine granular substance which stains bluish-purple and contains embedded in it larger granules, which are of almost the same size as the chromatin granules of the nucleus, but differ from them in staining properties. This granular matter is quite evenly distributed throughout the cell, but is nowhere so dense as in the preceding stages.

The nuclei are at the middle of the cell or in the proximal half. The finely divided granular substance which was present in the nucleus has all disappeared at this stage, but the chromatin of the nucleus continues to increase. It exists in the shape of spherical and rod-like masses at the surface of the nucleus (Fig. 5). I have not seen any cases where I thought that solid particles were passing from the nucleus into the

cytoplasm. On the contrary I think there is evidence that the nucleoproteids leave the nucleus in a fluid condition; because the nuclei at this stage have a swollen and distorted appearance (Fig. 6). In a few cases a vacuole surrounds the nucleus. A nucleolus is present in nearly every case.

Apparently some of the contents of the subepidermal cells are used in the formation of diastase, because those cells in the vicinity of the epidermal layer are showing more signs of depletion than any others; in fact those in the vicinity of the young plant show little sign of depletion. The content of starch has increased over any previous stage.

Morphology of the cells at the end of the fifth day of activity. At this time there is a still greater depletion of the granular substance in the cytoplasm of the epidermal cells, which causes it to appear lighter coloured in all instances. The greatest scarcity of granules is at the proximal end of the cells. The nuclei are large and contain a large amount of chromatin in the form of spherical masses at the surface of the nucleus. They show a difference from the last stage in being found no longer in the proximal half of the cell, but in the distal half, a short distance above the centre. The nucleolus has diminished in size until it is no longer visible. Apparently there is some relation existing between nucleolus and chromatin, because as one increases the other decreases. Some later experiments give more light on this subject. The same condition was found in *Drosera* by Rosenberg ('99), although there were numerous exceptions.

The morphology of the cells on the ninth day of activity (Fig. 7). The condition of the cells in the epidermis is quite similar to that described in the last stage, except it is very evident that increasingly greater quantities of proteid grains are disappearing. The nuclei are quite similar to those last described, both in appearance and position. The proteid granules which remain are most abundant in the distal end of the cell.

The morphology of the cells after thirteen days of activity (Fig. 8). The endosperm of the seeds from which this material was taken was nearly exhausted. The cytoplasm of the scutellar epidermal cells is compact and fills nearly the entire cell-cavity. It contains a small number of flocculent granules of a different sort from those appearing in the cells when enzymes are being actively secreted. The nuclei are situated near the centre of the cell. The nucleo-plasm stains but slightly different from the cytoplasm, making it difficult to determine the exact boundary between the two. Large nucleoli and a small amount of chromatin are the only substances which can be distinguished in the nuclei.

This condition of affairs suggests that the cells have ceased their active metabolism and are at this time in a passive state, and that perhaps even at this time a few have been partly absorbed by the growing plant. From this time on there is probably very little enzyme produced.

Taking a general survey of the morphological changes, we see that

at first the cytoplasm of the secreting-cells contains fine, granular proteid material, which shows an affinity for basic stains. The nuclei of these cells contain finer granules, which stain differently from those in the cytoplasm and disappear much sooner. With the high power they can easily be distinguished from the chromatin. The amount of chromatin is small in the earliest stages, but the nucleoli are quite prominent.

At the beginning of the second day of activity the cytoplasm is densely filled with granular material, which is distributed on the cytoplasmic reticulations. A comparison of Figs. 2 and 3 will show the relative increase in size of the cell during the first day's activity. From this time forward there is a constant depletion of the granules of the cytoplasm, accompanied by a slow elongation of the epidermal cells. The nuclei are almost invariably nearer the proximal end of the cell. After two days of activity, one can notice that part of the starch of the endosperm has disappeared. In the nuclei of the epidermal cells there are two noticeable changes—a continued increase in the amount of chromatin and a decrease in the size of the nucleolus, which from this time forward is very inconspicuous.

The processes described above continue without much variation until the endosperm is exhausted. On the third or fourth day the nuclei move from their position in the proximal end of the cell toward the centre, and on the fifth or sixth day are found in the distal end of the cell (Fig. 7). The cytoplasm is nearly free from granules on the tenth day, and now shows an affinity for acid stains. Just before the final dissolution occurs, the cytoplasm of the epidermal cells becomes abundant and stains similarly to the nucleus.

C. The Effect of Inhibited Growth upon the Secreting-Cells.

Seedlings which had grown at a temperature of 23° C. for fifty hours, and whose roots had attained a length of 3 to 4 cm., were transferred to a temperature of 8–10° C., after having removed and fixed a number of scutella. When they had remained twenty-four hours at the low temperature, the amount of growth shown by the roots was very small. At this time more material was removed and fixed in different killing fluids; the remaining seedlings were then placed at a temperature of 20° C. for forty hours longer. At the expiration of this time the roots showed that growth was progressing normally again. The different lots of material were then sectioned and stained for microscopical study.

The sections showed that the cells of the epidermal layer were in an active condition when transferred to the cold room. They were full of fine granules, which stained blue with Mann's Eosin-Toluidin Blue. The densest accumulation of granular material was in the distal end of the cells. The nuclei, which were in the proximal end of the cell, contained chromatin in

the form of fine granules. The nucleoli were spherical, well-defined bodies which stained dark purple.

After the growth had been checked by low temperature for twenty-four hours there was a marked change in the appearance of the cells. The cytoplasm contained large lumps which stain dark purple. The staining property of these aggregations was more intense than that occurring in ordinary secretion. The nuclei had lost most of their chromatic substance and appeared quite clear. They contained at least one large nucleolus which had lost neither in size nor staining qualities.

When normal conditions were restored, the cells appeared to regain activity. The fixed and stained material showed two things very clearly: (1) the aggregations had almost entirely disappeared from the cytoplasm, leaving it homogeneous purple ; and (2) the nuclei contained numerous large granules of chromatin, but the nucleoli had disappeared. It hardly seems possible that any of the nucleo-proteids are absorbed by the nucleus when activity is resumed.

The fact that the nucleus contains granules only during resting periods, or periods of arrested growth, and is large, hyaline, and intimately connected with the cytoplasm during the time of most active secretion, indicates to my mind that the nucleus is not a storehouse of diastase in any form, but is the source of energy by which the diastase is produced. The view here taken is that the diastase is manufactured from other proteids and turned into secretion by the activity of the epidermal cells, in which processes the nuclei probably perform the largest part of the work.

My own work fails to confirm many of the observations made by Torrey ('02). I did not find, at the beginning of secretion, the nuclei filled with dark staining granules. Nor did I find the granules being extruded in a solid state into the cytoplasm through breaks in the nuclear membrane. There were, it is true, in the resting stage, fine granules in the nucleus ; but they disappeared during the first two days and did not reappear. By far the greater amount of proteid granules was found in the cytoplasm of the epidermal and subepidermal cells before secretion began. Moreover, the granules in the cytoplasm differed both in size and staining qualities from those in the nuclei, and gradually disappeared as secretory activity progressed.

I likewise failed to find that the process of secretion in *Zea* is an intermittent one, in which periods of activity alternate with periods of rest. If the seedlings are grown at a constant temperature, the process of secretion is continuous while enzymes are being produced. In repeating Torrey's methods I found that the use of Iron Haematoxylin as a stain was the cause of many differences in our observations, because it is not reliable in differentiating the various cell-constituents. As his own paper states, it stains everything alike in a very deceptive manner, and is the cause of

many *artefacta* in the sections. When part of the material was stained with Mann's Eosin-Toluidin Blue and part with Iron Haematoxylin, the resulting sections gave very different appearances. The preparations made with the former stain were relied upon because of their similarity to the sections of living cells.

IV. Observations on the Absorbing Organ of *Phoenix dactylifera.*

The absorbing organ is a button-shaped structure which is located in the date-seed on the side opposite the furrow. By means of the enzyme it produces, it dissolves the hard, ivory-like endosperm of the seed, and absorbs the soluble material for the use of the young plant. At first the absorbing organ is about the size of the head of a pin, but as germination progresses it enlarges and finally fills all the space formerly occupied by the endosperm. If we make a longitudinal section of this organ after the radicle has begun to protrude, it will be found to have a mushroom shape; under the microscope it is seen to be composed of thin-walled parenchyma-cells with large intercellular spaces. In the radicle the cells are elongated, but they approach a spherical shape in the head of the absorbing organ. Near the margin of such a longitudinal section the cells are smaller and have contents different from the other cells. The entire surface of the head of the organ is covered by a layer of short, columnar cells.

A. Studies of Living Material.

Cells in the resting seed. The cells of the epidermal layer contain, in addition to the large spherical nuclei, fine hyaline granules in the cytoplasm. The other cells of the absorbing organ contain similar small granules and, in addition, numerous larger granules, all of which give the test for proteid with re-agents. The epidermal cells do not contain as much granular material as the other cells of the absorbing organ.

Observations upon seedlings five days old. At the end of this time the absorbing organ has increased to nearly twice its original size. The epidermal cells contain approximately the same amount of proteid matter as before, but in the form of larger granules. The nuclei of these cells are large and distinct, and each is at the centre of the cell.

Observations upon seedlings twelve days old. The radicles have not yet appeared outside of the seed. Except for continued enlargement, the sections of the absorbing organs are much the same as in the previous stage. The epidermal cells contain more proteid in the form of fine granules, but the nuclei are unchanged.

Observations upon seedlings twenty-two days old. The radicles of the seedlings average 2·5 cm. in length. The epidermal cells of the absorbing organ

have lost most of the granules which they contained in the previous stage. Those which remain are small and quite evenly distributed in the parietal layer of cytoplasm. The nucleus is smaller than in previous cases. It is situated near the centre of the cell, and connected to the lateral walls of cytoplasm by radiating strands. The deeper-lying layers of cells are nearly empty of granular material at this time. The elements of a fibro-vascular system are beginning to appear among the hypodermal cells.

Observations upon seedlings twenty-nine days old. The parts of the embryos outside the seed average 4 cm. in length. The cells and cell-contents are much the same as in the last stage, except that they are more depleted of granular material. The granules which yet remain are nearly all confined to the epidermal and first hypodermal layers.

Observations upon seedlings thirty-three days old. The scanty granular material which yet remains in the cells of the absorbing organ exists in the form of large granules. Each epidermal cell contains a large vacuole surrounded by a thin layer of cytoplasm. The nuclei of these cells are smaller than in previous stages.

Observations upon seedlings fifty days old. The cells of the absorbing organ, both epidermal and hypodermal, are empty of granules, so far as can be ascertained by staining with iodine or methylene blue. The cytoplasmic body and nucleus retain the same size and relative position as in the previous stage.

B. Studies of Microtome Sections.

The morphology of cells in seedlings six days old (Fig. 9). There are very few changes from the condition which has been described for the resting stage in the living material. The epidermal cells of the absorbing organ show no elongation as yet. The cytoplasm contains a large amount of fine granular material which may represent the zymogen, because it disappears as enzymes are formed. The spherical nuclei, whose average diameter is about two-thirds the width of the cell, are usually situated near the centre of the cell. When stained with Kleinenberg's Haematoxylin, the chromatin is demonstrated as very small grains on the nodes of the linin network at the surface of the nucleus. The karyolymph does not contain granular matter as in the case of *Zea*. A small nucleolus is present in each nucleus.

The morphology of cells in seedlings nine days old. The cells show certain well-marked changes from the conditions described in the preceding stage. The zymogen granules in the cytoplasm have not only increased in size, but in their affinity for stain. All the cells of the embryo are filled with proteid granules which stain more cyanophil than at any subsequent time. The densest accumulation of granules is in the hypodermal layers of cells. There is also a larger amount of chromatin present in the nucleus, the difference being due to an increase in the size of the

granules already present rather than to an increase in their number. The nucleoli show a slight increase in size.

The morphology of cells in embryos fourteen days old (Fig. 10). With the exception of a few layers of marginal cells, the granular contents of the absorbing organ have disappeared, and from this time forward there is no indication of metabolic activity in any except the surface-layers of cells. The epidermal cells are not only increasing in size, but numerous examples of karyokinetic division indicate that they are increasing in number. The staining reactions indicate that they are not so cyanophil as in the stage last described. The cytoplasm is beginning to show a diminution in the amount of granular material present. The finer granules seem to be the first to disappear. After their number has been diminished, it can be seen that the granules lie on the cytoplasmic network, not in its meshes.

The nuclear chromatin does not increase as fast with increased activity as in the case of *Zea*, yet there is an increase. The nucleolus, instead of diminishing, has up to this time retained its original size, and shows a strong affinity for stain.

The morphology of cells in seedlings eighteen days old (Fig. 11). The cells are much the same, except for continued elongation, as in the preceding stage. Their staining qualities indicate that they are becoming more erythrophil.

The morphology of cells in seedlings twenty-six days old (Fig. 12). At this stage the cells of the absorbing organ are practically free from granular material. There are a few erythrophil granules in the marginal cells. The deeper-lying cells which originally contained proteid material have enlarged to several times their former size, and the protoplasm remaining forms a thin parietal layer. The elements of a vascular system have begun to make their appearance among the marginal cells of the absorbing organ.

There are three morphological differences between the nuclei of this and preceding stages—(1) they are smaller in volume; (2) the nucleoli are also smaller and often surrounded by a vacuole; (3) there is an increased amount of chromatin in the nucleus, which occurs in the form of small grains on the linin network at the surface of the nucleus.

The morphology of cells in seedlings thirty-three days old. At this age the absorbing organs are white elliptical disks about 8 or 10 mm. diameter and 3 mm. thick. The epidermal cells have lost much of the cytoplasm which they previously contained, many of them containing only a parietal layer. The most noticeable change is in the number of granules present in the cytoplasm (Fig. 13). It may be that part of the protoplasm has broken down to form an enzyme. The nuclei are not much smaller, but the nucleoli are diminished in size. The amount of chromatin is less also.

The morphology of cells in seedlings four months old. The seedlings from which the material for this study were obtained were grown in earth

in the plant-house. At the end of four months I found that the reserve cellulose of the seed was entirely consumed, and that the absorbing organ filled all the space occupied by the cellulose. The amount of cytoplasm in the cells is very small; in none is there more than a thin parietal layer, and in many the nuclei have broken down and been absorbed. In the epidermal cells the amount of cytoplasm is very small. Strands radiate from the nucleus to different parts of the cell. The layer of cytoplasm on the distal wall is usually thicker than on the other walls. In some cells the cytoplasm has begun to break down into a disorganized mass of granules.

The nuclei, situated in various parts of the cell, have a smooth hyaline appearance and are devoid of chromatin. They still have a small, distinct nucleolus, which is about the same size as when activity began. The whole condition of affairs suggests that the cells no longer possess the function of actively secreting enzymes, but are now breaking down and being absorbed by the growing plant. The cells in the centre of the absorbing organ were the first to disappear, but the process of dissolution goes on until finally the epidermal cells are reached.

Taking a general view of the changes in the secreting-cells in *Phoenix*, we first find them short and thick, containing large spherical nuclei and densely granular cytoplasm which is distinctly basophil. During the first five or six days the cells increase in size, due to the absorption of water, but the contents show scarcely any change in composition. When secretion begins, the nuclei contain small granules of chromatin and small nucleoli. As secretion progresses they increase in size slowly until near the end of the third week; then the nucleoli begin to diminish, followed a little later by the chromatin granules. The cells and their contents are strongly erythrophil at this stage, and the proteid granules have nearly disappeared from the cytoplasm. The cytoplasm itself begins to disappear at the end of the fourth week, and finally the cells contain only a disorganized mass of substance.

It is quite evident that if my observations have been correct there are some differences between the secreting cells of *Zea* and those of *Phoenix*. The nuclei of the epidermal cells in *Zea* contain, at the beginning of activity, varying amounts of granular substance which soon disappear, leaving only the chromatin and the nucleoli. The nuclei of similar cells in *Phoenix* show no such substance.

The position of the nucleus in the cells of the epidermal layer is different in the two cases. In *Phoenix* it is almost always found at the centre of the cell; in *Zea* it moves to the distal end of the cell as the activity of secretion progresses.

It may be that the behaviour of the nucleus in the latter instance is in accord with the views of Haberlandt ('87), Townsend ('97), Harper ('99), and others, that the nucleus is usually situated in that part of the cell where the most active metabolism occurs. But nothing definite can be stated

until more plants have been examined. The behaviour of the nuclei in *Phoenix* appears to contradict any assumption which could be made concerning *Zea*.

The nuclei in the two plants increase in size for a time as germination progresses. In *Phoenix* this increase ceases when the embryo is but a few centimetres long, and the nucleus then appears to become smaller, though it is probable that enzyme-formation is greater after the nucleus begins to diminish. The nuclei may increase in size merely because the cells increase in size.

There are also differences in the behaviour of chromatin and nucleoli in the nuclei of the two plants. In *Zea* the nucleoli are large when the activity of secretion begins, and diminish in size as it progresses until they are no longer visible. The quantity of chromatin, on the other hand, increases as the nucleoli diminish. In *Phoenix* the nucleoli are present in all stages of secretory activity, attaining their maximum size about the time the cytoplasmic granules begin to disappear. The chromatin, scanty at all times in comparison with *Zea*, shows slight changes in quantity analogous to those occurring in *Zea*.

In the secreting cells of *Zea* the greatest apparent activity was reached when the nucleoli had disappeared and the chromatin had greatly increased. When the activity ceased, there was a formation of large nucleoli simultaneous with a disappearance of chromatin (Fig. 8). When the activity of secreting cells was checked by means of low temperature, it was found that the chromatin disappeared and large nucleoli were formed.

If these facts be interpreted to mean that there is some kind of relationship existing between the chromatin and nucleolus, such as has been postulated by Rosen ('95), Dixon ('99), van Wisselingh ('00), Gardner ('01), and others, then the difference between chromatin and nucleolus appears to be one of degree rather than of kind. The observations would seem to indicate that the material in the form of nucleoli was in a less active state than when in the form of chromatin, and as the cell-metabolism increased the latent substances became active.

Our ideas of the relations of the nucleolus to the other constituents of the cell are, as yet, entirely hypothetical. There are opportunities for studying the nucleolus of secreting-cells which seem capable of yielding better results than those hitherto obtained during the process of mitosis, because the former are performing their normal metabolism instead of being interrupted by the process of division.

The fact that these cells are both absorbing and secreting organs gives added importance to a point raised by Rosenberg ('99) concerning the causes for an increase of chromatin during secretory activity. He thought it might follow either as the result of the formation of an enzyme or as the result of absorbing abundant nutrition. The ease with which the scutellum of *Zea* may be separated from the endosperm suggests an experiment (which,

unfortunately, has not been performed) for determining which of the two hypotheses (if either) is correct.

Concerning the nature of the various granules in the cells little can be said with any degree of certainty. They appear to arise as products of cellular activity, yet Müller ('96) believes that they are the elementary organs of the cell and are capable of growth and division. It is hoped that a wider application of the methods of physiological chemistry will supplement the results thus far obtained by the methods of histology.

The extrusion of solid substance from the nucleus has been reported by many observers, but I have not been able to find any indications of it in my study. In every case it appeared as though the exchange of substances was accomplished when they were in a liquid state. It must be borne in mind that the soluble proteids would be precipitated by the reagents used in killing and dehydrating, and therefore granules in the prepared sections are not necessarily indicative of their presence in the living cell.

Throughout the progress of the work I have been impressed by the similarities between enzymes and protoplasm in their manner of origin, action, and ultimate dissolution. Many of the similarities have been pointed out by Bokorny ('00). The two substances react similarly to most stimuli, except that enzymes show a greater resistance to destructive agents such as temperature, light, chemicals, &c. The decomposition of certain substances, e. g. sugar, by enzymes and protoplasm is very similar.

The passage of two currents through the epidermal cells, one toward the endosperm and one toward the seedling, is undoubtedly brought about by osmosis modified by the selective action of the protoplasmic membrane. The existence of starch in the scutellum of *Zea Mais* shows that very little diastase passes toward the young plant.

V. Summary.

1. The results obtained in fixed and stained material are dependent to some extent upon the *technique*.

2. In the resting condition the secreting-cells of both *Zea* and *Phoenix* are crowded with relatively small proteid granules. As secretion begins these granules gradually disappear. In *Zea* this disappearance coincides closely with the consumption of the endosperm ; in *Phoenix*, however, the granules disappear long before the endosperm is dissolved.

3. The chromatin of the nuclei is small in amount at the beginning of secretion and increases as germination progresses. The nucleolus diminishes in size as germination progresses. These changes are more noticeable in the case of *Zea* than in *Phoenix*.

4. There is no evidence that solid matter is extruded from the nucleus.

5. At the close of secretory activity the protoplasm of the secreting-cells breaks down and the products of disintegration disappear from sight.

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An asterisk denotes that I have not examined the original paper.

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EXPLANATION OF FIGURES IN PLATE XX.

Illustrating Mr. Reed's paper on Enzyme-secreting Cells.

All figures were drawn with Abbé's camera lucida, Zeiss 1/16 oil immersion objective, and compensating oculars 8, 12, and 18. Figs. 1–8 were drawn from the epidermal cells of the scutellum of *Zea Mais*. Figs. 9–13 were drawn from the epidermal cells of the absorbing organ of *Phoenix dactylifera*.

Fig. 1. Nucleus in the resting condition. Saturated solution of mercuric bichloride. Kleinenberg's Haematoxylin. × 1550.

Fig. 2. Cell after absorbing water for three hours. Mann's Picro-corrosive killing fluid. Mann's Eosin-Toluidin Blue. × 1550.

Fig. 3. Cell after thirty hours' activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1550.

Fig. 4. Nucleus after forty-eight hours' activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1550.

Fig. 5. An unusually swollen nucleus after three days of activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1550.

Fig. 6. Normal nucleus after three days of activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1550.

Fig. 7. Cell after eight and one-half days of activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1600.

Fig. 8. Cell after thirteen days of activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1600.

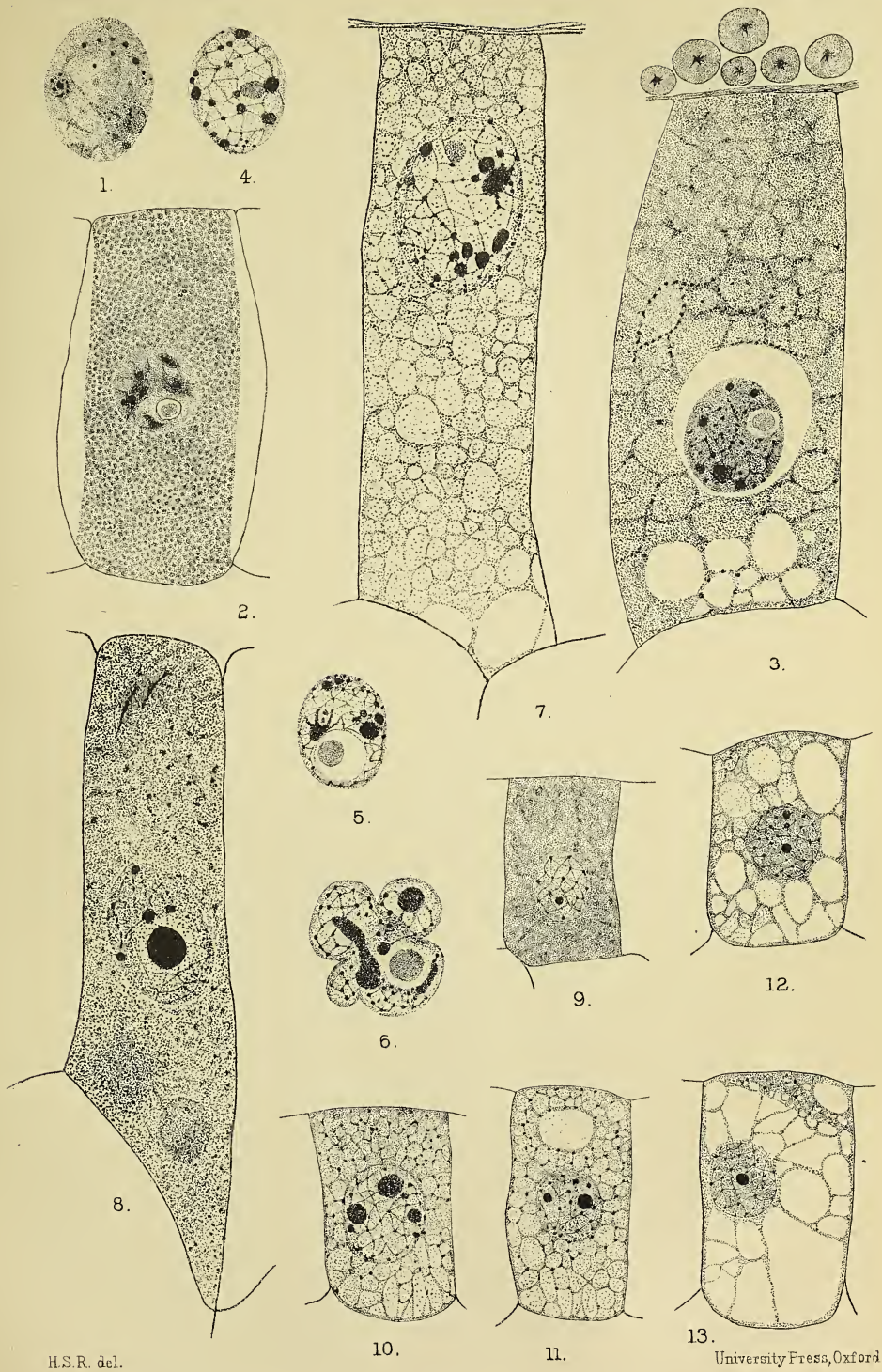
Fig. 9. Cell after six days of activity. Worcester's killing fluid. Kleinenberg's Haematoxylin. × 1550.

Fig. 10. Cell after fourteen days of activity. Worcester's killing fluid. Kleinenberg's Haematoxylin. × 1550.

Fig. 11. Cell after eighteen days of activity. Worcester's killing fluid. Mann's Eosin-Toluidin Blue. × 1550.

Fig. 12. Cell after twenty-six days of activity. Mann's Picro-corrosive fluid. Kleinenberg's Haematoxylin. × 1550.

Fig. 13. Cell after thirty-three days of activity. Worcester's killing fluid. Flemming's triple stain. × 1550.



H.S.R. del.

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REED.—ENZYME-SECRETING CELLS.

The Proteases of Plants.

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SINCE the publication in these pages (June, 1903) of my last paper (1) on this subject, I have been continuing my investigations, and I have also come across several important papers by other observers, so that a considerable amount of further information has accumulated of which some account may now well be given.

Before discussing the new facts, I propose to indicate very briefly the desirability of somewhat modifying the current method of describing the phenomena of proteid-digestion, and to suggest a terminology more in harmony with the present state of knowledge. Hitherto the proteases of both plants and animals have been classified as 'peptic' or as 'tryptic,' in accordance with their general resemblance to either the pepsin or the trypsin of the animal body; and a digestion has been described as 'peptic' when it went no further than the conversion of the higher proteids into albumoses and peptones, and as 'tryptic' when the peptones formed were decomposed into non-proteid bodies such as leucin, tyrosin, &c. But with the discovery of erepsin by Cohnheim, this simple classification of the proteases has become inadequate, for erepsin is neither 'peptic' nor 'tryptic.' Of the two, it is more nearly allied with trypsin than with pepsin, inasmuch as it actively decomposes peptones: but it differs widely from trypsin in that it cannot peptonize the higher proteids, such as albumin and fibrin. It is, in fact, a representative of a new, third, class of proteases, which may be described as 'ereptic.'

Now as to the terms employed in describing the digestive process. The word 'proteolysis' is in common use, but not always in the same sense: it is sometimes applied to peptonization by pepsin: at other times, and more accurately, it is applied to the disruption of the proteid-molecule into non-proteid substances, and it is in this sense that I have used it in my more recent papers. But the most appropriate use of the word is its application to the sum-total of the processes involved in proteid-digestion, to all the changes determining the conversion of the higher proteids into such substances as leucin, tyrosin, &c. Accepting this connotation of

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'proteolysis,' the successive stages of the process may, I would suggest, be conveniently distinguished as—(*a*) *peptonization*, the conversion of the higher proteids into albumoses and peptones; and (*b*) *peptolysis*, the decomposition of peptones into nitrogenous but non-proteid substances.

This terminology offers the prospective advantage of simplifying the classification of the proteases. But before attempting this, it is necessary to draw attention to a recent paper by Vernon (2) in which he announces the important discovery that the peptolytic activity hitherto attributed to trypsin is largely due to an ereptic enzyme associated with it. This enzyme, which may be distinguished as pancreato-erepsin, is not identical with the entero-erepsin found by Cohnheim in the small intestine, though it belongs to the same group, other members of which will no doubt be discovered in due time. The effect of this discovery is somewhat to alter the position of trypsin proper—that is, trypsin free from pancreato-erepsin—in a classification of the proteases, bringing its peptonizing activity into relatively greater prominence. Taking this into account, and neglecting the somewhat conflicting views as to the possible peptolytic activity of pepsin—which may, after all, be due to an associated erepsin hitherto undiscovered—the proteases of the animal body may be classified as follows :—

A. Actively peptonizing, but not at all peptolytic: pepsin.

B. Actively peptonizing and peptolytic : trypsin.

C. Feebly peptonizing, actively peptolytic : erepsins.

There is a question bearing upon the relation between trypsin and erepsin that requires special consideration. Trypsin, it is well known, forms tryptophane as one of the products of its peptolytic activity: but does erepsin produce this substance? It is not inconceivable that a peptolytic enzyme might produce leucin and tyrosin without, however, forming tryptophane; and if this were found to be true of any form of erepsin, it would afford a clear distinction between tryptic and ereptic proteases. It is unfortunate that, so far as I have been able to ascertain, the available information on this important point is not altogether conclusive. Cohnheim's account of the products of digestion by entero-erepsin conveys the impression that tryptophane was not among them: but it does not appear that the presence or absence of this substance was made the subject of special investigation. On the other hand, Dr. Vernon informs me by letter that he has detected tryptophane in a digestion of peptone by entero-erepsin. For the present, at any rate, I accept the positive rather than the negative evidence, adopting the view that tryptophane is a product of peptolysis by erepsin as well as by trypsin.

I have not included the proteases of plants in this survey, as I propose to discuss their nature in the concluding section of this paper.

In dealing with the papers on proteolysis in plants, to which I have alluded, I will take first those relating to cases that I have not myself

examined. There is, to begin with, an elaborate investigation by Butkewitsch (3) into the digestive action of certain of the lower Fungi (*Aspergillus niger*, *Penicillium glaucum*, and species of *Mucor*, *M. stolonifer*, *M. racemosus*, *M. Mucedo*) upon proteids. The Fungi in question were cultivated, in previously sterilized vessels, on a substratum consisting of proteid matter (Witte-peptone or fibrin) either with or without cane-sugar, together with a small proportion of suitable mineral ingredients acidified with phosphoric acid. The duration of an experiment varied from five days to over a month. The results show that these Fungi can peptolyse Witte-peptone, with formation of leucin and tyrosin, and can proteolyse fibrin, thus confirming the observations of earlier observers such as Malfitano (4) and others. A remarkable feature of the proteolysis effected by *Aspergillus* was the formation of a large proportion of ammonia (NH_3), though it was much smaller in the presence than in the absence of cane-sugar in the culture.

There is, further, a laborious research by Weis (5) into the nature of the proteolytic enzymes of malt. The author recognizes that in the germination of barley both peptonization and peptolysis take place—or as he puts it, there is a 'phase pepsique' and a 'phase trypsique'—whence he infers the presence of two distinct proteases which he respectively terms peptase and tryptase. The peptic action is apparently rapid, and soon comes to an end, whilst the tryptic action is slower and continues until the complete decomposition of the products of the peptic stage. The tryptic action was found to be only slight, at most, in a neutral liquid; rapid in the presence of a small quantity of added acid (e.g. lactic acid 0·2%; HCl 0·04%), and much retarded by the addition of alkali. The author is of opinion that the effect of acid and alkali upon the activity of proteolysis is to be explained, in accordance with the views of Fernbach and Hubert (Comptes rendus, t. 131, 1900, p. 293), who regard the primary (acid) and secondary (basic) phosphates present in the malt-extract as determining the course of proteolysis, the former promoting, the latter retarding it.

The author found both the peptic and the tryptic activity of malt to be interfered with by certain antiseptics, such as thymol, chloroform, formol, whilst toluol had but a slight effect. In the paper already referred to (1) I also have drawn attention to the influence of antiseptics on proteolysis in the special case of papaïn.

It was further ascertained that the proteases of malt-extract could digest various vegetable proteids other than the glutin of wheat; such as its own proteids, proteids of ungerminated barley, of rye, and of oats, legumin, vegetable casein; as also, among animal proteids, the fibrin of ox-blood, whilst the action on egg-albumin was slight.

Yeast (*Saccharomyces Cerevisiae*).

In a previous paper (6) I expressed the opinion, as the result of a few experiments, that yeast contains a proteolytic enzyme which is active in neutral and in acid liquids but not in alkaline. At that time I had not seen the paper in which Hahn and Geret (7) have given a full account of their very thorough investigation of this subject: their results are of such interest that a brief *résumé* of them will not be out of place. They worked with the expressed juice of fresh yeast, a liquid that contains a considerable amount of proteid coagulable on boiling, and is also spontaneously coagulable on being kept in the incubator at 37°C. for two hours. They ascertained that this liquid digested fibrin within twenty-four hours; but their investigation was directed more especially to the self-digestion (autolysis) of the liquid. Their method of estimating digestive activity was the comparison of the weights of the coagulum obtained from a given quantity of juice before and after digestion. For instance, in one case the weight of coagulum obtained before digestion was taken as 100, the weight after autolysis for twenty hours was 9·1. By this means they ascertained (*a*) that the natural acid juice digests actively; (*b*) that its activity is diminished, though not to any great extent, by such antiseptics as chloroform, thymol, toluol, salicylic acid, and hydrocyanic acid (HCN); (*c*) that it is increased by the presence of neutral salts, such as NaCl 3%, KNO_3 1%, KNO_3 10%; (*d*) that it is increased by the addition of HCl from 0·05% up to 0·3%, 0·2% HCl being the optimum, and that it is diminished in the presence of 0·5% HCl, and almost destroyed by HCl 1%; (*e*) that the activity is diminished by neutralization, and still more so by alkalinity of 0·2–0·5% NaHO. The inferences that they draw as to the nature of the proteolytic enzyme will be discussed in the concluding section of this paper.

Since the publication of the paper by Hahn and Geret, the only other contribution to the study of yeast-proteolysis is, so far as I am aware, that of Bokorny (8). He investigated the action of dried yeast, used in the solid form, upon various proteids either of animal or of vegetable origin: his experiments were made exclusively with liquids containing from 0·2–2% of added acid, chiefly phosphoric, without, apparently, any antiseptic, their duration varying from three hours to three days. The measure of digestive activity was the amount of the precipitate obtained on treating the concentrated digestion-liquid with excess of alcohol: the nature and relative quantity of the products was determined by dissolving the alcohol-precipitate in water, when any albumose present could be precipitated by saturation with ammonia sulphate or zinc sulphate, and any peptone by precipitation with phosphotungstic acid from the filtrate obtained after the separation of the albumoses.

The main conclusion at which Bokorny arrives is that the acid reaction is essential to the digestive activity of yeast, and that the degree of acidity has an important influence upon the character of the digestive process as indicated by the products: thus, when the acidity is less than 0·5 %, only a little albumose is formed, but a relatively large quantity of substances that are not precipitated by zinc sulphate or ammonia sulphate.

It can hardly be said that the paper adds material facts to existing knowledge of digestion by yeast, nor can the conclusion as to the relation between acidity and proteolysis be regarded as convincing. In the first place, the objection may be raised that no antiseptic was used; and though it may be urged that in many experiments the amount of acid present (0·5–1 %) was sufficient to prevent bacterial action, yet in those cases where the acidity was less strong, and the digestion prolonged (24–48 hours), the possibility of such action is obvious: in one case, indeed (Expt. 1), an offensive odour was noted and the development of mould. In the second place, no account is taken, in estimating the digestive products, of the proteids contained in the yeast itself. I have found that a watery extract of dried yeast, after boiling, filtering, and concentrating, yielded a mainly proteid precipitate with alcohol amounting to something like 20 % of the original weight (see p. 298): hence, when it is borne in mind that in Bokorny's experiments the weight of yeast employed amounted to 10, 20, or even 40 % of the proteid supplied for digestion, it is clear that the omission to take the yeast itself into account is a serious one. Finally, it is doubtful if any material amount of proteolysis was effected when the proportion of added acid was 0·5 % or more: for, as Hahn and Geret have pointed out, and as I have myself found, the digestive activity of yeast rapidly diminishes with increasing proportions of added acid (see p. 302).

I give now a selection of my experiments to illustrate the digestive activity of yeast under various conditions. I have employed fresh brewers' yeast, also yeast that I myself preserved in the dry state, but chiefly the dried yeast that is now obtainable as an article of commerce (prepared by the Granular Yeast Company Limited, London, E.C.), which is convenient to use, with the great advantage that it is possible to make a number of experiments with uniform material. The experiments include observations on self-digestion (autolysis), on the peptolysis of Witte-peptone, and on the proteolysis of fibrin. The test applied in the autolytic and peptolytic experiments was that for tryptophane, the presence of this substance being taken as evidence of peptolysis. When the experiments were comparative, the test had to be applied with certain precautions. Thus, in each set of observations, it was necessary to ascertain in some one case what quantity of chlorine-water had to be added to a given quantity of the digested yeast-liquid in order to produce a tryptophane-reaction

of maximum intensity; thus a standard of comparison for the other experiments in the same set was obtained. The quantity of chlorine-water required varies considerably, in relation, apparently, with the amount of tryptophane present: since an excess of chlorine destroys the reaction, it may be concluded that the more chlorine-water required, the greater the amount of tryptophane present. For instance, in an autolysis of a watery liquid containing 5 % dry yeast, I found that the addition of an equal volume of chlorine-water (say 5 cc. of each) gave the maximum tryptophane reaction, when the digestion had been short (say 4–6 hours): but when the digestion had been more prolonged (say 24 hours), it required twice the volume of chlorine-water to obtain a reaction as intense as that given as the result of the shorter digestion. It is also necessary to allow the tested liquid to stand for several minutes before estimating the intensity of the reaction, for it takes an appreciable time to develop. The liquid to be tested must, of course, have an acid reaction.

In the experiments with fibrin, the main object was the determination of peptonizing activity: accordingly the crucial test was the total disappearance of the fibrin, which was consequently supplied in small quantity (usually about 0·5 grm. to 100 cc. of liquid). The fibrin had been preserved in 50 % glycerin.

The dried yeast, previously to an experiment, was ground to a fine powder in a hand-mill, and was then thoroughly triturated in a mortar with the water necessary to prepare the required digestive liquid. The resulting mixture was then either used as it was, or it was filtered at a low temperature so as to prevent autolysis during the somewhat lengthy process, and the clear filtrate was employed. Toluol, to about 1 %, was found to be the most unobjectionable antiseptic, though I obtained good results with others, such as chloroform, sodium fluoride, and hydrocyanic acid. It is important to state definitely that in no case did the freshly-prepared yeast-liquid, whether mixture or extract, give any tryptophane-reaction, thus proving that the yeast used contained no tryptophane to begin with.

Autolysis.

The fact that, under certain circumstances, the yeast can digest its own proteids is a familiar one. My object in experimenting upon it was to ascertain something more definite as to the conditions determining the activity of autolysis, and as to the nature of the enzyme by which it is effected. The following experiments will give an idea of the method adopted and of the results attained.

Experiment 1. 1 grm. dried yeast placed in each of 3 bottles with 40 cc. distilled water: to No. 1 nothing further was added; to No. 2 was added 0·5 grm. precipitated chalk, to neutralize any free acid present; to No. 3, 0·1 grm. citric acid (= 0·25 %).

After about 20 hours in the incubator (temp. 38–40° C.) the tryptophane-reactions were: No. 1, marked; No. 2, strong; No. 3, faint.

A repetition of the experiment, using chloroform-water as the liquid, gave the same results.

A somewhat similar experiment was made with the object of ascertaining if so prolonged a period of digestion were necessary for autolysis.

Experiment 2. 40 cc. of an intimate mixture of 20 grms. ground yeast with 200 cc. of distilled water were placed in each of 5 bottles, to each of which toluol (1%) was added; the contents of the bottles were then varied as follows:—No. 1, nothing further added; No. 2, HCl to 0·05 %; No. 3, HCl to 0·1 %; No. 4, HCl to 0·2 %; No. 5, Na_2CO_3 to 0·5 %.

After $2\frac{1}{2}$ hours in the incubator the tryptophane-reactions, on treating 5 cc. of the liquids with equal vol. of chlorine-water, were:—No. 1, very strong; No. 2, distinct; No. 3, marked; No. 4, distinct; No. 5, which was distinctly alkaline, strong: further addition of chlorine-water did not intensify the reaction in any case.

The following morning, after 20 hours more in the incubator, the tryptophane-reaction of Nos. 2 and 4 had become strong.

From this it appears that autolysis is a rapid process, a conclusion confirmed by another experiment in which one of two bottles, each containing 2 grms. dried yeast and 40 cc. distilled water, was kept for one hour in the incubator at 38°C., whilst the other bottle remained on the laboratory table at about 11°C. At the end of this time the contents of the former gave a distinct tryptophane-reaction, whilst those of the latter gave no reaction.

It appears, further, that autolysis can proceed within a wide range of alkalinity and acidity. The limits of this range were more nearly approached in the following experiment, which was of short duration:—

Experiment 3. 40 cc. of a mixture of 20 grms. dried yeast with 400 cc. distilled water, and toluol to 1 %, were placed in each of 9 bottles, the contents of which were varied as follows:—No. 1, nothing further added; No. 2, added 2 grms. precipitated chalk; No. 3, added Na_2CO_3 to 0·5 %; No. 4, added Na_2CO_3 to 1 %; No. 5, added Na_2CO_3 to 2 %; No. 6, added HCl to 0·05 %; No. 7, added HCl to 0·1 %; No. 8, added HCl to 0·2 %; No. 9, added HCl to 0·5 %.

After 4 hours in the incubator at 38° C., 5 cc. of each of these various liquids, treated with an equal volume of chlorine-water, after acidification with acetic acid where necessary, gave the following tryptophane-reactions:—Nos. 1 and 2, very strong; No. 3, which was neutral before acidification, gave a strong reaction, as did also No. 4, which was alkaline; No. 5, which also was alkaline before acidification, gave only a distinct reaction; Nos. 6 and 7 gave a strong reaction; No. 8 a distinct reaction; No. 9 no reaction.

The limit of acidity is here definitely indicated, the absence of the tryptophane-reaction proving that proteolysis did not take place in the presence of HCl added to 0·5 %. The limit of alkalinity was not actually reached, though the retarding effect of 2 % Na_2CO_3 was sufficiently

marked to justify the inference that a small further addition of alkali would arrest autolysis altogether.

As, however, the time of digestion in this experiment was short, it was necessary to ascertain whether similar results were obtainable with more prolonged digestion. In the case of HCl, a repetition of the foregoing experiment showed that no tryptophane-reaction was developed in a 0·5 % HCl liquid after digestion for 20 hours, and only a slight reaction after 72 hours. In yet another experiment, with 5 % yeast-mixtures containing respectively 0·2 %, 0·5 %, 0·8 %, and 1 % HCl, the tryptophane-reactions were—at the end of 24 hours' digestion—strong in the first, faint in the second, and none in the third or fourth; and at the end of 72 hours, strong in the first, distinct in the second, and still none in either the third or the fourth. Hence it appears that autolysis was much retarded by 0·5 % HCl, and altogether inhibited by 0·8 % or 1 %. In fact the limit of proteolytic activity in the presence of HCl lies between 0·5 % and 0·8 %, probably about 0·6 %, for a mixture containing 5 % yeast.

With regard to alkali, I found that a similar yeast-mixture, to which 2 % Na_2CO_3 had been added, gave no tryptophane-reaction after digestion for 72 hours. In this case I endeavoured to ascertain as nearly as possible the minimum time of exposure to the action of alkali required to arrest proteolytic action, by the following method:—

Experiment 4. 50 cc. of a 5 % yeast-mixture, with toluol, were placed in each of 3 bottles, Nos. 1, 2, and 3, to which Na_2CO_3 was added to the extent of 1 %, 2 %, 3 % respectively. The bottles were then placed in the incubator for a certain time. At the end of this time the contents of each bottle were divided into two equal portions, one of which was left alkaline, whilst to the other half HCl was added to slight acidity, and the 6 bottles were then returned to the incubator for any required number of hours, after which the tryptophane-reactions were compared.

In the decisive experiment of this kind, the 3 alkaline bottles were digested for 2 hours, when the tryptophane-reactions were, in No. 1, distinct; in No. 2, none; in No. 3, none. Half of the contents of each bottle having been acidified, the 6 bottles were further digested for 22 hours, when the reactions were:—

No. 1, still alkaline,	faint	acidified,	strong:
„ 2, „	none	„	distinct:
„ 3, „	none	„	none.

This experiment showed that digestion for two hours with 3 % Na_2CO_3 entirely destroyed proteolytic activity. A similar experiment, in which the period of exposure to this degree of alkalinity was only one hour, showed that this time did not suffice to destroy proteolytic activity, though it was much diminished.

The foregoing experiments were made with mixtures containing usually 5 % dried yeast: in none was the proportion less. It seemed

important to determine whether or not the results given by such a mixture apply equally to others containing less yeast, and it was found that they do not apply.

The following experiments were made with mixtures containing 2 % dried yeast:—

Experiment 5. *Acid.* 50 cc. of the mixture were placed in each of 3 bottles, Nos. 1, 2, and 3, acidified respectively with 0·1 %, 0·2 %, and 0·5 % HCl. After 3 hours in the incubator none gave any tryptophane-reaction; after 24 hours the reaction was faint in No. 1, none in either of the others.

Alkaline. In a similar experiment, in which the contents of the 3 bottles had been rendered alkaline by the addition of 1 %, 2 %, and 3 % Na_2CO_3 respectively, no tryptophane-reaction was obtained after digestion for 4 hours, or for 24 hours.

Here proteolytic activity was destroyed by 0·2 % HCl, as also by 1 % Na_2CO_3, degrees of acidity and alkalinity which produced no such effect in mixtures containing 5 % yeast. It may be concluded that there is a definite ratio between the amount of yeast present in a mixture and the amount of acid or alkali necessary to prevent autolysis.

On the evidence of the tryptophane-reaction, it results from the foregoing observations that autolysis is very active at the natural acidity of the yeast-mixture: anything more than a slight addition of either acid or alkali tends to diminish it. The acidity of yeast is partly due to the presence of organic acids; but not chiefly, for I have observed that it is impossible to neutralize a mixture or extract of yeast by adding excess of chalk. In view of the large proportion of phosphoric acid (about 50 %) and of potash (about 35 %) in the ash of yeast, it may be concluded that the acidity is mainly due to the presence of acid phosphate of potash. Naegeli (9) has in fact suggested that the cell-sap contains KH_2PO_4 and K_2HPO_4. Repeated digestions of mixtures to which excess of chalk had been added (see Experiment 3) have shown me that autolysis is even more active when the free organic acid present has thus been neutralized than at natural acidity. The conclusion to be drawn is that the most favourable degree of acidity is that afforded by the acid phosphates, a conclusion agreeing with that of Weis (see p. 291) in the case of malt. The influence of added acid on autolysis would appear to be, in accordance with the views of Fernbach and Hubert with regard to malt, that it is favourable so long as it merely suffices to convert the dibasic (K_2HPO_4) into monobasic (KH_2PO_4) phosphates, but unfavourable when free acid begins to accumulate. Similarly, the action of added alkali is favourable so long as it merely neutralizes any free acid present, but unfavourable when it begins to convert the monobasic into dibasic phosphates.

The study of autolysis necessarily involves the consideration of the proteids contained in the yeast-cell. I am not aware of any more recent

investigation in this direction than that of Naegeli (10), who stated the proteid content of yeast containing 8 % of nitrogen as follows:—

Ordinary albumin	36 %.
Gluten-casein, soluble in alcohol . . .	9 „
Peptone, precipitated by lead acetate . .	2 „

This statement is not altogether clear. The first item probably means that 36 % of the dry weight of the yeast consisted of coagulable proteid. The significance of the second item is doubtful: it is not impossible that it may really be albumose, or perhaps a mixture of albumoses and peptones. For some albumoses are relatively soluble in alcohol, precipitation only beginning with an alcoholic strength as high as 80 %; moreover, some of them possess the property, specially mentioned as characteristic of Naegeli's 'gluten-casein,' of readily giving off sulphuretted hydrogen when treated with caustic soda or potash. Again, peptone is to some extent soluble in alcohol when at all dilute; in fact one form (amphopeptone B) of it is soluble in 96 % alcohol. Finally, the substance described as 'peptone, precipitated by lead acetate,' is possibly not 'peptone' at all, since peptone proper (amphopeptone) is only partially precipitated by lead acetate: it is more probably one of the albumoses which are readily precipitated by this reagent.

In view of the rapidity with which autolysis took place, as indicated by the tryptophane-reaction, it seemed probable that the dried yeast used in my experiments contained albumoses or peptones, or a mixture of these, to begin with; and I have only so far investigated the proteids as to determine this point. A filtered watery extract was slightly acidulated and then boiled, when a precipitate of the coagulable proteids (albumin, &c.) was obtained. The filtrate was concentrated by evaporation and then treated with excess of alcohol, when a considerable precipitate was given. The precipitate was filtered off, dried, and dissolved in water on a filter; the solution was then saturated with ammonic sulphate, after the method of Kühne, in both alkaline and acid reaction, giving a considerable precipitate which consisted of albumoses. The filtrate, after appropriate treatment, still gave the biuret-reaction, indicating the presence of amphopeptone. Hence it is clear that the dried yeast contained both albumoses and peptones, the former in larger quantity than the latter. What still remains to be done is to determine the nature of the proteids that are coagulated on boiling.

Since there is evidence that the dried yeast contained albumoses and peptones, and since the test of proteolysis was the presence of tryptophane, my experiments do not throw light upon the peptonization of the higher proteids of the yeast in the course of autolysis. The conclusion to be drawn from them is that there is present in yeast a peptolytic enzyme which is most active at or near the natural acidity of a watery mixture or extract, which is due to the presence of acid phosphates.

Peptolysis.

Inasmuch as the foregoing experiments on autolysis were gauged by the tryptophane-test, they were essentially experiments in peptolysis. Nevertheless, I thought it necessary to institute experiments as to the peptolytic action of yeast upon added albumoses and peptones, as contained in the substance known as Witte-peptone: the results, as might perhaps be expected, were similar to those of the autolysis-experiments.

In the first place it was ascertained that a filtered watery extract of yeast was always peptolytically active, however short the period of extraction might be, even when the quantity of yeast used was small; and further, that peptolysis was rapidly effected. The following experiment, in which the period of extraction was limited to the time necessary for filtration, illustrates both these points:—

Experiment 1. 2 grms. of the dried yeast were extracted on a filter with 100 cc. of distilled water containing 1 % toluol: within an hour 50 cc. of liquid were obtained, to which 0·5 grm. Witte-peptone was added, and were then placed in the incubator. The liquid gave no tryptophane-reaction.

After digestion for one hour the liquid gave a marked tryptophane-reaction.

Here, notwithstanding the short duration of both extraction and digestion, a dilute extract gave unmistakable evidence of peptolytic activity.

The next experiment was made with the object of demonstrating the effect upon peptolysis of various strengths of acid and alkali.

Experiment 2. 20 grms. of dried yeast were extracted with 400 cc. of toluol-water (1 %), and left to filter for several hours in a cold room. The filtered liquid, which gave a faint tryptophane-reaction, was distributed as follows:—40 cc. were put into a bottle (No. 1) without further addition; in the remainder of the liquid 10 grms. of Witte-peptone were dissolved, and 40 cc. of the solution were put into each of 8 bottles (Nos. 2–9): to No. 2, nothing more was added; to No. 3, added 2 grms. of precipitated chalk; to No. 4, Na_2CO_3 to 1 %; to No. 5, Na_2CO_3 to 2 %; to No. 6, Na_2CO_3 to 3 %; to No. 7, HCl to 0·1 %; to No. 8, HCl to 0·2 %; to No. 9, HCl to 0·5 %.

After 4 hours in the incubator the tryptophane-reactions were:—No. 1, distinct; No. 2, marked; No. 3, strong; all three being acid: No. 4, distinct; No. 5, faint; No. 6, none; all three being alkaline: No. 7, strong; No. 8, marked; No. 9, distinct.

After 25 hours in the incubator the reactions were:—No. 1, distinct; Nos. 2 and 3, strong; No. 4, marked; No. 5, distinct; No. 6, none; Nos. 7 and 8, strong; No. 9, marked.

These results are in general agreement with those of the corresponding autolysis experiment (p. 295).

It was further ascertained that here also the retarding or inhibiting effect of added acid or alkali was the more marked the more dilute the yeast-extract.

Experiment 3. *Acid.* 4 grms. dried yeast were extracted for several hours in the cold with 200 cc. toluol-water (1 %): the filtered liquid gave a faint tryptophane-reaction: 2 grms. of Witte-peptone were dissolved in the liquid, 50 cc. of which were then put into each of 3 bottles acidified as follows:—No. 1, HCl to 0·1 %; No. 2, HCl to 0·2 %; No. 3, HCl to 0·5 %.

After 3 hours in the incubator the tryptophane-reactions were:—No. 1, distinct; No. 2, faint; No. 3, none: after 29 hours they were—No. 1, marked; No. 2, distinct; No. 3, none.

Alkali. 50 cc. of an exactly similar yeast-extract, containing the same percentage of Witte-peptone, were placed in each of 4 bottles: to No. 1, Na_2CO_3 to 1 % was added; to No. 2, Na_2CO_3 to 2 %; to No. 3, Na_2CO_3 to 3 %; to No. 4, nothing.

After 2 hours' digestion the tryptophane-reactions were:—in Nos. 1, 2, 3, faint; in No. 4, marked: the reactions were the same after the bottles had remained in the incubator for 25 hours.

On comparing the results of Expt. 3, where the strength of the yeast-extract may be taken as 2 %, with those of Expt. 2, where the strength of the extract may be taken as 5 %, it appears that the retarding action of added acid and alkali was more marked in the former than in the latter: for instance, in the case of the 2 % extract, peptolysis was inhibited by the addition of HCl to 0·5 %, and by all strengths of added alkali; whereas the 5 % extract peptolysed actively with HCl 0·5 % and with 1 % Na_2CO_3. This is very much the same relation as that indicated by the corresponding autolysis-experiments: such differences as exist are due to the different chemical composition of the liquids in the two sets of experiments.

The fact that the retarding action of added acid and alkali is less marked in peptolysis than in autolysis is clearly brought out by a comparison of the results obtained by a peptolytic experiment on the same lines as the autolytic experiment (Expt. 4, p. 296), which had as its object to determine the effect of exposure to the action of alkali of different strengths for a short time, and showed that autolysis was inhibited by treatment for two hours with 3 % Na_2CO_3 liquid at 38°C.: complete inhibition was not produced in the peptolytic experiment under similar conditions.

Experiment 4. 10 grms. of dried yeast were extracted for several hours with 200 cc. toluol-water (1 %) and filtered: the filtered liquid gave faint tryptophane-reaction. 50 cc. of the liquid were placed in each of 3 bottles, to which Na_2CO_3 was added to 1 %, 2 %, 3 % respectively, and then the bottles were kept in the incubator for 2 hours. The contents of each bottle were then divided into 2 equal parts, in separate bottles, and one half was slightly acidified with HCl, whilst the other half remained alkaline: 0·2 grm. of Witte-peptone was added to each bottle, and a little more toluol.

After 24 hours' digestion the tryptophane-reactions were :—

	Na_2CO_3 1 %	2 %	3 %
Alkaline . . .	faint	faint	none
Acid	strong	strong	marked

24 hours later they were :—

Alkaline . . .	marked	faint	none
Acid	very strong	very strong	strong.

These experiments with Witte-peptone confirm the conclusion arrived at from the autolysis-experiments (see p. 298)—that yeast contains an actively peptolytic enzyme, most active at or near the natural acidity of the extract, becoming less active, to total arrest, on the addition of either acid or alkali. Further, they show that this protease can be very readily extracted with water, and that the peptolytic action of a watery extract is marked as well as rapid, even when (as in Expt. 1) the extract is dilute (2 %).

Peptonization.

Having ascertained that yeast is actively peptolytic, I proceeded to investigate its peptonizing capacity, the test being the complete disintegration of a small quantity of fibrin. The experiments were made with (*a*) solid yeast substance, (*b*) aqueous extracts, (*c*) extracts made with 2 % NaCl solution.

(*a*) *Experiments with solid yeast substance.* The first of these experiments was of a general character, with the object of ascertaining definitely if digestion of fibrin were effected by yeast, and how digestion would be influenced by added alkali and acid.

Experiment 1. In each of 6 bottles were placed 40 cc. distilled water and 5 grms. of partly dried brewers' yeast, with toluol as the antiseptic; 0·5 grm. of fibrin was added to each, and the bottles were severally treated as follows :—to No. 1, nothing further was added ; to No. 2, 1 grm. chalk (reaction remained acid); to No. 3, Na_2CO_3 to 0·5 % ; to No. 4, HCl to 0·04 % ; to No. 5, HCl to 0·1 % ; to No. 6, HCl to 0·2 %.

After 20 hours in the incubator the fibrin had not disappeared in any bottle, though in some it had diminished; 24 hours later it had disappeared in Nos. 1 and 2 ; 24 hours later it had disappeared in No. 3, whilst most of it remained in the others.

In a repetition of this experiment (omitting bottle 6), with 10 grms. of yeast (25 %) in each bottle, the fibrin disappeared in all the bottles within 48 hours.

These experiments show that yeast can digest fibrin; and that the activity of any given mixture, as also its resistance to the retarding action of added acid or alkali, depends upon the amount of yeast that it contains. These two points were then further investigated. The material used in the following experiments was the dried ' granular ' yeast already mentioned.

With regard to the relation between the digestive activity of a mixture and the quantity of yeast contained in it, I found to begin with that 50 cc. of a mixture of chloroform-water with 1·25 % yeast did not digest 0·2 grm. fibrin in 70 hours. This relation, as well as the action of acid and alkali, is further determined in the following comprehensive experiment:—

Experiment 2. Mixtures were prepared of toluol-water (1 %) with 2·5 %, 5 %, 10 %, and 20 % dried yeast respectively. 40 cc. of each of these mixtures were put into each of 3 bottles, to one of which nothing was added, to the second Na_2CO_3 to 2 %, to the third HCl to 0·5 %, and 0·3 grm. fibrin to each bottle. After 22 hours' digestion the results were:—

	Added *nothing*.	*Na_2CO_3*.	*HCl*.
20 % bottles; fibrin	gone	gone	not gone
10 „ „ „	gone	gone	not gone
5 „ „ „	not gone in any:		
2·5 „ „ „	not gone in any:		
after further digestion for 25 hours—			
20 % bottles; fibrin	—	—	not quite gone
10 „ „ „	—	—	not gone
5 „ „ „	gone	not gone	not gone
2·5 „ „ „	gone	not gone	not gone
after further digestion for 28 hours—			
20 % bottles; fibrin	—	—	gone
10 „ „ „	—	—	not gone
5 „ „ „	—	not gone	not gone
2·5 „ „ „	—	not gone	not gone

24 hours later, when the experiment closed, the results were the same.

These results suffice to indicate the relation between the digestive action on fibrin of yeast-mixtures of different strengths, and the degree to which digestive activity is affected by fairly strong acid and alkali in each case. Those afforded by the bottles containing 0·5 % HCl are of special interest in relation to Bokorny's experiments, in which, as I have already pointed out (see p. 293), the conditions seem to have been such as to prevent any digestion at all of the added proteid. This criticism applies more particularly to those of his experiments (Nos. 1–8) in which the amount of yeast present was 5 %, the strength of acid 0·5–1 % H_3PO_4, and the proteid to be digested (ten times the weight of the yeast employed) the meat-residue from the preparation of Liebig's extract. The facts upon which I base this criticism are supported by other results, subsequently described, obtained in experiments with yeast-extracts. It is more difficult to criticize Bokorny's further experiments (Nos. 9–15), in which proteids of vegetable origin (prepared from Pea-flour, Soja-bean-meal, and Rape-cake) were the material to be digested, since the quantitative relations are not clearly stated: but, in view of the small amount of digestive products obtained,

and the possibility that a considerable proportion of these may be attributed to the relatively large quantities of dried yeast added, amounting to 30% or more of the weight of the proteid to be digested, they appear to be open to the same objection as the others.

In all the foregoing experiments unboiled fibrin was used, so that a possible source of error was present. In order to eliminate this, some experiments were made in which the fibrin had been boiled for a few minutes. I found that 50 cc. of both a 10% and a 20% mixture of yeast with toluol-water digested 0·3 grm. fibrin in four days.

(*b*) *Experiments with aqueous extracts.* The foregoing experiments with solid yeast afforded no information as to the solubility of the digestive protease; so I had recourse to filtered watery extracts in the first instance. It was soon ascertained that active extracts can be obtained under suitable conditions. The following experiment proves this, and gives some indication of the effect of acidity and of alkalinity:—

Experiment 1. 12½ grms. of dried yeast were extracted (24 hours) with 250 cc. distilled water (yeast = 5%). 50 cc. of the filtered liquid were put into each of 4 bottles, with 0·2 grm. fibrin, and treated thus:—to No. 1, nothing further added; to No. 2, Na_2CO_3 to 0·5%; to No. 3, HCl to 0·05%; to No. 4, HCl to 0·2%.

After 21 hours' digestion in the incubator the fibrin in Nos. 1 and 3 showed diminution, in Nos. 2 and 4 it was not affected: 23 hours later it had disappeared in Nos. 1 and 3, but remained unaffected in Nos. 2 and 4.

In the next place I tested the digestive activity of stronger yeast-extracts, whether of natural acidity, or alkaline, or with added acid.

Experiment 2. Extracts of 10 grms. and of 20 grms. dried yeast with 100 cc. of toluol-water (1%) were prepared and filtered. 30 cc. of each extract were put into each of 3 bottles with 0·3 grm. fibrin, one bottle of each having nothing added, another bottle having Na_2CO_3 added to 2%, and the third bottle HCl to 0·5%.

After 27 hours' digestion in the incubator the condition of the fibrin was—

	Nat. acid.	*Na_2CO_3.*	*HCl.*
bottles 10% yeast	gone	unaltered	unaltered
„ 20% „	gone	attacked	gone
21 hours later it was—			
bottles 10% yeast	—	unaltered	attacked
„ 20	—	attacked	—

48 hours later the fibrin was still unaltered in the 10% extract with Na_2CO_3, and had not disappeared in either the 10% extract with HCl or the 20% extract with Na_2CO_3.

Hence it appears that both 10% and 20% watery yeast-extracts actively digest fibrin, and that, as might be expected, the latter are less affected by added acid and alkali than the former. Comparing the results of this experiment with those of the corresponding experiment (see p. 302)

with solid yeast, it is clear that the resistance of solid yeast to acid and alkali is greater than that of the filtered extracts.

The following experiment shows that a dilute and rapidly prepared watery extract has little or no action on fibrin:—

Experiment 3. 2 grms. of dried yeast were extracted on a filter with 100 cc. toluol-water (1 %), the whole process being completed in 2 hours. 50 cc. of the filtrate were put into a bottle with 0·2 grm. fibrin. The fibrin had not disappeared after digestion in the incubator for 5 days.

Having observed that the digestion of boiled fibrin took place in the presence of solid yeast, I made an experiment of this kind with yeast-extract, and with the same result: 60 cc. of a 20 % yeast-extract digested 0·2 grm. fibrin in 4 days.

(*c*) *Experiments with* 2 % *NaCl extracts.* It occurred to me that it should be possible to prepare yeast-extracts that would digest fibrin more actively than did the aqueous extracts, by the use of some solvent other than distilled water. I found such a solvent in a 2 % solution of common salt (NaCl): extracts made with this liquid, even when rapidly prepared, can be depended upon to digest fibrin, and are therefore specially suitable for the investigation of the digestive action of yeast upon this proteid.

The following experiment, with a 10 % yeast-extract, gives a general indication of the effect of various antiseptics and of HCl upon the digestion of fibrin:—

Experiment 1. 20 grms. of dried yeast were extracted for several hours on a filter with 200 cc. of 2 % NaCl solution. 25 cc. of the filtrate were placed in each of 6 bottles, treated respectively as follows:—No. 1, nothing further added; No. 2, added HCN to 0·2 %; No. 3, added NaF to 1 %; No. 4, added chloroform to 0·5 %; No. 5, added toluol to 0·5 %; No. 6, added HCl to 0·2 %: to each added 0·2 grm. fibrin.

After 26 hours in the incubator the fibrin had disappeared in all the bottles except No. 6, where it seemed to be quite unaltered.

The following experiment demonstrates the inhibiting action of added acid and alkali:—

Experiment 2. 40 grms. of dried yeast were extracted for several hours with 400 cc. of 2 % NaCl solution containing 1 % toluol. 40 cc. of the filtered liquid, with the addition of a little more toluol, were put into each of 9 bottles with 0·3 grm. fibrin: the further additions were—to No. 1, nothing; to No. 2, 2 grms. precipitated chalk; to Nos. 3, 4, 5, HCl to 0·1 %, 0·2 %, 0·5 % respectively; to No. 6, H_3PO_4 to 0·5 %; to Nos. 7, 8, 9, Na_2CO_3 to 1 %, 2 %, 3 % respectively.

After 5 hours in the incubator the fibrin in Nos. 1, 2, 3 showed signs of solution. After 24 hours it had disappeared in Nos. 1, 2, 3, and 7: it had not perceptibly diminished in any of the others, nor had it done so 72 hours later.

These results show that addition of Na_2CO_3 to 2 %, or of H_3PO_4 to 0·5 %, or of HCl to 0·2 %, inhibits the digestive action of a 10 % yeast-extract made with 2 % NaCl solution. On comparing them with those obtained in the corresponding experiments with 10 % solid yeast (p. 302), and with 10 % aqueous extract (p. 303), there is complete agreement as regards the effect of added acid, a matter of importance in relation to Bokorny's experiments; and as regards the effect of added alkali, the only preparation that withstood the action of 2 % Na_2CO_3 was that containing solid yeast.

The inhibiting effect of added alkali was further investigated by the method employed in the corresponding experiments in autolysis (p. 296) and peptolysis (p. 300).

Experiment 3. 10 grms. of dried yeast were extracted on a filter for 3–4 hours with 200 cc. 2 % NaCl solution containing 1 % toluol. 50 cc. of the filtrate were placed in each of 3 bottles, to which Na_2CO_3 was added to 1 %, 2 %, 3 % respectively; the bottles were then placed in the incubator for 2 hours. On being removed, the contents of each bottle were divided into 2 equal parts, one of which remained alkaline, the other being made slightly acid with HCl. There were then 6 bottles, 3 acid and 3 alkaline, each containing 25 cc. of liquid: to each was added 0·2 grm. fibrin and a little more toluol.

The 6 bottles were placed in the incubator, together with another bottle, No. 7, containing 25 cc. of the original extract, which had not been treated with Na_2CO_3, and 0·2 grm. fibrin.

After 20 hours' digestion the fibrin had disappeared in bottle No. 7 (natural acidity): but it had not undergone any apparent change in Nos. 1–6, nor had it done so after 48 hours' digestion.

Thus treatment for two hours with even 1 % of Na_2CO_3 sufficed to destroy the digestive activity of a 5 % yeast-extract made with salt-solution, a result that more closely defines the action of alkali than those of the preceding experiments, in which it was ascertained that neither a 5 % yeast-mixture (p. 302) nor a 10 % watery extract (p. 303) digested fibrin in the presence of 2 % Na_2CO_3.

But it has not yet been made clear what is the advantage of a NaCl extract over a watery extract of yeast. When the extracts are strong, say 10 %, the advantage is not very marked: the digestive action of both is vigorous and rapid, more especially when the period of extraction and filtration has been prolonged for many hours. When, however, dilute and rapidly prepared extracts are employed, the greater activity of the NaCl extract is most apparent, as the following experiment shows.

Experiment 4. 1 grm. of dried yeast was treated with 50 cc. toluol-water (1 %); 1 grm. of yeast was treated with 50 cc. of 2 % NaCl solution: the mixtures were at once filtered, so that the preparation of the filtered liquids did not last more than 2 hours. To each bottle 0·2 grm. fibrin was added, and then they were both put into

the incubator. After 20 hours' digestion the fibrin in the watery extract was unaltered, whilst that in the NaCl extract was much diminished, and 4 hours later had disappeared. The fibrin in the watery extract had not disappeared after digestion for 4 days longer.

In another experiment in which extraction was prolonged for several hours, the activity of stronger aqueous and 2 % NaCl extracts of yeast was compared. It was found that 30 cc. of a 10 % yeast NaCl extract digested 0·2 grm. fibrin within 18 hours, and the same quantity of 5 % yeast NaCl extract digested the fibrin in 24 hours; whereas the times of digestion by the corresponding aqueous extracts were 46 and 66 hours respectively.

The object of the next experiment was to ascertain in what way NaCl affects digestion. Does it directly promote it, or does it do so indirectly by dissolving out of the yeast something that distilled water fails to extract or extracts less completely?

Experiment 5. 3 grms. of dried yeast were extracted with 60 cc. of 2 % NaCl solution (toluol 1 %); 6 grms. were also extracted with 120 cc. of toluol-water: extraction and filtration occupied about an hour and a half. 40 cc. of the NaCl extract were put into a bottle (No. 1) with 0·2 grm. fibrin: of the aqueous extract, 40 cc. were put into a second bottle (No. 2), and other 40 cc. into a third bottle (No. 3), to which NaCl to 2 % was added, as also 0·2 grm. fibrin to both 2 and 3.

After 18 hours in the incubator the fibrin in No. 1 had disappeared: this was not the case in either No. 2 or No. 3, nor had it disappeared after digestion for 24 hours.

This result, in the first instance, confirms the conclusions as to the superior activity of NaCl extracts as compared with aqueous extracts; and, in the second place, it gives an explicit answer to the question propounded above. It clearly shows that the presence of NaCl is of importance in the process of extraction rather than in the process of digestion; and it may be inferred that the NaCl solution dissolved out of the yeast something, no doubt a protease, that water alone failed to extract.

An experiment was made to test the action of a NaCl extract on boiled fibrin. It was found that 60 cc. of a 20 % yeast-extract with NaCl digested 0·2 grm. fibrin in 3 days: the action was slow, but it was more rapid than that of a watery extract of the same strength (see p. 304).

Summary of Experiments on Yeast.

Evidence has been adduced to prove that yeast can effect both peptolysis and peptonization. The fact that these processes can be carried on by filtered extracts makes it clear that they are not due to the yeast-plant as a living organism, but to one or, perhaps, more substances that can be dissolved out of it; and there can be no doubt that the active substance is, in any case, a protease. An important issue is thus raised

that may be expressed in the two questions—(1) is there, as is now generally held, a single protease in yeast, or is there more than one, and in the latter case, how many? (2) What is the nature of the protease or proteases? The results of my experiments will be briefly considered with a view to a reply.

Peptolysis. The most important fact that has been brought to light is the rapidity with which this process is effected: thus in an autolysis-experiment (p. 295) it was found to have proceeded actively in 2½ hours; and in a Witte-peptone experiment (p. 299) in 1 hour. Moreover, in the latter experiment the watery-extract was very dilute (2 %) and the time of extraction very short (1 hour): therefore the protease concerned is readily soluble in water.

It has been shown, further, that peptolysis is most active at or near the natural acidity of the liquid, at a degree of acidity determined by the presence of acid phosphates. It is retarded, and eventually arrested, by any deviation from this degree of acidity, in the direction either of alkalinity or of increased acidity: the effect of added alkali or acid varies with the amount of solid yeast present, or with the strength of the extract, as also with the length of the exposure to its action. Thus, in the case of a 5 % yeast-mixture, peptolysis was found to be inhibited in the presence of either about 0·6 % HCl, or 2 % Na_2CO_3, for 24 hours, as also by exposure to 3 % Na_2CO_3 for 2 hours (p. 296). Similar results were obtained with 5 % watery extracts acting on Witte-peptone (p. 299).

Peptonization. Under this heading I include the experiments upon the digestion of fibrin.

It has been made clear, in the course of these experiments, that peptonization takes place much less rapidly than peptolysis. Even with relatively strong yeast-extracts several hours were required for the digestion of a small quantity of fibrin: thus 40 cc. of a 5 % yeast-mixture did not digest 0·3 grm. fibrin at all in 22 hours, though the fibrin disappeared within 24 hours more (p. 302).

The next point of importance is the relation between watery extracts of yeast and NaCl extracts. When the extracts were strong (5 % and upwards) and the time of extraction long, the difference in the activity of the two kinds of extracts was not found to be important; but when the extracts were dilute and the time of extraction short, the difference was striking. A rapidly prepared 2 % watery extract did not digest fibrin at all (p. 304), whilst a similarly prepared NaCl extract (p. 305) digested the fibrin in about 24 hours. The inference to be drawn is that the peptonizing enzyme is not readily soluble in distilled water, but is readily soluble in 2 % NaCl solution.

Peptonization was found, like peptolysis, to proceed most actively at or near the natural acidity of the liquid, and to be arrested or retarded by the addition of either acid or alkali. But a comparison of the results

shows that the two processes do not exactly agree in the latter respect: it appears that the range of reaction is rather more limited for peptonization than for peptolysis. Thus, with regard to the action of added acid, the only case in which digestion of fibrin took place in the presence of as much as 0·5 % HCl was one in which the mixture or extract was very strong (20 %, see p. 302); on the other hand, 0·5 % HCl did not inhibit peptolysis in a 5 % yeast-mixture (p. 296) or in 5 % yeast-extract (p. 299). Similarly, with regard to the action of added alkali, whilst it is true that in the experiments in which solid yeast was used, peptonization and peptolysis were equally affected by the addition of Na_2CO_3 to 2 % (compare Expt. 2, p. 302, with Expt. 4, p. 296), there was a marked difference in favour of peptolysis when extracts were employed (compare Expt. 2, p. 303, and Expt. 2, p. 304, with Expts. 2, p. 299, and 4, p. 300). In the two former digestion of fibrin by 10 % or 20 % extracts was inhibited, whilst in the two latter peptolysis was effected by 5 % extracts treated with the same amount of alkali. In Expt. 3, p. 305, digestion of fibrin was inhibited by 1 % Na_2CO_3.

Conclusions. The chief results of the investigation are these:—

(1) dilute yeast-mixtures or aqueous extracts rapidly effect peptolysis, as indicated by the tryptophane reaction, but do not digest fibrin;

(2) dilute NaCl extracts of yeast readily digest fibrin;

(3) peptolysis and peptonization are influenced in the same manner, but not in the same degree, by the addition of acid or alkali.

I infer that these two digestive processes are not effected by one and the same protease. On the contrary, the facts indicate the presence of two proteases: the one exclusively peptolytic, readily soluble in water; the other peptonizing, less soluble in water, but readily soluble in 2 % NaCl solution.

The Mushroom.

Agaricus (Psalliota) campestris.

The discovery of proteases in Basidiomycetous Fungi seems to have been made by Hjort (11), who found that watery extracts of them digested fibrin. In the case of *Agaricus (Pleurotus) ostreatus*, digestion was most active when the liquid was neutral; less active when acidified with 0·5 % oxalic acid, and was altogether inhibited by alkalinity. The fibrin entirely disappeared in 40 hours; the digested liquid then giving no biuret, but strong tryptophane-reaction, and containing leucin and tyrosin. In the case of *Polyporus sulfureus*, the naturally acid extract readily digested fibrin, as did also extracts acidified with HCl to 0·2 % or with oxalic acid to 0·25 %; but neutralized or alkaline extracts did not digest at all. In a 12-hours' digestion the liquid contained albumoses and peptones, but no amido-acids or hexon-bases.

Shortly afterwards the matter was investigated by Bourquelot and Hérissey (12). They found that a filtered watery extract of *Agaricus* (*Amanita*) *muscarius* digested five-sixths of the caseinogen of skim-milk within four days, and they detected tyrosin in the digested liquid. Similar results were obtained with *Polyporus sulfureus*.

In the course of a few experiments with the mushroom, I obtained evidence (13) that the tissue can both peptonize fibrin and peptolyse the lower proteids, thus confirming in a general way the conclusions of my predecessors.

Somewhat more recently the matter has been taken up by Delezenne and Mouton (14), and with widely different results. They prepared extracts, using normal saline solution (0·8 % NaCl) with chloroform or toluol, of the dried pilei of various species (the Mushroom, *Amanita muscaria, Amanita citrina, Hypholoma fasciculare*), which readily peptolysed peptone, and digested gelatine and casein, but had no action on fibrin. This last result seems so contradictory to previous observations that I have thought it necessary to make some further experiments to test its accuracy.

Peptonization.

The test applied was the disappearance of a relatively small quantity of fibrin. The laminae were in all cases removed from the pileus.

In the first instance the actual tissue, reduced to a pulp, was made use of: provided that the material was mature, the pileus being fully expanded, the result was that digestion of fibrin took place.

Experiment 1. 10 grms. fresh mushroom-pulp were digested with 100 cc. distilled water containing chloroform (0·25 %) and 2 grms. fibrin for 26 hours; at the end of this time the fibrin was completely disintegrated.

Experiment 2. 15 grms. fresh mushroom-pulp were digested with 100 cc. chloroform-water (0·5 %) and 2 grms. fibrin: after 24 hours' digestion the fibrin was dissolved.

Experiment 3. In an experiment similar to and contemporaneous with the preceding, where the antiseptic was HCN (0·2 %), the fibrin was disintegrated and mainly dissolved.

When a watery extract of the fresh ripe pileus was used, from which the solid matter had been removed either by straining through muslin or by filtering through paper, the result was less certain: in most cases the fibrin seemed to be somewhat diminished in quantity, but rapid and complete solution was not constant. The following are some of the more successful experiments:—

Experiment 4. 1 grm. of fibrin was digested with 50 cc. of a watery extract of 40 grms. mushroom-pulp with 200 cc. distilled water, with HCN to 0·2 %: within 24 hours the fibrin was completely disintegrated.

Experiment 5. In this experiment 0·2 grm. of fibrin was completely digested by 50 cc. mushroom-extract, to which NaF to 1 % had been added, in 18 hours.

These results clearly indicated that watery extracts of the Mushroom digest fibrin, in agreement with those of Hjort. However, I was not altogether satisfied, as digestion of fibrin did not occur in every experiment. With the object of obtaining active extracts with greater certainty, I had recourse to the method of extraction with 2 % NaCl solution that had proved serviceable in the case of yeast, and with the same success.

Experiment 6. 120 grms. fresh mushroom-pulp were extracted with 300 cc. 2 % Na Cl solution for 21 hours; the liquid was then strained off through muslin, and placed in 8 bottles each holding 40 cc. with 0·2 grm. fibrin. The treatment of the bottles was—No. 1, nothing added; No. 2, liquid boiled; No. 3, added HCN to 0·2 %; No. 4, added NaF to 1 %; No. 5, added chloroform to 0·5 %; No. 6, added toluol to 0·5 %; No. 7, added HCl to 0·1 %; No. 8, added HCl to 0·2 %.

After 20 hours' digestion in the incubator the fibrin had completely disappeared in Nos. 1, 3, 4, 5, 6; it had not undergone any apparent diminution in Nos. 2, 7, 8.

I found that it is also possible to obtain active extract from dried Mushroom.

Experiment 7. 8 grms. of dried pileus (had been kept for over 6 months) extracted for 4 hours with 100 cc. 2 % NaCl solution; filtered, and added toluol to 1 %. 30 cc. of this liquid had completely digested 0·2 grm. fibrin within 19 hours.

I incidentally observed that 20 cc. of the expressed juice of fresh Mushroom digested 0·2 grm. fibrin within 24 hours in the presence of 1 % toluol.

In another experiment a comparison was instituted between the relative activities of aqueous and 2 % NaCl extracts which were (*a*) of natural acidity, or (*b*) acidified to 0·1 % HCl, or (*c*) made alkaline by adding Na_2CO_3 to 0·5 %, in the presence of toluol.

Experiment 8. 60 grms. fresh mushroom-pulp were extracted for about 24 hours with 250 cc. distilled water; a similar quantity of pulp was extracted for the same time with 250 cc. 2 % NaCl solution. 40 cc. of the filtered aqueous extract were put, with some toluol and 0·2 grm. fibrin, into each of 3 bottles: to No. 1, nothing was added; to No. 2, HCl to 0·1 %; to No. 3, Na_2CO_3 to 0·5 %. 40 cc. of the NaCl extract were also put into each of 3 bottles, and similarly treated.

After 24 hours in the incubator (38–40°C.) the result was that the fibrin had not been digested, or apparently diminished, in any one of the bottles containing the aqueous extract, whilst it had disappeared in the bottle containing the NaCl extract alone, but not in either the Na_2CO_3 or the HCl bottles.

The superior activity of the NaCl extract is more marked, as was the case with yeast, the more dilute the liquids.

Experiment 9. 2·5 grms. of partly dried Mushroom were extracted (4 hours) with 50 cc. 2 % NaCl solution, and an equal quantity with 50 cc. distilled water.

25 cc. of the NaCl extract were put into each of 2 bottles with the addition of some toluol and 0·2 grm. fibrin, the fibrin having been previously boiled in one case: 25 cc. of the watery extract were put, with toluol and unboiled fibrin, into each of 2 bottles, and to one of them 1 grm. NaCl was added.

After 19 hours in the incubator, the unboiled fibrin in the bottle containing NaCl extract had disappeared; the fibrin had not disappeared in any of the other three.

This experiment demonstrates not only the superior activity of the NaCl extract, but also the solvent action of the NaCl (compare Yeast, p. 306).

The next experiment relates to the action of acid and alkali upon a rapidly prepared NaCl extract.

Experiment 10. 100 grms. fresh mushroom-pulp were extracted with 300 cc. of 2 % NaCl solution containing toluol, and filtered, the whole process of preparation occupying about an hour. 40 cc. of the extract were put into each of 5 bottles, with 0·2 grm. of fibrin, treated thus—to No. 1, nothing further added; to No. 2, Na_2CO_3 to 1 %; to No. 3, Na_2CO_3 to 2 %; to No. 4, HCl to 0·1 %; to No. 5, HCl to 0·2 %.

After 24 hours in the incubator, the fibrin had disappeared in No. 1, was unaltered in Nos. 2 and 3, and seemed to be attacked in Nos. 4 and 5: 24 hours later the fibrin had not disappeared in any one of these four bottles.

These results, as also those of Expts. 6 and 8, show that the peptonizing activity of a mushroom-extract, of the strengths employed, is destroyed by the addition to the naturally acid liquid of 0·1 % HCl, or of 0·5–1 % Na_2CO_3.

So far it has been assumed that the disappearance of the fibrin in the experiments implied peptonization. In order that there might be certainty on this point, the following experiment was made:—

Experiment 11. 60 cc. of NaCl extract (toluol 1 %) were put to digest 2 grms. of fibrin: in 20 hours the fibrin had disappeared, and the liquid, after boiling and filtering, gave a well-marked biuret-reaction. At the commencement of the experiment, a sample of the extract gave no biuret-reaction.

The results of these experiments on fibrin are such as to lead inevitably tothe conclusion that the mushroom contains a peptonizing enzyme capable of digesting fibrin: it is therefore remarkable that Delezenne and Mouton (see p. 309) should have expressed the contrary opinion. The reason for this contradiction is that these observers used *boiled* fibrin in their experiments. This precaution, it is true, obviates a possible source of error by eliminating any self-digestion of the fibrin: but it is doubtful if this advantage compensates for the disadvantage involved, the disadvantage of missing altogether the presence of the peptonizing enzyme. Proteids coagulated by heat offer, as is well known, considerable resistance to the digestive action even of animal proteases; so that it is not surprising

that the protease of the mushroom should have failed to act upon them. This precaution is not, however, absolutely indispensable: for it is a simple matter to check possible self-digestion of the fibrin by control-experiments. Thus, in the foregoing Expt. 6, digestion of fibrin took place in Nos. 1, 3, 4, 5, 6, but not in No. 2 where the liquid (but not the fibrin) had been boiled: had the results given by Nos. 1, 3, 4, 5, 6 been due to self-digestion, then the probability is that the same result would have been given by No. 2, which was not the case. All the fibrin used in these experiments was prepared and preserved at one time and in the same manner: hence the fibrin may be regarded as a constant factor, all the variations being due to the mushroom-liquids, as affected by the various substances added to them. I may add that I have not succeeded in observing digestion of boiled fibrin, though strong mixtures and extracts were used, and the experiment was continued for a week.

Peptolysis.

There is already a certain amount of evidence that the mushroom contains an actively peptolytic enzyme, which is to be found in all the papers that I have previously cited (Nos. 11, 12, 13, 14). My main object in making further experiments has been not so much to establish this fact, as to determine the conditions that affect the activity of the protease and so to arrive at some conclusion as to its nature. I may say, however, that I have never failed to obtain from mushrooms, whether ripe or immature, and with great facility, a watery extract—in some cases a glycerine-extract—that readily peptolysed Witte-peptone, as indicated by the tryptophane-reaction. It should be explained that, on account of the deep colour of the liquids, it was not possible to apply the tryptophane-reaction directly: the liquids had first to be boiled with animal charcoal and then filtered.

The following experiment gives a general idea of the method employed and of the results obtained:—

Experiment 1. 90 grms. of fresh mushroom-pulp were extracted for 18 hours with 200 cc. chloroform-water: on straining through muslin, a red, opalescent, acid liquid was obtained.

40 cc. of the liquid were placed in each of 6 bottles, with 0·5 grm. Witte-peptone and a little toluol, the bottles being then treated as follows: added to 1, nothing further; to 2, 1 grm. chalk to neutralize any free acid; to 3, Na_2CO_3 to 1·25 %; to 4, HCl to 0·04 %; to 5, HCl to 0·1 %; to 6, HCl to 0·2 %.

After 23 hours in the incubator the tryptophane-reactions were—1, marked; 2, marked; 3, strong; 4, marked; 5, strong; 6, faint.

There was thus evidence of active peptolysis having taken place within a distinctly alkaline and a distinctly acid range of reaction, and of its arrest in the presence of stronger acid.

The next experiment gives some idea of the rapidity with which peptolysis was found to be effected by a dilute mushroom-extract, and shows further that peptolysis is a much more rapid process than is peptonization.

Experiment 2. 5 grms. fresh mushroom-pulp were extracted on a filter for about an hour with 100 cc. 1 % toluol-water. The filtered liquid gave no tryptophane-reaction. 50 cc. of it were put into a bottle with 0·3 grm. fibrin, and 50 cc. into another bottle with 0·5 grm. Witte-peptone.

In 1 hour the contents of the Witte-peptone bottle gave a distinct tryptophane-reaction: 24 hours later the reaction was strong. In the same time the fibrin in the other bottle had not disappeared; but it disappeared within the next 24 hours.

The following experiment brings out clearly the relative rapidity of peptolysis and of peptonization, and of the effect of added acid and alkali on these processes respectively:—

Experiment 3. 5 grms. of dried powdered mushroom were extracted with 200 cc. toluol-water (1 %): 5 grms. were also extracted with 200 cc. of 2 % NaCl solution containing 1 % toluol. 40 cc. of the NaCl extract were put into each of 3 bottles, with 0·2 grm. of fibrin; also 40 cc. into each of 3 bottles with 0·5 grm. Witte-peptone: to 1 fibrin bottle and 1 Witte-peptone bottle (Nos. 1), nothing was added; to another pair of bottles (Nos. 2) Na_2CO_3 to 1 % was added; to a third pair (Nos. 3), HCl to 0·1 % was added. An exactly similar series of bottles containing the aqueous extract was prepared.

After 24 hours' digestion the results were—

	NaCl ext.			*Aq. ext.*		
Nos.	1	2	3	1	2	3
fibrin	gone	not gone	not gone	not gone	not gone	not gone
tryptophane	strong	strong	marked	very strong	strong	strong.

Hence it is apparent that peptolysis and peptonization are independently affected by the addition of acid and alkali.

Conclusions.

Although my investigation of the mushroom has not been so minute as in the case of yeast, the results obtained suffice to draw similar conclusions.

In the first place, the conclusion is justified that the mushroom contains a peptolysing enzyme which is readily extracted by water and acts with rapidity. Secondly, it is equally clear that the mushroom contains a peptonizing enzyme capable of digesting fibrin.

As in the case of yeast, so here, the question arises as to whether both processes are effected by one and the same protease, or whether there are not two proteases in the mushroom, the one especially peptolytic, the other especially peptonizing.

The observed facts are, on the whole, favourable to the latter conclusion. To begin with, the results of the peptolysis-experiment No. 2, suggest that the rapid peptolysis and the slow peptonization should be interpreted as being due to the presence of two proteases, the one readily soluble in water, the other less soluble. Again, the superior peptonizing activity of NaCl extracts compared with watery extracts (see p. 310), suggests that the protease concerned is more readily soluble in 2 % NaCl solution than in distilled water. The importance of NaCl as a solvent is demonstrated in Expt. 9 (p. 310).

The inference drawn from these observations on solubility is supported by the observations upon the effect of added acid and alkali on peptolysis and peptonization respectively. Taking the limits of peptonization as HCl 0·1 % and Na_2CO_3 1 % (p. 311), those of peptolysis are less restricted, extending beyond these limits in both directions.

The Nature of the Proteases.

The generally accepted opinion with regard to the two Fungi in question is that they contain a single protease. In the case of yeast, Hahn and Geret, Bokorny, and others, regard this protease as a trypsin: and Hjort has made the same suggestion in the case of the Basidiomycetous Fungi investigated by him. My observations, as already explained, lead me to the conclusion that two proteases exist in these plants, the one peptolytic, the other peptonizing. It remains now to consider what the nature of these proteases may be.

At a meeting of the Linnean Society of London, on November 20, 1902 (Proceedings, 1902–3, p. 42), I announced the discovery in many plants and different parts of plants of a peptolytic enzyme analogous to the recently discovered entero-erepsin of the animal body: a more complete account of my researches was soon afterwards (January, 1903) published in this periodical (13), the mushroom having been one of the plants investigated.

The further observations, of which an account has now been given, confirm me in the conclusion that the mushroom contains an erepsin, that is, a peptolytic enzyme which is unable to peptonize the higher proteids such as fibrin and albumin; and they justify the extension of the conclusion to yeast.

This vegetable erepsin is not, however, identical in properties with either the entero-erepsin discovered by Cohnheim or the pancreato-erepsin discovered by Vernon (see p. 290). The action of both these animal erepsins is limited to neutral or feebly alkaline liquids, whilst I have found that vegetable erepsin can act through a fairly wide range of acid and alkaline reaction, its greatest activity being manifested when the reaction of the liquid is at or near natural acidity. Hence vegetable erepsin affords a new type of ereptic action.

Now as to the nature of the peptonizing enzyme. It may be either a pepsin or a trypsin, but the question is, which? This question is more easy to put than to answer, because there is at present no method by which the enzyme can be obtained free from the associated erepsin; and until that is done, no direct answer can be forthcoming. But it is possible to form an opinion upon indirect evidence. It should be borne in mind that, as I have elsewhere stated, there is at present no well-established instance of the occurrence of a merely peptonizing enzyme in the Vegetable Kingdom. A more important point is, however, that of the reaction of the liquid in which the protease will work. The activity of animal pepsin is limited to acid liquids; whilst animal trypsin, though most active in a distinctly alkaline liquid, can nevertheless work in a neutral or even in a slightly acid liquid. In its range of reaction, the vegetable peptonizing enzyme resembles animal trypsin rather than pepsin; but with this difference, that whereas animal trypsin is most active in a distinctly alkaline liquid, the vegetable protease is most active in a distinctly acid liquid. It seems therefore probable that the protease in question may be a trypsin of a new type, characterized by its activity in an acid, rather than an alkaline, liquid.

On these grounds it is suggested that the yeast and the mushroom contain two associated proteases, vegetable erepsin and vegetable trypsin, an association that finds its analogue in the pancreatic secretion of animals which, as Vernon has recently shown (2), contains both erepsin (pancreato-erepsin) and trypsin proper. The term 'vegetable trypsin' is already in common use, but in a wider sense than that in which I have just employed it. Hitherto it has been applied to the vegetable proteases without taking the presence of erepsin into account, whereas I limit the term to the peptonizing enzyme apart from the erepsin. Vernon's results have introduced the same distinction into animal physiology; formerly the term 'trypsin' was applied to the protease of the pancreas, but this, as he has shown, is really a mixture of pancreato-erepsin with true trypsin.

A few lines may be devoted, in conclusion, to the consideration of the question as to how far these views are applicable to plants in general. It can hardly be doubted that, at some period in their existence, all plants and all parts of plants contain a peptolytic enzyme concerned in promoting the distribution of proteids in the temporary form of amido-acids, &c. But it is not clear that a peptonizing enzyme is of such general occurrence: on the contrary, as I have already pointed out (13, p. 262), many parts of plants failed to digest fibrin in my experiments. It is possible that in those experiments the precise conditions most favourable to peptonization were not provided: it may be that, for instance, the use of NaCl extracts that have given such good results with the Yeast and the Mushroom, will give similar results in other cases. I have not yet had time to make

extended investigations in this direction; but what already I have done is, I think, of sufficient interest to be mentioned here.

In a previous paper (13, p. 254), I gave an account of some peptonizing experiments with the bulbs of the hyacinth, the tulip, and the onion, the bruised bulb-tissue being employed. The results were not conclusive, but indicated that whilst the onion did not digest fibrin, the hyacinth and the tulip did so to some extent in a slightly alkaline liquid.

I have since resumed these experiments, using watery or NaCl extracts of the bulbs, and taking the disappearance of a small quantity of fibrin as the test of digestion, following the method adopted in the investigation of the yeast and the mushroom, with interesting results.

Experiment 1. A hyacinth bulb, weighing about 75 grms., was reduced to pulp and extracted for two hours with 100 cc. of 2 % NaCl solution: the liquid was strained through muslin. 30 cc. were put into each of 3 bottles with 0·2 grm. fibrin and some toluol: to No. 1, nothing further was added; to No. 2, HCl to 0·1 %; to No. 3, Na_2CO_3 to 0·5 %.

After 23 hours in the incubator, the fibrin had disappeared in Nos. 1 and 3, and was attacked in No. 2: the tryptophane-reactions were, strong in No. 1, distinct in No. 2, marked in No. 3: 28 hours later the fibrin had disappeared also in No. 2.

Experiment 2. Similar extracts were prepared of the tulip and onion bulbs: 40 cc. of the extract were in each case put, with 0·2 grm. fibrin, into each of 4 bottles, with the following additions: to No. 1, nothing; to No. 2, HCl to 0·05 %; to No. 3, HCl to 0·2 %; to No. 4, Na_2CO_3 to 1 %.

Within 48 hours the results were: tulip, fibrin gone in No. 1; nearly gone in Nos. 2 and 4; unaltered in No. 3: onion, fibrin unaltered in all. All the bottles gave more or less strong tryptophane-reaction.

The positive results given by the hyacinth and the tulip point to the probability that a peptonizing enzyme is more generally present in plants than is at present recognized. But the negative result given by the onion is even more suggestive; clearly peptolysis (autolysis) had occurred in this experiment without peptonization of the added fibrin. It appears, therefore, that erepsin is present in the onion without any other protease. If this be so, it is important evidence in favour of the existence of an ereptic protease in plants, and strengthens the conclusion, already expressed, that in those plants that can digest fibrin there is also present a distinct peptonizing enzyme.

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NOTES.

ON THE ORIGIN OF PARASITISM IN FUNGI[1].—Up to the present no definite explanation has been offered as to why a given parasitic Fungus is often only capable of infecting one particular species of plant. This, however, is well known to be the case, for although the spores of Fungus-parasites germinate freely on the surface of any plant when moist, infection only takes place when the spores germinate on the particular species of plant on which the Fungus is known to be parasitic. This apparently selective power on the part of the Fungus I consider to be due to chemotaxis.

An extensive series of experiments was conducted with various species of Fungi, including saprophytes, facultative parasites, and obligate parasites, and the results are given in tabulated form in the full paper. The chemotactic properties of substances occurring normally in cell-sap were alone tested; among such may be enumerated saccharose, glucose, asparagin, malic acid, oxalic acid, and pectase. In those instances where the specific substance, or combination of substances, in the cell-sap assumed to be chemotactic could not be procured, the expressed juice of the plant was used.

These experiments proved that saprophytes and facultative parasites are positively chemotactic to saccharose, and this substance alone is sufficient in most instances to enable the germ-tubes of facultative parasites to penetrate the tissues of a plant, unless prevented by the presence of a more potent negatively chemotactic or repellent substance in the cell-sap.

As an illustration, *Botrytis cinerea*, which attacks a greater number of different plants than any other known parasite, cannot infect apples, although saccharose is present, on account of the presence of malic acid, which is negatively chemotactic to the germ-tubes of *Botrytis*.

In the case of obligate parasites the cell-sap of the host-plant proved to be the most marked positive chemotactic agent. Malic acid is the specific substance that attracts the germ-tubes of *Monilia fructigena* into the tissues of young apples; whereas the enzyme pectase performs the same function for the germ-tubes of *Cercospora cucumis*, an obligate parasite on the cucumber.

Immune specimens of plants belonging to species that are attacked by some obligate parasite owe their immunity to the absence of the substance chemotactic to the parasite.

Purely saprophytic Fungi can be educated to become parasitic, by sowing the spores on living leaves that have been injected with a substance positively chemotactic to the germ-tubes of the Fungus experimented with. By a similar method of procedure, a parasitic Fungus can be induced to attack a different species of host-plant.

[1] Abstract, reprinted from the Proceedings of the Royal Society.

These experiments prove what has previously only been assumed, namely, that parasitism in Fungi is an acquired habit.

A series of experiments prove that infection of plants by Fungi occurs more especially during the night, or in dull, damp weather. This is due to the greater turgidity of the cells, and also to the presence of a larger amount of sugar and other chemotactic substances present in the cell-sap under those conditions.

GEORGE MASSEE, Kew.

CULTURAL EXPERIMENTS WITH 'BIOLOGIC FORMS' OF THE ERYSIPHACEAE[1].—In the introductory remarks the author points out that through specialization of parasitism 'biologic forms' have been evolved in the Erysiphaceae which, both in their conidial (asexual) stage and ascigerous (sexual) stage, show specialized and restricted powers of infection. The powers of infection, characteristic of each 'biologic form,' are under normal conditions sharply defined and fixed, and hitherto the result of the experiments of numerous investigators—both in regard to the present group of Fungi and to the Uredineae, where the same specialization of parasitism occurs—has been the accumulation of evidence tending to emphasize the immutability of 'biologic forms.'

The second part of the paper gives the result of cultural experiments with 'biologic forms' of *Erysiphe Graminis* DC., carried out during the past summer in the Cambridge University Botanical Laboratory. It has been found that *under certain methods of culture, in which the vitality of the host-leaf is interfered with, the restricted powers of infection, characteristic of 'biologic forms,' break down.*

In the first method of culture adopted, the leaf, which was either attached to a growing plant, or removed and placed in a damp chamber, was injured by the removal of a minute piece of leaf-tissue. In this operation the epidermal cells on one surface, and all or most of the mesophyll tissue, were removed at the cut place, but the epidermal cells on the other surface (opposite the cut) were left uninjured. Conidia were sown on the cuticular surface of the uninjured epidermal cells over the cut. In a few experiments the conidia were sown on the internal tissues of the leaf exposed by the cut, and these gave the same results.

Using this method of culture, over fifty successful experiments, of which details are given, were made. In these the conidia of certain 'biologic forms' were induced to infect 'cut' leaves of host-species which are normally immune against their attacks.

The experiments proved that the range of infection of a 'biologic form' becomes increased when the vitality of a leaf is affected by injury, and also that species of plants 'immune' in nature can be artificially rendered susceptible.

Further experiments showed that *the conidia of the Fungus produced on a 'cut' leaf are able at once to infect fully uninjured leaves of the same host-species.*

In other experiments, a method suggested by Professor H. Marshall Ward with the object of avoiding lesion of the leaf, was adopted. Leaves were injured by touching the upper epidermis for a few seconds with a red-hot knife, and conidia were

[1] Abstract, reprinted from the Proceedings of the Royal Society.

sown on the injured place. It was found that the cells immediately surrounding the place of injury were rendered susceptible to the attacks of a 'biologic form' which is unable to attack uninjured leaves of the plant in question.

In the third part of the paper, dealing with general considerations, the following hypothesis is advanced as to the actual manner in which the injury to a leaf causes it to become susceptible to a 'biologic form' otherwise unable to infect it. It is supposed that the leaf-cells of each species of host-plant contain a substance or substances—possibly an enzyme—peculiar to each species which, when the leaf is uninjured and the cells are vigorous, are able to prevent the successful attack of any mildew except the *one* 'biologic form' which has become specialized to overcome the resistance. When the vitality of the leaf, however, becomes affected by injury, this substance is destroyed, or becomes weakened, in the leaf-cells in the neighbourhood of the injury, so that the conidia of *other* 'biologic forms' are now able to infect them.

The author suggests that injuries to leaves, caused in nature by hail, storms of wind, attacks of animals, &c., may produce the same effect as the artificial injuries described above in rendering the injured leaf susceptible to a Fungus otherwise unable to infect it. Conidia produced on these injured places would be able to infect uninjured leaves, and would spread indefinitely. Such may be the explanation of a common phenomenon—the sudden appearance of disease caused by parasitic Fungi on plants hitherto immune.

A case is described which, it is believed, gives evidence that the injuries produced by *Aphides* caused leaves previously 'immune' to become susceptible.

In the concluding remarks, reference is made to the antagonistic forces concerned in the evolution of a 'biologic form,' viz. 'specializing factors' and 'generalizing factors.'

Attention is also drawn to the close parallel between (1) the behaviour of the Fungus in the experiments in which the conidia were sown on the tissues of the leaf exposed by the cut; and (2) the biological facts obtaining in the class of parasitic Fungi known as 'wound parasites' (*Nectria*, *Peziza willkommii*, &c.), which are able to infect their hosts only through a wound.

ERNEST S. SALMON, Cambridge.

ON THE STRUCTURE OF THE PALAEOZOIC SEED LAGENOSTOMA LOMAXI, WITH A STATEMENT OF THE EVIDENCE UPON WHICH IT IS REFERRED TO LYGINODENDRON.—The present communication deals with the structure of *Lagenostoma Lomaxi*, a fossil seed from the lower coal-measures, and with the evidence upon which the authors refer it to the well-known carboniferous plant, *Lyginodendron*.

It is found that this species of *Lagenostoma*, especially in its young form, was enclosed in a husk or cupule, borne on a short pedicel.

The seed, which is of Cycadean character, is fully described, and its relation to other fossil and recent seeds discussed.

[1] Abstract, reprinted from the Proceedings of the Royal Society.

The cupule enclosing the seed was borne terminally on a pedicel; it formed a continuous, ribbed cup below, and divided above into a number of lobes or segments. Externally, both pedicel and cupule were studded with numerous prominent multicellular glands of capitate form. The anatomy indicates that the whole organ was of a foliar nature.

A comparison with the vegetative organs of *Lyginodendron Oldhamium*, with which the seeds are intimately associated, demonstrates a complete agreement in the structure of the glands and in the anatomy of the vascular system. Where vegetative and reproductive organs, presenting identical structural features, not known to occur in other plants, are thus found in close and constant association, the inference that the one belonged to the other appears irresistible.

As regards the position of the seed on the plant, two possibilities are discussed; the cupule, with its pedicel, may either represent an entire sporophyll, or a modified pinnule of a compound leaf. Either view is tenable, but various comparative considerations lend a somewhat greater probability to the second alternative.

In the concluding section of the paper, the systematic position of *Lyginodendron* is discussed. On the whole of the evidence, the position of the genus as a member of a group of plants transitional between Filicales and Gymnosperms appears to be definitely established. While many Filicinean characters are retained, the plant, in the organization of its seed, had fully attained the level of a Palaeozoic Gymnosperm. There are many indications that other genera, now grouped under Cycadofilices, had likewise become seed-bearing plants. It is proposed to found a distinct class, under the name *Pteridospermae*, to embrace those Palaeozoic plants with the habit and much of the internal organization of Ferns, which were reproduced by means of seeds. At present, the families Lyginodendreae and Medulloseae may be placed, with little risk of error, in the new class, Pteridospermae.

F. W. OLIVER and D. H. SCOTT.

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On the Fertilization, Alternation of Generations, and General Cytology of the Uredineae[1].

BY

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With Plates XXI–XXIV.

THE question of the sexuality of the Uredineae has been a vexed one ever since the suggestion put forward by Meyen (34), more than sixty years ago, that the spermogonia and aecidia represented the male and female organs. This view seemed to receive support from the later observations of Tulasne (53), and De Bary (3, 4), who showed that not only were spermogonia and aecidia closely associated in a large number of forms, but also that the spermatia produced in the spermogonia were apparently wanting in any power of germination. It appeared, then, possible that the aecidium was the result of the fertilization by a spermatium of a definite female reproductive organ. All workers, however, failed either to trace this process or even to produce any evidence of the existence of sexual organs at any stage in the development of the aecidium. These results, together with the observation that aecidia were sometimes produced in the entire absence of spermogonia (see De Bary (4); also Klebahn (27), p. 194), seemed to negative the view of any actual fertilization by the spermatium.

Owing in part to these observations and to the discovery by Cornu (12), in 1875, that the spermatia were capable of germinating to a slight degree in nutritive solutions, and also to the later observations of Möller (36) that the spermatia of some lichens could develop a mycelium under similar conditions, Brefeld (10) was led about 1889 to put forward the view that the Uredineae (and all the higher Fungi) were without any trace of sexuality and that the group should be considered as a family of the Basidiomycetes. In his view the spermogonia and spermatia, in this group and in the lichens,

[1] A short preliminary account of the chief results of this work appeared in the New Phytologist, vol. iii, 1904, pp. 24–27.

[Annals of Botany, Vol. XVIII. No. LXXI. July, 1904.]

are nothing more than accessory asexual reproductive organs to which the terms pycnidia and conidia should more aptly be applied. These ideas were followed by Van Tieghem (54) and by Vuillemin, and have of late years gained considerable acceptance [1].

The great objection, however, to the view that the spermatia are of conidial nature is that they seem quite incapable of causing infection; the fact that they appear about the same time as the aecidiospores is also an argument against this view, as will be shown later.

Although it is clear that a careful cytological study would most likely throw great light on the vexed question of sexuality in this group, yet for a very long time our knowledge of the cell structure of the Uredineae was confined to a few scattered observations by Schmitz (49) and Rosen (45). About ten years ago, however, the researches of Poirault and Račiborski (43) and of Sapin-Trouffy (48), a pupil of Dangeard's, threw very considerable light on the cytology of this group. The former observers showed that in many stages of the Uredineae the nuclei were to be found closely associated and dividing in pairs (the *conjugate nuclei* of these authors), each pair in a separate cell. Sapin-Trouffy carried the matter further, and showed that the following very interesting cycle of nuclear development was to be observed in the forms possessing an aecidium (*Eu-* and *-opsis* forms). The mature teleutospore is always uninucleate and gives origin to four uninucleate sporidia from which a mycelium arises in which the nuclei are arranged singly, usually in separate cells. In the aecidium, borne by this mycelium, the nuclei, however, become paired; the aecidiospores thus contain two nuclei, and the paired condition of the nuclei is retained throughout ensuing mycelia and uredospores (if present) up to the teleutospores, which in the young state are binucleate, but at maturity become uninucleate by the fusion of their nuclei. In all cases of division of the paired nuclei the two are very closely associated, and a half of each nucleus goes to the new cell, so that the two nuclei in the cells produced by division are never daughter-nuclei.

These very striking observations throw no light on the nature of the spermatia, for though the latter were shown to be uninucleate, like the cells of the mycelium on which they are borne, Sapin-Trouffy assumed their conidial nature and paid little attention to them. For him the most important stage in the cycle just described was the fusion of nuclei in the teleutospore, a fusion which he considered to be of the nature of a true sexual process [2], and to be comparable with the fusion in the ascus and in the basidium. On the facts observed, however, there does not seem sufficient evidence for such a view; for a process of fusion which takes place in a cell

[1] A full account of the older observations on the nature of the spermatia and the sexuality of the group is given by Klebahn (27, pp. 194–202).

[2] Dangeard and Sapin-Trouffy (13) had earlier considered it as a 'pseudofécondation.'

which shows no signs of specialization as a female reproductive cell, and one in which the ancestors of the fusing nuclei have been associated together in the same cell for many thousands of generations, lacks all of the characters of a sexual process except that of mere nuclear fusion.

It would, in fact, seem obvious that the critical point for investigation in relation to the question of sexuality is the early development of the aecidium, for it is there, in the full life-cycle, that the two nuclei first become associated, and the transition from the single to the paired condition takes place. Sapin-Trouffy, however, imbued with the idea of the importance of the fusion in the teleutospore, paid little attention to this point, but merely states that from the mycelium with single nuclei, binucleate hyphae grow up which cut off a series of binucleate aecidiospore-mother-cells, from which by division the binucleate aecidiospores are derived.

The other results obtained by this worker, such as those on the details of nuclear division (in which he describes the regular presence of two chromosomes and the absence of a spindle) and on chromosome-reduction, left much to be desired, owing to the insufficiency of the methods employed. The figures which he gives are also of a very diagrammatic nature. In spite, however, of the obvious need of further work, the only later contributions of importance are those of Maire (29, 30), published in part after this work was in progress. Maire accepts the results of Sapin-Trouffy, but states that the cells of the aecidium which give origin to the aecidiospore-mother-cells are, in *Endophyllum Sempervivi*, De Bary, and in *Puccinia Bunii*, DC., at first uninucleate, but later become binucleate by a process which he believes to be one of simple division.

The result of cytological observations, as far as they go, is thus to suggest that the Uredineae are totally wanting in any ordinary form of sexuality or of sexual organs, and that the aecidiospores are produced by a structure of the nature of a conidiophore, in which, however, a peculiar association of nuclei takes place. The spermatia, however, still remain as very puzzling structures on which these investigations throw no light, for they are apparently completely wanting in function.

It is interesting to note that during last year Arthur (1), while admitting our ignorance of the question of sexuality in the group, has put forward the view that the aecidium is 'a device to restore vigour to the fungus,' as after this stage the parasite usually spreads and develops very rapidly; the sexual nature of the aecidium is thus suggested on physiological grounds.

An investigation for the purpose of studying the vexed question of sexuality and for settling some of the points left doubtful by Sapin-Trouffy had been projected ever since the appearance of Sapin-Trouffy's work, and was carried out during the last two years. It was clear that what was required was not so much a rapid survey of the whole group, as had been done by Sapin-Trouffy, but the careful investigation of a few

forms with special attention to aecidium-development and the character of the spermatia. With this object there were chosen as likely to be favourable objects, *Phragmidium violaceum*, Wint.[1], a common autoecious *eu*-form found on the various forms of *Rubus fruticosus*, and *Gymnosporangium clavariaeforme*, Rees, a heteroecious form with the spermogonial and aecidial stage on various species of *Crataegus*, and the teleutospore stage (uredospores are wanting) with a perennial mycelium on *Juniperus communis*, L. Material of the last-named form was obtained in abundance from the neighbourhood of Crockham Hill, Kent, where the infected Hawthorn and Juniper are often to be found in close proximity.

A careful study of these forms, especially of *Phrag. violaceum*, shows very clearly that a definite *fertilization* is to be observed in the aecidium, that the Uredineae show a well-marked alternation of sexual and asexual generations, and that the fusion in the teleutospore, the nature of which has been so much disputed, is relatively of unimportance, being a mere preliminary to reduction.

Methods.

The material used was fixed chiefly with either Flemming's fluid (usually the weak formula) or with acetic alcohol which was allowed to act only for a short time. All material, except that for the study of teleutospore-germination, was fixed in the field, an air-pump being used in the case of the non-alcoholic fluids to remove air from the surface of the pieces and so facilitate the penetration of the fluid. The material after fixing, washing, and dehydrating was preserved in equal parts of alcohol, glycerin, and water. For staining, Flemming's triple stain, Benda's iron-haematoxylin, and brazilin were all found very useful; for the latter stain it was found convenient to use, instead of the ordinary alcoholic iron-alum solution, which goes bad very quickly, a solution made by diluting Benda's '*Liquor ferri*' with about nine parts of 70 % alcohol. This mixture remains unprecipitated for many months.

For the study of teleutospore-germination *G. clavariaeforme* was found very favourable. The masses of teleutospores will retain their power of germination in a cool place for over a month. If pieces of the bark with the masses attached are placed for a few seconds in water under an air-pump so as to get them thoroughly wet, and then in Petri-dishes over damp filter-paper at a temperature of 20° C., germination takes place very readily and sporidia are formed in less than three hours. Very good results in the study of nuclear divisions in the germ-tube were obtained by the use

[1] The characters which distinguish this species from that of *Phrag. Rubi*, Wint., are very unsatisfactory and the two forms should probably be united. The form here investigated is obviously the same as that studied by Sapin-Trouffy, which he calls *P. Rubi*, but as there are usually four cells to the teleutospore and only a slight projection at the apex the other name seems preferable.

of Flemming's weaker fluid diluted with an equal quantity of water. After fixing and washing, the material was brought up to the clearing fluid by the method of Overton (41). It was first placed in 10 % glycerin, which was allowed to evaporate either in a warm place or in a desiccator over calcium chloride; the glycerin was then removed with absolute alcohol and the material placed in a 10 % solution of cedar oil in alcohol. After the alcohol had evaporated in a desiccator the material was obtained in cedar-wood oil without the least distortion of the delicate germ-tubes, and with far less trouble and risk than by the passage through various strengths of alcohol, and mixtures of alcohol and clearing fluid. The material can then either be treated by the method of fixing to the slide (see Blackman, 8), or by imbedding small teased pieces in hard paraffin very successful sections can then be made through the teleutospores and germ-tubes. By the latter method alone can the details of nuclear division be successfully studied. For staining the cell-walls of the hyphae a 1 % watery solution of Congo-red, either neutral or slightly alkaline, was found of great use, as it stains the walls of the hyphae without affecting the host-cells.

I have to thank Miss H. C. I. Fraser for very considerable help in preparing material during the latter part of this work.

TELEUTOSPORE AND PROMYCELIUM.

Phragmidium violaceum.

The peculiar nuclear cycle with its sudden transition in the aecidium, as described by Sapin-Trouffy, was fully confirmed in the case of the two forms investigated. It will thus be convenient to start with the first stage of the series with single nuclei—the mature teleutospore—and then work by way of the spermogonia and aecidia to the series with paired nuclei, and after dealing with the uredospores end with the development of the teleutospores and the fusion of the two nuclei into one.

Mature Teleutospore. The teleutospore of this form appears towards autumn, and is to be found in groups on the leaves which remain attached throughout the winter. It is a large structure consisting usually of four thick-walled cells, and a stalk which is enlarged below, this part of the stalk being capable of swelling up in water to a great extent (Fig. 1). The wall of the uppermost cell is provided with a rounded peg-like projection of cell-wall substance which is sometimes perforated by a narrow canal connected with the cavity of the cell below. The whole spore is covered with a thin cuticular layer which is raised into numerous rounded bosses (Figs. 1 and 2). Each cell possesses a delicate endosporium, a continuous, thick mesosporium, and an outer exosporium, which is to be found only as

a sheath round the two middle cells, but exists also round the upper part of the top cell and the base of the lowest cell (Fig. 2). The stalk has a narrow lumen which widens out below (Figs. 5 and 86 *c*); its wall shows only slight differentiation of layers. Each cell has usually four pores which are closed on the outside by the cuticle only, the protoplasm and endosporium projecting slightly into the cavity in the mesosporium and exosporium (Fig. 2). Each cell contains a single central nucleus with a well-marked nucleolus, and dense cytoplasm containing a quantity of yellow oily material which gives to it an orange colour. In the dry state very little can be made of the structure of the nucleus, and except for the nucleolus it appears almost homogeneous.

Germination. Spores taken in March from the 'wintered' leaves germinate readily in moist air or water, but will not do so earlier. The first beginning of the germ-tube (promycelium) is the welling out through one of the pores of a spherical mass of yellow cytoplasm surrounded, of course, with a thin wall. This stage (Fig. 1) is very striking, and is also to be observed in *G. clavariaeforme*. From this spherical mass the cylindrical germ-tube grows out away from the spore (Fig. 5).

The nuclei undergo an interesting series of changes when the spores have been lying in water some time. After twelve hours in this condition the nucleus increases in size, and there is to be observed a somewhat flattened nucleolus pressed close against the nuclear membrane, which often projects at this point. The faintly staining, and hitherto almost homogeneous, mass of the nucleus begins to stain more darkly, and faint indications of a chromatin thread are to be seen (Fig. 3). Soon the chromatin part becomes clear as a single much-twisted thread, at first closely coiled; but as increase in size of the nucleus still goes on the coil becomes a more open and thinner spireme thread. This stage, however, is not a preparation for division, for it appears to last only a short time, and very soon the nuclei return to the ordinary condition and show a nucleus with a chromatin network, and, instead of a single large nucleolus, a few smaller ones (Fig. 4). The nucleus has a large cavity and is only partly filled with the network, so that in the living teleutospore examined in water before germination it appears as a distinct, clear 'vacuole' (Fig. 5), as it was termed by the older observers.

In some few cases *two* smaller nuclei are to be found in the cell of the teleutospore (Fig. 4 *a*) instead of one larger one. These are, no doubt, the original paired nuclei of the teleutospore, which for some reason have delayed their fusion. Their fate is unknown, as two nuclei were never observed to pass into the germ-tube; probably they fuse later. There was no evidence that they represented the results of a precocious division.

The nucleus passes into the germ-tube, being constricted in its passage through the pore, which is only of small diameter (Fig. 6). If the germ-tube

does not reach the air, it usually grows on continually (Blackman, 7), the protoplasm with the nucleus being found at the end of the tube (Fig. 7).

Division of nucleus. This form is not a favourable object for the study of this process on account of the irregularity of the germination and the difficulty of cutting the necessary sections. The divisions in the promycelium were studied fully in the form to be next described, but here it was merely observed that not two (as Sapin-Trouffy states), but numerous chromosomes were formed on division, and that there was a definite spindle with centrosomes.

Sporidia-formation. The four cells of the promycelium contain only small nuclei with a very small amount of chromatin. The sterigmata produced may either be short, as in the typical condition seen in Fig. 8 *a*; or elongated, with more of the appearance of germ-tubes (Fig. 8). The difference appears to depend upon the degree of moisture present (vide infra under *Gymnosporangium*). The whole of the protoplasm does not pass into the sporidia, but a small quantity remains in the promycelial cell (Figs. 8 *a* and 9). The sporidium is a globular or pear-shaped structure with a wall of some slight thickness and irregular in outline (Fig. 8 *a*), a nucleus which sometimes shows no nucleolus, but a well-marked chromatin network (Fig. 10).

The sporidia germinate very readily, sometimes while still attached to the sterigma (Fig. 9), and often form a secondary sporidium which seems always to be binucleate (Fig. 11), and in one case four nuclei were apparently to be observed (Fig. 13). This binucleate condition is also sometimes to be observed in the primary sporidium even while it is still attached to the sterigma (Fig. 12). The condition with two nuclei appears to be fairly common in the primary or secondary sporidia of the group. It has been described in *Puccinia Malvacearum* by Sapin-Trouffy (48), in *Endophyllum Sempervivi* by Maire (29), and for *Coleosporium Euphrasiae* by Poirault and Račiborski (43), and it is to be sometimes found in *G. clavariaeforme*. It appears to be entirely without significance and to be merely a precocious division of the nucleus in which the usual wall-formation is delayed. The nuclei are certainly not paired (conjugate), for in *Phragmidium* and the first two species mentioned above the sporidia have been observed to give rise to a mycelium with single nuclei as in all other known cases; in fact in *E. Sempervivi* Maire states that the two nuclei can be observed to pass into the infecting germ-tube and there become separated by a septum.

Gymnosporangium clavariaeforme.

Mature Teleutospore. The two-celled teleutospores of this form, with their very long gelatinous stalks by means of which the spores are held together in compact yellow masses, are well known (Fig. 14). As noticed by

earlier observers they are of two kinds, thick-walled and thin-walled, the latter being found more usually in the interior of the mass; it is possible, also, that there may be some connexion between the thickness of the wall and the time of year at which the spores are formed, for the first-formed spores seem to be almost all thick-walled [1].

Each cell of the teleutospore contains dense vacuolate cytoplasm with a quantity of yellow oily material which gives the colour to the spore. There is a single nucleus of considerable size with a chromatin network arranged superficially round the cavity and one or more small nucleoli (Figs. 14 and 15). The wall of the spores shows usually no clear distinction into layers. Near the septum, the wall of each cell (of the thick-walled spores) is thinner at one spot, and into the cavity so produced the protoplasm projects slightly (Fig. 14). It would seem that the wall is never thickened at these spots, for such thin places in the wall are found in comparatively young spores; they are thus to be considered of the nature of pores. These pits are not to be found in the thin-walled spores (where they are obviously unnecessary), but the naturally thin wall may project slightly at the corresponding points.

Germination. Under suitable conditions of moisture and temperature germination takes place very readily, in a moist atmosphere at 20° C. the promycelium being formed and the sporidia developed in less than three hours. The cytoplasm forces its way through the pore described above, and, after swelling out into a spherical mass, as in *Phragmidium*, attains a considerable length before the nucleus migrates into it (Fig. 16). The nucleus, very much constricted and condensed in its passage through the pore, takes up a position in the middle of the tube and is there to be seen as a narrow elongated body with a few small nucleoli, and granular chromatin which forms usually only an indistinct network. Under suitable conditions of air-supply the nucleus then proceeds to divide.

Nuclear division. The first sign of division is the gathering of the granular chromatin material towards one end or the middle of the nucleus, while at the same time a small, deeply staining, spherical body, the centrosome, is to be observed in the cytoplasm and connected with the nucleus by a portion of slightly differentiated cytoplasm or kinoplasm (Fig. 17). The chromatin then forms a closely coiled spireme thread in the middle of the nuclear area and the outline of the nucleus becomes very faint (Fig. 18); the centrosome was not traced at this stage. The thread then becomes apparently broken up into a number of narrow, elongated

[1] The suggestion put forward by Kienitz-Gerloff (Bot. Zeit., xlvi, 1888, p. 388) and Dietel (Hedwigia, xxviii, 1889, p. 99), that the thin-walled spores represent in this genus the endospores, cannot be accepted. Both kinds of spores are uninucleate, and both are able to form normal sporidia; the long, undivided tubes put out by the thin-walled spores were no doubt the result of special conditions of growth (see Blackman, 7).

chromosomes (Fig. 19 *a*), but they lie so close together that their exact number and length are difficult to make out; there are certainly at least ten. At this stage the spindle was first observed as a very short structure with well-marked centrosomes at each pole, lying among, but without any distinct relation to, the chromosomes (Fig. 19 *b*). From the fact that the centrosome with a mass of kinoplasm was first observed outside the nucleus, and from analogy with the second division, there can be no doubt that the spindle is formed in the cytoplasm, between two centrosomes which arise by division of the one observed earlier, and that it later comes into association with the chromosomes.

The spindle gradually increases in length, and the closely packed, ill-defined chromosomes come into close relation with it (Fig. 20). As the spindle lengthens the chromosomes become confined to the more central part of the spindle (Figs. 21, 22), which, if originally oblique (Fig. 22), takes up a position parallel to the long axis of the germ-tube. The spindle still continues to elongate and the chromosomes to spread out, so that when the spindle has attained its full length, they occupy usually two-thirds of its length (Figs. 23–5).

This stage, at which the spindle has reached its full length, seems to correspond with the metaphase, being the one most frequently met with; but no distinct equatorial plate is ever formed. At this stage the form of the chromosomes is usually almost completely lost, and they appear to have partly fused together, a few free ends (Fig. 23) or the presence of irregular lumps of chromatin (Figs. 24, 25) alone suggesting their existence.

The chromatin material then begins to move towards the poles, but rarely, as in Fig. 26, are two distinct polar groups to be seen. Usually this stage cannot be sharply distinguished from the last, and the chromatin lumps merely become strung out from pole to pole and often showing a tendency to form two elongated masses (Fig. 29). Sometimes two irregular twisted threads forming two clumps can be seen moving towards each pole (Fig. 28), and occasionally the masses moving towards the poles can be seen to have formed two chromatin networks (Fig. 27). After this stage, the suggestion of separate chromosomes becomes usually completely lost and the chromatin merely stretches out from pole to pole as two elongated dumb-bell-shaped masses (Fig. 30). The chromatin then collects at the poles, forming there one irregular mass (Fig. 31), or the distinction into separate masses may be clearly maintained at each pole (Fig. 32).

During these stages the centrosomes show clear but faintly staining polar radiations, which are often of very considerable length (Fig. 24), and in the case shown in Fig. 31 extended to the free end of the germ-tube. When the chromatin has collected at the poles the two (or four) masses move apart

and the spindle becomes stretched out and very narrow; the centrosomes and radiations are however still clearly visible (Fig. 31). The daughter-nuclei are then formed; each is a somewhat pear-shaped body and consists of a mass of perfectly homogeneous staining material. At the pointed end of each, the centrosome can be clearly observed; it is not attached directly to the nucleus, but is connected with it by means of a small portion of kinoplasmic material which seems to correspond with the end of the former spindle (Fig. 33). The centrosome shows distinct radiations (Fig. 33).

Under the conditions under which the teleutospores were germinated the two second divisions follow immediately on the first. The centrosome becomes divided into two (Fig. 34), and between them the spindle appears (Fig. 34 *a*). At the same stage the nucleus becomes larger and irregular in shape, and a few granules appear in it; in Fig. 34 *a* they are to be seen just where the spindle is in contact with the nucleus, over the edge of which it lies. The spindle then becomes arranged in the long axis of the germ-tube, and shows distinct polar radiations, while the nucleus, which has shown no definite wall from its first formation, is seen as a mass of chromatin lying in an irregular way over the spindle (Fig. 35). The chromatin then becomes more symmetrically arranged round the spindle, and is seen to be granular throughout (Figs. 36, 37). The chromatin then takes the form of a distinct *network*, which at first covers only part of the spindle (Fig. 38 *a*), but soon spreads over the whole of it, leaving only the centrosomes visible (Fig. 38 *b*). The network of chromatin then becomes drawn apart as two portions towards the poles (Fig. 39). In a later stage the threads which connected the two main masses may remain visible for a time at the poles, and resemble chromosomes (Fig. 40). The chromatin then collects at each pole usually as a single mass (Fig. 41), but not infrequently it forms two distinct masses (Fig. 41 *a*). The four daughter-nuclei when they are first formed show, as in Fig. 42, a somewhat pear-shaped mass of lightly staining homogeneous material, which bears at the pointed end a mass of kinoplasm and a distinct centrosome, as in the corresponding stage of the first division. The promycelium then becomes divided into four cells, and the nuclei gradually take on a normal appearance, a chromatin network becoming visible and the centrosome apparently disappearing (Fig. 43).

It is clear that though the division in the promycelium of *Gymnosporangium* is much more typical than Sapin-Trouffy supposed, yet there is certainly no chromosome-formation in the second division, and in the first division, though distinct chromosomes appear to be present, they seem soon to lose their individuality, so that of their splitting or regular separation there is very considerable doubt.

The two chromatin-groups, which are often to be observed both in

the first and second division, probably represent the chromatin derived respectively from the two nuclei which fused in the teleutospore. It was these two masses which Sapin-Trouffy mistook for two chromosomes, as a comparison of his figures with Figs. 30, 32, and 41 *a* clearly show.

The method of spindle-formation, in which the spindle is formed free in the cytoplasm between two centrosomes and later comes into relation with the dividing nucleus, is of great interest. It is clearly of the type described, in animals, by Hermann and others for the *Centralspindel.* The only case in plants with which it is at all comparable is the peculiar method of spindle-development described by Lauterborn (28) for Diatoms [1].

Since the work of Sapin-Trouffy, who failed to discover either a spindle or chromosomes in the divisions in the promycelium, it has been shown by Juel (25), in *Coleosporium Euphrasiae*, that a spindle and polar radiations were present, and that the division was of a much more typical nature than Sapin-Trouffy had described. He was not, however, able to determine the behaviour of the chromatin part of the nucleus. When this work was complete a paper appeared by Holden and Harper (22), in which it was shown that in the divisions of the developing teleutospore of a form of *Coleosporium* on Callistephus, &c., to which they give the name *C. Sonchi-arvensis*, a spindle with centrosomes, polar radiations, and showing numerous chromosomes was to be observed, as in *Gymnosporangium.* They did not, however, trace the origin of the spindle, and they give but a few figures of the behaviour of the chromatin, for *Coleosporium* does not seem to be nearly so favourable an object of study as the form here investigated. They are of the opinion that both divisions are typical in nature.

Sporidia-formation. There is nothing worthy of special comment in the normal formation of sporidia. A primary sporidium is shown, in section, at Fig. 25.

In some cases the original four cells of the promycelium may round themselves off, separate, and put out germ-tubes, thus behaving like sporidia. Long germ-tubes may also be put out by the four cells while they are still attached, as shown in Fig. 44. Although the exact conditions were not investigated, this method of shortened development seems to be dependent on conditions of moisture, and is probably a response to growth under water or at least in a very moist atmosphere. It has been shown in an earlier paper (7) that promycelia actually growing under water develop to a great length, but rarely divide, and never form sporidia. A single case was there figured for *Phragmidium*, where the promycelium had divided

[1] P. Denke (Beihefte z. Bot. Centralbl., xiii, 1902, p. 182) has described the development of an extra-nuclear spindle, though, of course, without centrosomes, in the division of the microspore and megaspore mother-cell of *Selaginella.*

under water, and one of the four cells was putting out a germ-tube[1]. Inside the gelatinous mass of teleutospores access to free air must be difficult, and it is probably in such a situation that the promycelial cells tend to germinate directly.

Such cases as these of *Gymnosporangium* and *Phragmidium* show that *the real germinating unit is the promycelial cell*, and that under certain conditions it can become a separate spore, put out a germ-tube and behave as a sporidium, and no doubt cause infection. When growing normally, however, in air the development of the germ-tube is arrested, and it becomes a mere sterigma, on the end of which the primary sporidium is developed. The primary sporidium thus appears to be merely the arrested and swollen germ-tube put out by the promycelial cell, just as the secondary sporidium is merely the arrested and swollen germ-tube of the primary sporidium. The sporidia are probably nothing more than special adaptations to the development of the promycelium in air; in the absence of air such a development would be unnecessary, for there would be no chance of the wind-dispersal of the sporidia. The primary sporidium is thus really secondary in nature, and bears the same relation to the promycelial cell (the true primary spore) as does the so-called secondary sporidium to the first-formed one.

It is evident that the formation of the so-called promycelial cells is really nothing more than the division of the contents of the teleutospore into four spores, which may separate as such, but usually remain united and form four secondary spores—the sporidia. As far as is known, the teleutospore is quite incapable of forming a mycelium; it seems then time that the term *promycelium* should be dropped, and the process of development considered merely as one of spore-formation.

That the later development of the teleutospore is really a process of spore-formation has been obscured not only by the formation of sporidia (really the secondary spores), but also by the fact that, owing to the thickness of the spore-wall, a process of germination is a necessary preliminary to division. In *Coleosporium*, however, where the teleutospores are thin-walled and develop *in situ*, there is no process of germination, but the spores become directly divided into four cells, which normally put out each a sterigma and form sporidia. Holden and Harper (23), however, in this genus have lately observed cases in which the divisions of the teleutospore rounded themselves off and became directly spores. In such cases, the teleutospore, its real nature no longer obscured either by the process of germination or by the formation of sporidia, is clearly seen to be *a spore-mother-cell, which undergoes a tetrad division to form four spores.*

[1] A somewhat different interpretation was placed upon this case at the time, but a comparison with *Gymnosporangium* gives the clue to its real nature. Sapin-Trouffy (48) has described a case in *P. Malvacearum* in which submerged promycelial cells separate and put out germ-tubes, which become sterigmata (and bear sporidia) only if they reach the air.

MYCELIUM AND SPERMOGONIA.

Phragmidium violaceum.

The mycelium which appears in the leaf soon after germination of the teleutospores is derived by infection from the sporidium, and soon bears the spermogonia; it consists of numerous hyphae with *single* nuclei, which appear to be always enclosed in separate cells. Most of the hyphae are intercellular; some penetrate the cells, but they appear to have no definite connexion with the nuclei of the host-cells, as in the haustoria described by Sapin-Trouffy for some forms.

The spermogonia are found usually on the upper side only of the leaf. Like the aecidia they are of indefinite extent, and are to be found as simple layers of parallel spermatial hyphae developed beneath the cuticle. Each irregular layer or spermogonium can be distinguished into a number of slightly projecting 'hummocks,' above which the cuticle is perforated to form the ostiole. One of these 'hummocks' is shown in section in Fig. 46. The spermogonia have usually no paraphyses, but occasionally one or two spermatial hyphae may grow out as sterile threads and project through the aperture in the cuticle.

The spermogonia are formed by hyphae which grow up between the epidermal cells, and form beneath the cuticle a mycelial bed, or plectenchyme, of uninucleate cells. From this there arises a compact mass of parallel spermatial hyphae. The spermatial hyphae of any given group all point their free ends towards the aperture in the cuticle in the centre of the hummock (Fig. 46). Each group of spermatial hyphae with its ostiole should probably be looked upon as a single spermogonium, several spermogonia being collected together to form a composite structure. The cuticle, which is of course pushed up by the growth of the hyphae, becomes very much thickened at these spots, and stains very deeply with the safranin of Flemming's triple stain and with iron-haematoxylin (Fig. 46).

Each spermatial hypha contains a single, usually elongated, nucleus with a chromatin network, one or two small nucleoli, and an indistinct membrane. The spermatia are budded off from the tips of the hyphae, a projection being first pushed out from the apex of the hypha, and when it has reached its full size the nucleus passes into it. The end of the hypha has a ring of special cell-wall, staining deeply with Congo-red, as in *Gymnosporangium*, which offers a much more favourable object for studying the development of the spermatia.

The mature spermatia are minute uninucleate cells more or less oval in shape. Each contains a comparatively very large nucleus, which shows a dense chromatin network but no nucleolus; surrounding this is a thin

layer of very finely granular cytoplasm with apparently no reserve-material, and the whole is enclosed in a very thin cell-wall.

The spermatia make their escape through the ostiole, and are found spread out on the leaf in the immediate neighbourhood. They took no part in aecidium-formation or in any other process, and numbers of them when observed on the leaf-surface appear to be in a disorganized state, in which the distinction between nucleus and cytoplasm is almost completely lost.

Gymnosporangium clavariaeforme.

The mycelium in the leaves of the Hawthorn which bears the spermogonia shows clearly the *single* nuclei apparently always enclosed in separate cells. They are of small size, and usually show no distinct nucleolus, and their nuclear membrane is often indistinct. The spermogonia appear very early after infection, in about seven to ten days.

The spermogonia are flask-shaped structures of the usual type, and are developed beneath the epidermis. They arise from a layer of small-celled 'tissue' (plectenchyme), which gives origin to a number of upwardly directed and parallel hyphae, the spermatial hyphae. Certain of these hyphae, chiefly the peripheral ones, grow out to form the paraphyses which project through the ruptured epidermis. The others bud off each a series of spermatia, which collect in the cavity of the flask, and later become extruded (Fig. 48).

This form is a favourable object for studying the exact development of the spermatia. The spermatial hyphae are narrow, elongated cells with a central elongated nucleus, which shows a granular chromatin network and one or more small nucleoli; the nuclear membrane, however, is indistinct (Fig. 49). The hypha contains only finely granular cytoplasm. It is furnished at the free end with a curious ring of thickening, which is easily rendered visible by the fact that it takes the Congo-red with more avidity than the rest of the cell-wall (Figs. 49–54).

The first beginning of the development of a spermatium is the pushing out of a finger-like projection from the free end of the hypha, thus displacing the last-formed spermatium (Fig. 49). This projection contains exceedingly finely granular protoplasm. When it has attained its full size, or a little earlier, the nucleus of the spermatial hypha begins to undergo a process of condensation (Fig. 50), so that it is transformed into a homogeneous and deeply staining chromatin mass (Figs. 51 and 55), the nucleolus or nucleoli being apparently squeezed out into the general cytoplasm (Fig. 55 *a*). The condensation of the nucleus may begin at one end and progress downward, as shown in Fig. 50. The mass of chromatin then becomes drawn apart into two masses (Figs. 52, 56), which remain

connected by a thread of kinoplasmic substance. The two become finally separated, and the upper one, passing through the thickened girdle, moves into the spermatium (Fig. 53). The lower chromatin mass passes back into the resting state (Fig. 54), and the process is again repeated. The spermatium becomes cut off from the hypha by a wall formed just above the thickening ring, which may be connected with the disjunction of the spermatium.

As in the divisions to be described later, the nuclei sometimes show not a single chromatin mass, but *two* masses, which are drawn out separately towards the respective poles (Fig. 57).

The only trace of spindle-formation was the connecting strand between the two separated chromatin masses. It may be that a rudimentary spindle is present as in the case of the conjugate divisions, but is obscured by the chromatin mass. The whole method of division is obviously of an exceedingly simple type.

When the spermatium separates from the spermatial hypha its nucleus is an almost homogeneous and deeply staining mass (Fig. 58), but in the mature state becomes larger and shows a dense network without a nucleolus. As in *Phrag. violaceum*, the mature spermatia (Fig. 59) are small cells with a large, dense nucleus, very little cytoplasm, a thin cell-wall and apparently no reserve-material.

The spermatia, when extruded from the spermogonium, collected on the leaf in apparently sticky masses. Many of them seemed, as in *Phragmidium*, soon to undergo a process of degeneration, in which the staining distinction between nucleus and cytoplasm became lost, and in one case this process of disorganization was observed in some of the spermatia while they were still enclosed in the spermogonium.

The spermatia were never observed germinating or taking any part in aecidium-formation.

The formation of spermatia has been investigated by Sapin-Trouffy (48) in *Uromyces Erythroni*, DC., and other forms, and in *Puccinia Liliacearum*, Duby, by Poirault and Račiborski (43), and also by Maire (29, 30); the observations here given confirm and supplement their results. Both Sapin-Trouffy and Maire, however, describe two chromatin masses as always present on nuclear division, and consider them to be of the nature of chromosomes. These two structures are not always present in *G. clavariaeforme*, and they certainly cannot be considered of the nature of true chromosomes, as a comparison with the divisions in the promycelium shows, for these chromosomes are formed and are much more numerous. This matter will be discussed later in dealing with conjugate division.

AECIDIUM-DEVELOPMENT.

Phragmidium violaceum.

The so-called aecidium in this genus, like that of *Caeoma* (=*Melempsora* in many cases), is characterized by a very simple structure, for unlike that of the other genera of the group it is neither definite in shape nor bounded by a thick-walled pseudoperidium. In the form investigated the aecidium is nothing more than a group, indefinite in extent, of aecidiospore-bearing cells, bounded at the periphery by a number of thin-walled paraphyses, which are, however, sometimes wanting. It is clear that this aecidium is no more a definite organ than is the sorus of uredospores or teleutospores, which has an exactly similar arrangement—a group of spore-bearing cells surrounded by paraphyses. The aecidiospores are developed immediately beneath the epidermis, and not deeper down in the tissues as in the typical aecidium.

The first beginning of the aecidium is the massing of hyphae beneath the epidermal cells of the leaf, usually on the lower side. The hyphae form there a layer of uninucleate cells, two or three cells thick. The cells immediately beneath the epidermis increase somewhat in size, and soon become divided by a transverse wall, parallel with the surface of the leaf, into an upper and lower cell, each with a single nucleus. The upper cell remains more or less cubical, and shows a vacuolate protoplasm and a small nucleus, which has no nucleolus and sometimes remains as a dense structure without returning completely to the resting state (Fig. 66). The lower cell elongates considerably, and shows abundant granular protoplasm and a large nucleus with a well-marked nucleolus and a clear chromatin network (Figs. 61, 66, 70). The upper cell is a *sterile cell*; its nucleus becomes disorganized, and it is soon destroyed by the upward growth of the cell below. It is from the lower cell, which may be called the *fertile cell*, that the aecidiospores arise; for after a pause in its development it becomes *binucleate*, and proceeds to elongate (Fig. 61) and cut off a series of binucleate aecidiospore-mother-cells (Fig. 60), the pair of nuclei dividing together by the process of *conjugate division*.

The discovery of a sterile cell is alone a very important fact, for it shows that the aecidiospore-producing cell or hypha, at least in *Phrag. violaceum*, is a very specialized reproductive cell and not simply of the nature of an ordinary conidiophore.

The process by which the fertile cell became binucleate was naturally closely investigated, but all attempts to observe the single nucleus of the fertile cell in any stage of division were quite unavailing, in spite of the fact that other divisions were met with fairly frequently, and that in passing from the periphery to the centre of an aecidium of an appropriate

age one can observe the transition from the uninucleate to the binucleate condition. This failure led to a close examination of the fertile cells at about the point of transition, and it was observed that in a number of cases the fertile cell was occupied by two nuclei of different size and structure (Figs. 62–65). One was usually a larger nucleus with the characters given above for the original nucleus of the cell, the other, usually a smaller, denser nucleus containing no nucleolus or only a small one, and having, in fact, more the characters of a nucleus of an ordinary cell of the mycelium.

The differences in size and structure of these two nuclei was hardly compatible with the view that they were sister-nuclei, and when this fact was further considered in conjunction with the absence of all stages of the necessary division, there seemed no escape from the view that the smaller, denser nucleus must have had some other origin. This view was amply confirmed by the discovery of a number of cases in which *a nucleus was actually found passing into the fertile cell from one of the smaller cells of the mycelium at its base* (Figs. 66–70)[1]. The migrating nucleus is reduced to a narrow thread during the process, the actual aperture through which it passes being very small; neither before nor after its passage could a pit in the wall be observed[2].

In many cases there is to be found lying below the fertile cell a cell which obviously belongs to the same cell-row; it may be called a *basal cell*, though it is in no way specially differentiated. When this basal cell is present it is often from it that the migrating nucleus comes; and it is interesting to note that the nucleus seems to pass more often into one of the neighbouring fertile cells (Figs. 66, 67, and 71), with which it is in contact at the sides owing to the irregularity in length of these cells, rather than into the fertile cell immediately above it (Figs. 68 and 69). In the former case the relationship between the two nuclei which meet in the fertile cell may be considerably distant, while in the latter case the two nuclei are separated in origin only by one division, that which cuts off the sterile cell.

In some cases in which the fertile cell contained two dissimilar nuclei, the smaller, denser one lay in the upper part of the cell, and at the same time the sterile cell was without a nucleus (Fig. 65). It seemed then possible that the nucleus of the sterile cell might have migrated into the fertile cell below; but no direct evidence could be obtained for this view, and it is rendered improbable by the fact that a similar state of affairs was

[1] More than twenty-four cases were observed in which the fertile cell contained two dissimilar nuclei and more than fifteen cases in which a nucleus was in a stage of actual migration.

[2] The migration of nuclei in the tissues of Phanerogams which have been placed under abnormal conditions has been described by several observers (cf. Koernicke, Ber. d. Deutsch. Bot. Ges. xxi, 1904, p. 100).

to be observed in the fertile cell while the nucleus of the sterile cell above was still in position (Figs. 63 and 64). The disappearance of the nucleus of the sterile cell is doubtless to be accounted for by early disorganization, and the peculiar position in the fertile cell of the small nucleus by a change in position after migration, or by the fact of its having entered at the side.

It would appear that the two nuclei of the fertile cell soon become similar in size and shape, for it is only occasionally that a dissimilarity can be observed. Very soon after this condition has been attained the fertile cell proceeds to elongate, pushing its way through the sterile cell (Fig. 61) and soon completely destroying it. The paired nuclei divide by the process which has been termed conjugate division, and the fertile cell cuts off a series of cells (aecidiospore-mother-cells), which do not develop directly into aecidiospores, but each cuts off a small cell below, known as the intercalary cell (Figs. 71–74). It has been suggested that the function of this cell, which becomes disorganized and disappears soon after its formation, is to act as a disjunctive apparatus, and so bring about the complete separation of the aecidiospores. A mature aecidiospore is shown in Fig. 75. Most of the fertile cells become binucleate and develop further, for in older stages of the aecidium only a few of the fertile cells are found, here and there, still uninucleate. It is no doubt these cells, which never achieve the binucleate condition, that are found crushed and distorted in still later stages[1].

Although two is the usual number of nuclei in the fertile cell, the number *three* is nearly always to be found in one or two cells of each aecidium, and in one case it was found no less than four times in a single aecidium; but in comparison with the normal number it is of course very uncommon. The three nuclei divide together by a process of conjugate division (Fig. 77), and trinucleate aecidiospore-mother-cells (Fig. 76) and aecidiospores are produced, the fate of which is unknown. In one case also a fertile cell with four nuclei was to be observed.

How this multinucleate condition is brought about is not quite clear; it may be due to the migration of more than one nucleus into the cell, or the division of one or both of the nuclei without cell-wall formation. In a fertile cell which was yet undivided one of the nuclei was observed to be apparently undergoing division while the other was in the resting state. When three or four nuclei were observed in an undivided fertile cell it was interesting to note that their size was usually considerably less than that of the normal paired nuclei.

[1] The fertile cells are often in contact with several of the cells of the tissue below, owing to the irregularity in length of the former, and the usually narrow form of the latter when they do not form distinct *basal cells*; a more than sufficient number of nuclei is thus at hand to supply all the *fertile cells*.

The development of the aecidium is centrifugal, and when it has reached a certain size the peripheral cells, instead of forming ordinary sterile and fertile cells, grow out into uninucleate paraphyses: these may, however, in some cases may be absent.

The process of nuclear migration, by means of which the fertile cell is stimulated to rapid growth and continued division, is clearly a process of *fertilization*, the exact relation of which will be discussed later[1].

Gymnosporangium clavariaeforme.

The first beginning of the aecidium takes place deep down in the hypertrophied tissue of the leaf, &c., after the majority of the spermogonia have faded. The aecidium in this genus is of the typical form, with a definite pseudoperidium. Unfortunately the very early stages, which are much more difficult to obtain than in *Phragmidium*, were not observed, so that the presence or absence of sterile cells and the exact behaviour of the nuclei could not be investigated. It was clear, however, that the transition from the condition of single to that of paired nuclei took place, as described by Sapin-Trouffy, in connexion with the aecidiospore-bearing cells (fertile cells); for while in the whole mycelium, and even in the dense layer of fungal cells surrounding the actual aecidium, the nuclei were single, yet the fertile cells and their products showed clearly two nuclei in the paired (conjugate) condition.

It is evident that a comparative study of aecidium-development in the Uredineae generally, with special relation to the existence of sterile cells and the origin of the binucleate condition, is much to be desired. The only other forms of which we have any information are *Endophyllum Sempervivi*, De Bary, and *Puccinia Bunii*, DC., in which Maire (29, 30) is of opinion that the two nuclei of the fertile cell are daughter-nuclei arising by division of the original single nucleus. The migration of nuclei, however, is very easily overlooked unless special attention is drawn to such a point; it is interesting to note that Maire does not appear to have observed in *Puccinia Bunii* the actual division, for he merely states, 'A little later, it can be seen that the terminal cells [fertile cells] contain each two nuclei' (30, p. 39). It is of course possible that in the aecidium of *Phragmidium* with its simple structure we have a more primitive condition, and that in some other forms the association of nuclei of two different cells may have been replaced by association of daughter-nuclei of the same cell; but judgement must be suspended in the matter.

[1] Whether any protoplasm passes over with the migrating nucleus could not, of course, be determined.

MYCELIUM AND DEVELOPMENT OF UREDOSPORES.

Phragmidium violaceum.

The uredospores appear on the leaves about June and replace the aecidia, which are only short-lived. After passing the aecidial stage the parasite appears to have gained greatly in energy, for the degree of infection is now much greater[1]. The mycelium in the leaf which gives origin to the uredospores shows, naturally, paired nuclei like the aecidiospores from which it was derived.

The details of uredospore-formation were not investigated in the leaf, but from some patches on older stem-portions where the mycelium was perennial and gave rise to uredospores early in the year, even before the aecidia were developed on the leaves. These patches were found to be much more favourable objects of study than those on the leaves, the general form of which has been figured by Sapin-Trouffy. The perennial mycelium was found to form a layer of considerable thickness, in which even thin sections show a considerable number of the nuclei arranged in pairs (Fig. 78), but as the two nuclei of these cells often separate for some distance, the paired condition cannot always be observed. A number of the hyphae penetrate the cells of the host, thus acting as haustoria (Fig. 99), but they did not appear to have that special relation to the nucleus of the host-cell described for several cases by Sapin-Trouffy.

The stalked uredospores are borne on somewhat rectangular cells which may be termed *basal cells*. They grow up from the free surface of mycelium and form a regular layer (Fig. 79). These cells are usually vacuolate, and show two nuclei which are larger than the nuclei of the mycelial cells and exhibit each a well-marked nucleolus and granular chromatin. The uredospore arises from the basal cell as a binucleate outgrowth (Figs. 80, 81), which soon becomes of a somewhat oval shape. The two nuclei then undergo the process of conjugate division (Fig. 82) and the outgrowth becomes divided into two cells (Fig. 83), the upper increasing much in size and forming the uredospore, the lower remaining narrow, but elongating later and forming the stalk.

The wall of the uredospore becomes much thickened, but a very definite pit through which there is distinct protoplasmic continuity connects, for some time, its cavity with that of the slightly thickened stalk (Fig. 83 *a*). In the mature state, however, before the uredospore separates from the stalk, the pit becomes obliterated.

[1] The discovery of a process of fertilization is, of course, a confirmation of the interesting view, put forward by Arthur (1, 2), on other grounds, 'that the aecidium is a device to restore vigour to the fungus.'

The nuclei of the free uredospore have sometimes a peculiar, irregular contour as seen in Fig. 84; this appears to be only a temporary phase of development.

DEVELOPMENT OF TELEUTOSPORES.

Phragmidium violaceum.

The teleutospores appear on the leaves towards autumn, and are at first mixed with the uredospores, but later arises in sori which consist of teleutospores only, surrounded by a layer of paraphyses. The mycelium on the leaf from which they arise shows a septate mycelium with a pair of nuclei in each cell. In Fig. 85 the paired nuclei can be distinctly seen in the haustoria in the cells marked * and in several of the other cells, but owing to the thinness of the section the nuclei appear single in a number of cases.

From this mycelium there grow up, when the teleutospore sorus is to be developed, a number of rectangular, binucleate basal cells closely packed beneath the epidermis. It is from these special cells (which Sapin-Trouffy has figured in a diagrammatic way) that the teleutospores are developed. The young teleutospore appears first as an elongated, binucleate, cylindrical outgrowth from the basal cell. It increases in diameter, and soon cuts off from the apex downwards a series of three or four superposed cells (Figs. 86, 86 *a*), which form the body of the teleutospore, the lowest cell becoming the stalk. The upper cells take on the characteristic shape, and the walls become thickened. The upper cell grows out into a short, narrow projection (Fig. 86 *b*), the cavity of which soon becomes completely or almost completely obliterated by the great increase in thickness of its wall; there is thus produced the knob-like projection characteristic of the mature spore.

The lowest cell, the stalk, increases very greatly in length, and its cavity becomes almost completely obliterated by the thickening of its walls, except in the lowest part where it is inserted on the basal cell. Here there is a cavity of considerable size in which the two disorganized nuclei are to be found (Fig. 86 *c*). Some evidence was obtained of a pit connecting for some time the end of the stalk with the basal cell (as in the case of the uredospore and its stalk); the absence of thickening at this point, and the peculiar widening of the cavity shown in Fig. 86 *c*, strongly suggest such a connexion, though its existence was not established with certainty.

During the process of thickening of the wall of the spore the nuclei in the cells show each a single well-marked nucleolus and granular chromatin, and are usually to be found close together (Figs. 86, 86 *a*). When the wall is fully thickened the process of nuclear fusion begins. In the first stage the two nuclei are found in close contact (Fig. 87 *a*); in the second stage instead of two small nuclei one larger one is found, but the two

nucleoli remain for a time without fusion (Fig. 87 *b*), as described by Sapin-Trouffy. In the next stage the nucleus has increased somewhat in size, the two nucleoli have been replaced by one large one, and the chromatin, which has hitherto not been well marked, begins to stain more deeply (Fig. 87 *c*). The nucleus still increases in size, and the chromatin becomes resolved into a very well-defined thread which is apparently a single spireme (Fig. 88 *a*). This, however, is not the precursor of division, for the nucleus still increases in size and the chromatin returns to the condition of a fine network with the nucleolus as a somewhat elongated body flattened against the nuclear membrane (Fig. 88 *b*). The nucleus then goes into the resting state, in which it passes the winter, appearing as an almost homogeneous body except for the well-marked nucleolus (Fig. 2). The process of increase in size and of formation of a spireme thread seems to correspond with the changes included under the term *synapsis* in the higher plants and animals, but it is not followed by nuclear division but by a period of rest in which the spireme again disappears. A somewhat similar case has been described by Williams (60) for the tetraspore-mother-cell of *Dictyota*, where after *synapsis* a similar disappearance of the spireme thread and a period of rest was found to occur.

Gymnosporangium clavariaeforme.

The perennial mycelium which inhabits the stems of *Juniperus* and bears in spring the yellow masses of teleutospores shows very clearly the paired nuclei in the cells of hyphae, which are thick-walled as described by Sapin-Trouffy and others. As in the case of the teleutospores and uredospores of *Phrag. violaceum*, the teleutospores of this form are not borne directly on the mycelium but arise from comparatively large rectangular cells, which form a close-set layer on the surface of the mycelium, at the points where the teleutospores are developed. These each contain two nuclei which are somewhat larger than those of the ordinary cells of the mycelium, and show each a well-marked nucleolus (Fig. 89). They are similar to the teleutospore-bearing cells described by Sapin-Trouffy for *G. Sabinae*, but their side and lower walls are of considerable thickness. Each of these cells gives origin to a number (not more than three or four) of narrow outgrowths which develop into the stalked, two-celled teleutospores (Fig. 89). The outgrowths have paired nuclei (Fig. 90) and soon undergo two divisions, the first cutting off the stalk-cell, while the second divides the upper cell into the two cells of the teleutospore. The teleutospore then increases in size, the wall becomes thickened, and the cytoplasm vacuolar and filled with yellow, oily reserve-material.

The two nuclei in the teleutospore fuse, earlier than in *Phragmidium*, before the spore has obtained its full size or thickness of wall. The wall

at the point of contact of the two nuclei disappears and the two nuclei fuse, the two nucleoli being distinguishable for some little time after fusion (Fig. 91 *a*, *b*, and *c*). The fusion-nucleus then increases in size and goes through changes exactly similar to those of *Phragmidium*. A single large nucleolus appears, and the chromatin becomes resolved into a thick, continuous, spireme thread (Fig. 92). Here, also, this condition is merely temporary, for the thread after a time becomes replaced by a fine network, with usually only small inconspicuous nucleoli (Figs. 14 and 15), as described under the mature teleutospore. As in the case of *Phrag. violaceum*, these nuclear changes must be looked upon as a process of *synapsis*.

METHOD OF 'CONJUGATE' DIVISION.

The method of division of the paired nuclei is of the same simple type as that described for the single nuclei of the spermatial hyphae, but the two nuclei are always to be found in the same stage of division and in very close association during the process. This division can best be observed in the development of the aecidiospores, uredospores, and teleutospores.

The first step in the process is the disappearance of the nuclear membrane and the condensation of the chromatin of each nucleus into a homogeneous, irregular mass, with the natural result that the single nucleolus (which is always present at least in the nuclei of the cells connected with spore-formation) comes to lie free in the cytoplasm (Figs. 93 *a*, 95 *a*). Sometimes the chromatin can be observed in a state where it is not yet completely homogeneous and still retains in part the form of the original nucleus (Fig. 98 *a*). In the next stage the chromatin mass becomes more regular (Figs. 93 *b*, 95 *b*), and in favourable cases can be seen to be connected with a thread-like structure (Figs. 72, 93 *b*), which has the staining reactions of kinoplasm, and is no doubt to be considered as of the nature of a simple spindle. The chromatin mass then becomes elongated to form a thick rod which is apparently stretched out on the spindle, for the latter is completely obscured (Figs. 93 *c*, 95 *c*). Each chromatin rod then becomes drawn out into two more or less pear-shaped masses (Figs. 93 *d*, 95 *d*), which at first remain connected by a fine thread which has the staining reactions of kinoplasm (it takes the gentian in the triple stain), and is no doubt the drawn-out spindle (cf. divisions in promycelium). As the two masses move still further apart the connecting thread becomes very faint and ceases to be continuous (Fig. 93 *d*). At this stage, or somewhat earlier, the rejected nucleoli, which have hitherto lain almost unaltered in the cytoplasm, begin to decrease in size and finally disappear, though they may remain visible up to the stage in which the new nucleoli have begun to appear in the daughter-nuclei (Fig. 93 *e*).

In some cases, as in the case of the divisions of the single nuclei, it can clearly be observed that the chromatin does not form a single rod but a double rod (Fig. 96), which becomes drawn out into *two* pairs of chromatin masses, so that at this stage of the anaphase the nuclei show two opposite groups, consisting of four chromatin masses (Figs. 97 and 98). How far the presence of these two masses in the nucleus is of common occurrence it is difficult to say, for if the plane of separation of the two lies at right angles to the direction of view they would easily be overlooked. There would seem to be no doubt, however, that they are only occasionally present in the divisions in *Phragmidium*; in the teleutospore in *Gymnosporangium*, however, the double chromatin mass seems to occur more frequently.

It is the appearance of these two chromatin masses which led Sapin-Trouffy (48) and Maire (29, 30) to the belief that the nuclei in the Uredineae possess in all cases two chromosomes, while Poirault and Račiborski (43), observing the single mass, considered it to represent a single chromosome. It is obvious, however, that neither the origin nor behaviour of these structures is that of chromosomes, and the observation of numerous chromosomes, described earlier for the promycelial divisions, is sufficient to negative such a view. The real nature of these chromatin masses is, however, apparent on comparison of the divisions in the promycelium with the simpler divisions, either single or paired (conjugate), found in other stages of development. In both the promycelial divisions the chromatin (vide supra) shows at times a distinct segregation into two masses (Figs. 30, 32, 41 *a*). These masses are certainly not themselves chromosomes, and in the first division are clearly produced by the aggregation of numerous chromosomes; they probably represent, as suggested earlier, the chromatin derived respectively from the two nuclei which fuse in the teleutospore. It is not surprising then that nuclei which are the direct descendants of those in the promycelium should also show a similar tendency to a differentiation of their chromatin into two masses, though they have apparently ceased to form chromosomes. A comparison of Fig. 30 with Figs. 94 and 96 shows clearly the strong resemblance between the form taken by the chromatin in the two types of division.

NATURE OF SPERMATIA.

As the study of *Phrag. violaceum* clearly shows, the spermatia take no part in aecidium-development, and fertilization is regularly brought about in another way. This result is only in agreement with all earlier work, which has always failed to establish any direct relation between the spermatia and the aecidia. A fertilization of the *fertile cells* would obviously be difficult in *Phragmidium*, but it would be almost impossible in the typical aecidium, where the fertile cells are developed deeper down in the tissues.

As the spermatia have no power to act as male cells and appear quite incapable of causing infection, there would seem to be no escape from the view that they are now *functionless*[1]. It becomes evident then that a study of their structure is the only means by which one can hope to obtain evidence as to their primitive nature, for originally they must have acted either as male cells or as conidia. Strangely enough, their histological characters have never been considered from this point of view, though they appear to give very definite evidence for a decision of the disputed question.

As the observations detailed earlier show, the spermatia, in both the forms investigated, are small, uninucleate cells, with a thin wall, apparently no reserve-material, a very dense nucleus without a distinct nucleolus, and a very small amount of cytoplasm (Figs. 47, 59)[2]. That these characters are general throughout the whole group is shown by a glance at the figures of Sapin-Trouffy (48), diagrammatic as they are; though this observer, assuming that the spermatia were conidia, passed over these characters without particular comment. It is obvious, however, that these characters are not those of conidia nor of any regular asexual reproductive cells; on the other hand they are to a very striking degree the characters of *male* cells, in which, as is well known, there is usually little or no reserve-material, the nucleus is often very dense, and the cytoplasm is nearly always much reduced in amount.

The reduction in cytoplasm is very well marked in the small spermatia of *Phragmidium* and *Gymnosporangium*, but it is just as clearly shown in the comparatively large spherical spermatia of *Coleosporium Senecionis*, Fr., where, according to the figure of Sapin-Trouffy, the diameter of the nucleus is more than two-thirds of that of the whole cell. Such a structure is certainly without parallel in any known asexual reproductive cell, and sufficiently explains the inability of the spermatia to develop in water, for the volume of cytoplasm is insufficient to form a germ-tube of any length.

When one considers the peculiar structure of the spermatia of the Uredineae, their total incapacity, as far as is at present known, to bring about infection, their feeble power of development even in nutritive solutions, their usual close association with the aecidium, there seems no escape from the view that the *spermatia are male cells which have now become functionless*. The peculiar process of fertilization in the fertile cells of the aecidium of *Phrag. violaceum*, which one can hardly but consider as a reduced process, seems only explicable on the view that the spermatia formerly acted as fertilizing agents in connexion with the aecidia.

[1] It is satisfactory to note that Klebahn (27) in his recently published work, though unable to throw any light upon their nature, also arrives at the conclusion that they act neither as conidia nor male cells; he thinks they may in some way be useful to the plant as an excretory product.

[2] I have also observed the same structure in the spermatia of *Puccinia Poarum*, Niels, *P. Phalaridis*, Plowr., and *Uromyces Poae*, Rabh.

It must be pointed out in relation to the observations of Cornu (12), Brefeld (10), Plowright (42), Sapin-Trouffy (48), and others on the germination of the spermatia in nutritive solutions, that a complete absence of power of vegetative development is not a necessary character of male cells, as is shown by the well-known cases of the potential gametes of some algae which can develop either sexually or asexually. It must also be remembered that nutritive solutions are a highly artificial condition for the reproductive cells of such obligate parasites as the Uredineae; it is difficult to understand how the spermatia could act in nature as infecting organs (conidia) unless they are able to germinate in water like the other spore-forms of the group. Their appearance at about the same time as the *aecidiospores* is another argument against their conidial nature (as well as an argument in favour of their sexual nature), for, in the presence of such very effective infecting organs as the aecidiospores are known to be, there would seem to be absolutely no need for the production of conidia so structurally ill-equipped for directly carrying on the life-cycle as are the spermatia. The fact that most of the spermatia in the forms investigated are found to be disorganized soon after they are shed, and that rarely in *G. clavariaeforme* some of them degenerate while still in the spermogonium, is evidence against the conidial view and in favour of the view that they are abortive male cells.

There is of course a view which might be suggested, that the spermatia are degenerate conidia which have ceased to have any function. Such a view, however, may certainly be dismissed, for it is difficult to imagine the conditions in which the conidia would cease to be of value, and still more difficult to imagine a process of degeneration which brought about a reduction of the cytoplasmic portion of the cell, but left the nucleus untouched.

That the spermatia, though perfectly functionless, should still be produced by the majority of forms and in such great abundance is certainly a very striking phenomenon[1]. A somewhat similar process, apparently equally wasteful, is to be observed in certain animals, as in the gasteropod, *Paludina*, where two sorts of spermatozoa, normal and giant ones, are constantly produced, although the smaller ones only are functional.

The similarity of the spermogonia of the Uredineae to those of *Collema*, in which the earlier observations by Stahl on the fertilizing action of the spermatia have of late years been confirmed by Baur (5), is additional confirmation of the view that the spermatia in the Rusts are male cells.

It is true that Möller has shown that in certain cases the spermatia

[1] Even when the aecidium is dropped out of the life-history the spermogonium sometimes still remains as the first structure to be developed on the mycelium which arises from the sporidium, being later followed by uredospores or teleutospores; e. g. in the so-called *brachy-* forms.

of lichens can develop as conidia, but, as pointed out by E. Fischer (Bot. Zeit., 1888, p. 158) in reviewing Möller's work, and also later by Harper (20), this in no way proves that the spermatia are not potential male cells. That they do not usually develop except in nutritive solutions, that it is often months before they germinate, and that their growth is excessively slow, strongly suggest that they are not simple conidia; but these facts are quite in keeping with the view that they are male reproductive elements. The suggestion lately put forward by Metzger (33)—that the spermatia of lichens are male cells which have retained a certain power of vegetative development, and, now that in many cases the ascus fruit develops without their aid, they sometimes act as conidia, and may have become modified in that direction—seems to be the most satisfactory one[1].

There seem to be no data for a comparative cytological study of the spermatia of lichens, though it would be of great interest. The figure of the spermatium of *Buellia punctiformis* given by Istvanffi (24) shows a cell with a much smaller nucleus in proportion to the size of the cell than in the Rusts; but it is possible that only the nucleolus was observed.

FERTILIZATION IN THE AECIDIUM AND THE NUCLEAR FUSION IN THE TELEUTOSPORE.

It is very clear that the process which takes place in the young cells of the aecidium of *P. violaceum* is one which has most of the characters of an ordinary sexual process. A cell is to be observed, which after cutting off a sterile cell above, increases in size and becomes a specialized reproductive cell, exhibiting abundant protoplasm and a large nucleus with a well-marked nucleolus. A pause then occurs in its development, but it is later stimulated to further growth and rapid division by the entrance of a nucleus from without.

Such a series of phenomena leave no escape from the view that the fertile cell is a *female* reproductive cell which undergoes a process of *fertilization*. There are, however, two points in which the process differs from that of ordinary fertilization. One is, that the two nuclei which become associated in the fertile cell do not fuse, but retain their morphological individuality, though the closeness of their relationship is shown by their always dividing together in close juxtaposition; a fusion of nuclei does ultimately take place in some of their descendants, but it is confined to those pairs of nuclei which are to be found in the teleutospores. The second point of difference is, that the entering nucleus is derived from an un-

[1] Hedlund (22) has described two cases in which the spermatia of lichens germinated and developed a thallus in a state of nature.

differentiated vegetative cell and not from a specialized male cell or organ; this will be discussed in the next section.

A study of the cytological features of fertilization in various animals and plants shows clearly that *actual fusion of nuclei is not a necessary part of fertilization as ordinarily understood.* Although in Angiosperms the sexual nuclei fuse together before the egg develops further, yet in the Abietineae (Blackman, 6; Ferguson, 16), and in most animal eggs, development of the egg begins without the fusion of the nuclei, for the two begin to divide independently and only meet together on the first division-spindle. Again, in the egg of *Cyclops*, we have cases in which the two nuclei can be distinguished for several cell-generations[1]. Not only was Rückert (46) able to observe that in the first segmentation of the egg of *Cyclops* the two nuclei divide side by side in an association very little closer than that to be observed in the conjugate nuclei in the Uredineae; but Häcker (17, 18) even observed *double* nuclei in the cells of the embryo up to the sixteen-celled stage (at what stage actual fusion took place was not determined).

Again, in many cases of fertilization the fusion between the two nuclei is more apparent than real, for the chromatin and chromosomes derived from the male and female nuclei respectively have in a number of instances been traced as separate structures through several generations (eggs of *Ascaris*, *Cyclops*, &c., and the chromosomes in *Pinus*). Häcker (17) also believes that in the egg of *Cyclops* he has been able to trace the maternal and paternal chromatin as separate structures from the time of fertilization up to the formation of the next germ-cells. In fact all the cytological work of recent years tends to show that the chromosomes have a distinct 'individuality,' and that the nuclei of the cells of the higher animals and the nuclei of the sporophytic cells of the higher plants are really *dual* in nature, there being no real mixing of the chromatin from the two sources until the time of chromosome-reduction[2].

A study of the varieties of fertilization in the higher animals and plants shows clearly that three morphological stages are to be observed in connexion with the complete sexual cycle—*nuclear association within the*

[1] Conklin (11) also in the egg of *Crepidula* was able to observe the double character of the nuclei at the telophase in every cleavage up to the twenty-four-cell stage, and in several cleavages up to the sixty-cell stage.

[2] See for example the work of Van Beneden, Boveri, Herla and Zoja, and of later years that of Sutton (52), and the observations on chromosome reduction of Montgomery (37, 38), Sutton (51), and Farmer and Moore (15). Sutton describes a very interesting case in which the chromosomes (twenty-three in number) in the spermatogonium of an insect (*Brachystola*) are not only of eleven different sizes, those of the same size being arranged in pairs, but the majority of them in the resting state are each contained in a separate diverticulum of the sacculated nucleus, the 'accessory chromosome' lying in a separate closed vesicle and thus forming, virtually, a separate nucleus.

The results obtained in certain hybrids in which the sexual cells are apparently 'pure' in relation to certain maternal characters also suggest that the nuclear material from the two sources remains distinct from the time of fertilization up to the formation of the germ-cells.

same mass of cytoplasm and during division, nuclear reduction (fusion), and chromosome-reduction. These processes may either take place together or be separated by a considerable number of divisions. In the case of the higher plants the first two processes usually take place together, i. e. the sexual nuclei fuse in the resting state directly they meet[1]. In many animal eggs, and a few plants, the second process is somewhat delayed, and there is no nuclear fusion until the end of the first division. In the case of the egg of *Cyclops* we have a further stage, for nuclear reduction is put off for many cell-divisions, each of the cells showing two nuclei which are, however, closely associated together during division. In the Uredineae there is a still further stage of separation, for in this case nuclear reduction (or fusion) is put off until the stage corresponding with chromosome-reduction (vide infra), so, like that process, it is confined to the reproductive cells (teleutospores = spore-mother-cells) which are to carry on the life-history. The two nuclei, also, instead of dividing together on the same spindle divide on separate (rudimentary) spindles side by side.

Fertilization is, of course, a somewhat ill-defined term, and from a strict morphological point of view it is perhaps arbitrary to confine it to any one of the three processes just described, for sooner or later all of them must occur in the complete sexual cycle. A cell is, however, usually considered to be 'fertilized' directly the stimulus has been given to development and the number of chromosomes doubled by the entry of the male cell or nucleus, and for such a use of the term fertilization it is obvious that the essential point is the first stage—nuclear association in the same mass of cytoplasm and during division.

The time at which nuclear reduction (fusion) takes place is seen from the stages given above to be quite unimportant, but, like chromosome-reduction, it must occur sooner or later in the sexual cycle. The view which would consider fertilization as incomplete until nuclear fusion had taken place could hardly be accepted, as chromosome-reduction might equally be considered as the end of the process. It would also tend to divorce the morphological and physiological aspects of fertilization, for in *Cyclops* and *Phragmidium*, for example, we should have to consider the female cell as developing under the stimulus of fertilization, but with the process still incomplete. Besides, all recent work at present tends to show that nuclear fusion is mainly a nominal process (hence the term nuclear reduction is preferable), a mere change from association within the same cytoplasm to association within the same nuclear membrane.

The view of Dangeard and Sapin-Trouffy, which would consider the fusion in the teleutospore as itself a sexual process, was based on an

[1] It is generally believed that in many of the Thallophyta where there is no alternation of generations the *three* processes all take place together, chromosome-reduction occurring before the fusion-nucleus divides.

ignorance of the origin of the two nuclei and the exaltation of nuclear fusion as a test of fertilization. The fusion is the result of a sexual process (at least in *Phrag. violaceum*), but is clearly not in itself a fertilization. Račiborski (44), when he put forward the hypothesis that the conjugate divisions in the Uredineae represented a vegetative phase intercalated in fertilization between the stages of cytoplasmic fusion and nuclear fusion, was much nearer the truth, though, as has been pointed out above, it is not at all necessary to consider nuclear fusion as a stage in fertilization as usually understood. Račiborski, equally with Dangeard and Sapin-Trouffy, was quite ignorant as to the method by which the two nuclei became 'conjugate.'

Maire (29) had earlier recognized a similarity between the conjugate nuclei of the developing egg of *Cyclops* and the paired nuclei of the Uredineae, and applied the term 'synkaryon' to the two nuclei. He recognized that the fusion in the teleutospore was not in itself a process of fertilization, but considered it a mere process of 'mixie,' in which he includes both nuclear and chromosome reduction in the sense used above. He believed the 'synkaryon' to be brought about by the association of two daughter-nuclei of a cell, and at first considered it actually comparable to a sexual process, but in a later paper (30) he comes to the conclusion that between the fertilization in the higher plants and the formation of the 'synkaryon' in Uredineae (and Basidiomycetes generally) there are only 'des relations de cousinage.' He elaborated his views in some considerable detail, but the discovery of the origin of the two nuclei in the aecidium of *Phragmidium*, and the evidence here brought forward that the spermogonia and aecidia are in all cases to be considered as sexual organs, throws a completely new light upon the matter; his views need not therefore be discussed further.

ORIGIN OF THE PROCESS OF FERTILIZATION.

The process of fertilization as observed in the fertile cells of *Phrag. violaceum* might be looked upon as merely one of great simplicity in which the male cell remained undifferentiated. The two nuclei which take part in the process are not sister-nuclei though they may sometimes be closely related, and the process as a whole (except for the absence of obvious cytoplasmic fusion) is not much simpler that that of *Basidiobolus*, in which two neighbouring cells, after forming each a sterile cell, fuse together to form a zygospore.

Taking into account, however, on the one hand, the completely undifferentiated nature of the cell from which the acting male nucleus comes, and the peculiar method by which the nucleus passes from one cell to another, and on the other, the presence of the functionless spermatia with their special cytological characters, the only view that seems satisfactory to explain the facts is *that the primitive normal process of fertilization by*

means of the spermatia has been replaced by a fertilization of the female cell by the nucleus of an ordinary vegetative cell.

Such a view receives strong support from the observation of Farmer, Moore, and Digby (14) on apogamy in Ferns, published while this work was in progress. There seems no doubt that the process in the aecidium is to be looked upon as intermediate between that of normal fertilization, where both the cells are specially differentiated, and that observed in apogamous Ferns by these observers, where both acting male and female cells are represented by ordinary vegetative cells.

If the view be accepted that the process observed is a reduced form of ordinary fertilization, it seems very probable that the sterile cell also is reduced. Its position above the fertile cell would suggest that it formerly acted as a receptive cell pushing up between the epidermal cells as a trichogyne to which the sticky spermatia could be brought, for example, by insects. Some support is lent to such a view by the fact that occasionally cases are to be found in which the sterile cells do push up between the epidermal cells and swell out above, being merely covered by the cuticle (Fig. 77 *a*). If development were pushed one stage further and the cuticle pierced, a very effective receptive organ would be the result.

MORPHOLOGY OF THE AECIDIUM.

Although this subject cannot be treated fully in the absence of a comparative study of aecidium development in various members of the group, yet, as has been stated earlier, the aecidium of *Phragmidium* (and apparently that of *Caeoma*) is very different from the typical aecidium found in the other genera. It is merely a group of special reproductive organs, indefinite in extent, and merely bounded by paraphyses which are sometimes absent; and thus no more a definite structure than the sorus of uredospores or teleutospores.

In the light of our present knowledge the aecidium of *Phrag. violaceum* is thus to be considered as a *group or sorus of female reproductive organs*, each consisting of a female (fertile) cell below and a sterile cell above.

We probably have in this genus the most primitive form of the aecidium to be found in the group, but of the form of the aecidium in the still more primitive state when fertilization by the spermatia yet occurred, we can say nothing[1]. With the change to the reduced form of fertilization, with its obvious advantage of certainty, the number of female organs in the group could be indefinitely extended without the necessity of breaking through to

[1] It is of course possible that the spermatia may sometimes really act as fertilizing agents in *Phragmidium*, and perhaps in *Caeoma*, for they are occasionally found scattered over the surface of the leaf below which the aecidium is developing; and if a sterile cell should push through to the surface and come in contact with a spermatium fertilization might result: such a process, however, was never observed, and it can only be of very rare occurrence.

the surface. The absence of this necessity might also lead later to a development deeper down in the tissues in the host, as is found in the typical aecidium, where conditions of nutrition would perhaps be more satisfactory. With this deeper point of origin may also perhaps be associated the development of the pseudoperidium, which would protect the young structure while pushing its way to the surface.

There can be little doubt that the aecidium, when present, is always the seat of the process of transition from the condition of single to that of paired nuclei. Sapin-Trouffy (48) found this to be the case in all the forms (about fourteen) which he investigated. His observations have been confirmed by Maire (29 ; 30) for *Endophyllum Sempervivi* and for *Puccinia Bunii*, DC., and by the writer in the two forms here described, and also in *Puccinia Poarum*, Niels, *P. caricis*, Rebent, and in *Uromyces Poae*, Rabh. The conclusions as to the nature of the aecidium in *Phragmidium* must then apply throughout the group, and the aecidium in all cases must be considered as a group of female reproductive organs [1]. Whether in all cases the transition (fertilization) is brought about by a nuclear migration, or whether in some cases the two nuclei are formed in the cell by division (as Maire believes), must be left to future work to decide. Such a condition would, however, be merely a further stage of reduction in fertilization, comparable to the well-known case of fertilization by means of a sister-nucleus (the second polar body) observed in the 'parthenogenetic' eggs of *Artemia*.

The hypothesis put forward by Poirault and Račiborski, that the sporidium may become binucleate and so start a mycelium with paired (conjugate) nuclei, is quite untenable. The work of Sapin-Trouffy, of Maire, and the observations here detailed have shown that in all cases investigated the mycelium which arises from the sporidium has single nuclei. It is true that in certain cases, such as in *Coleosporium Euphrasiae*, investigated by

[1] In *Coleosporium* what are usually known as the uredospores are produced in chains and have intercalary cells between them, and are thus liable to be taken for the true aecidiospores though no pseudoperidium is present. In forms such as *C. Senecionis*, Fr., in which the full life-history is known (about fifteen species of this genus are now known to be heteroecious), there can be no confusion between the two, as the true aecidium is found early in the year on the one host, is associated with spermogonia, and has a definite pseudoperidium ; while the aecidiospore-like uredospores are produced later, on the other host, and are intercalated, like ordinary uredospores, in the life-cycle between the true aecidiospores and the teleutospores. Sapin-Trouffy has shown, as was to be expected, that in *C. Senecionis* the transition from the single to the paired nuclei takes place in connexion with the true aecidium, while the aecidiospore-like uredospores are borne on a mycelium with paired nuclei derived by infection from the aecidiospores. Owing to their position in the life-cycle, the histological nature of the mycelium which bears them, and the absence of a pseudoperidium, it is clear that the spores in question partake much more of the nature of uredospores than of aecidiospores, so the former name should be retained for them in spite of their development in chains. Bearing in mind the heteroecious nature of nearly all the forms of *Coleosporium* hitherto investigated the view put forward by Holden and Harper (23) can hardly be accepted, that the uredospores are really aecidiospores in the form to which they give the name *C. Sonchi-arvensis*, Lév., and of which they observed only the stage with uredospores and teleutospores.

Poirault and Raciborski, the sporidium does become binucleate, but this appears to be a mere precocious division of the nucleus without wall-formation, for, as mentioned earlier (see under section dealing with sporidia-formation), the sporidia of *Phrag. violaceum* and a few other forms have been found to give origin, in spite of their binucleate nature, to the normal mycelium with single nuclei. Poirault and Raciborski, however, observing that the mycelium which gave origin to the uredospores and teleutospores had paired nuclei and that the sporidium also was binucleate, concluded that the former arose from the latter. They completely, however, overlooked the fact that *C. Euphrasiae* had been shown by Klebahn (26) to be a heteroecious form and that the sporidium really gave rise to the aecidial stage, the so-called *Peridermium Pini*, which in the case of *C. Senecionis* Sapin-Trouffy had shown to have the typical structure, a mycelium with single nuclei. Holden and Harper (23) in a recent paper, observing that the sporidium becomes binucleate in a form to which they give the name of *Coleosporium Sonchi-arvensis*, Lév., have again put forward the view that the sporidium starts the stage with paired nuclei. Such a view, for the reasons just given, can hardly be accepted without direct evidence. The authors assume that the binucleated sporidium gives rise directly to the mycelium with paired nuclei which they investigated and found bearing the uredospores and teleutospores ; but it has been shown that the species in question, like that of *C. Euphrasiae*, is heteroecious (see Klebahn, 27), with its aecidial stage on *Pinus*, like nearly all the other species of the genus yet investigated. The aecidial stage in this form has therefore no doubt a mycelium with single nuclei like *C. Senecionis* and all the aecidial stages of the Uredineae hitherto known. The mere fact of the sporidium being binucleate cannot be considered as being of any special significance. It is true that the name *C. Sonchi-arvensis* has been applied loosely to a number of what are now known to be different forms, the original form being really confined to *Sonchus* in its second stage (see also Klebahn, 27). The *Coleosporium* on *Aster* and *Callistephus* will probably thus prove to be a new form, but there can be no reason to doubt its heteroecism, since nearly all the forms of the genus hitherto investigated appear to have two hosts.

There is no evidence that the sporidium of any form ever gives rise to anything but a mycelium with single nuclei. As stated earlier, all the work of Sapin-Trouffy, of Maire, and that here detailed goes to show that the condition with paired nuclei always starts in the aecidium when that structure is present. Even in the absence of the aecidium the paired nuclei only arise *later* in the mycelium in connexion with the development of the uredospores or teleutospores [1].

[1] Arthur (61), in reviewing the work on this subject, concludes that the sporidium regularly starts the condition of paired nuclei in the group, and is naturally at a loss to explain the fact that the spermatia are uninucleate. His views seem based on a misunderstanding of the work of Sapin-

NUCLEAR DIVISION.

From the observations of Poirault and Raciborski, Sapin-Trouffy, and Maire—although they did not observe all the details of the process and their interpretation is clearly at fault—there can be no doubt that the type of nuclear division here described, whether for single or conjugate nuclei, is general for all the Uredineae. It is clear then that in all of the divisions in the group, except those in the promycelium, we have to deal with a process of such a simple nature that chromosome-formation is in complete abeyance; so that the division actually partakes of the nature of direct division (amitosis). A comparison, in such a form as *G. clavariaeforme*, of the first and second divisions in the promycelium with the other simple divisions (whether single or conjugate), leaves no escape, however, from the view that the simple method of division is really a *reduced* form of *indirect* division. The first promycelial division is a fairly typical form of mitosis with a formation of chromosomes, though the absence of any regular equatorial plate, the fact that no splitting can be observed, and the early fusion of the chromosomes suggest that perhaps even here the process may be reduced from a halving of definite chromatin elements to the more or less direct separation of chromatin material as a whole. In the second division, however, though the spindle structure is perfectly typical, the chromatin instead of forming distinct chromosomes merely forms a network which becomes spread out on the spindle and later draws apart into two portions[1]. If this second method of division be still further reduced, so that the chromatin instead of forming a network forms a solid mass and the spindle is represented only by a fine, thread-like structure, we have the ordinary method of division characteristic of the nuclei, whether single[2] or paired, of the cells other than those of the promycelium.

It seems impossible to doubt that these three methods of division represent progressive stages in a process of reduction, for it is hardly

Trouffy, who showed clearly (and his work has been confirmed by Maire and in this paper) that the spermogonium was always borne on a mycelium with *single* nuclei. Arthur also states that it has been shown that both aecidiospores and uredospores arise from a binucleated mycelium in the usual vegetative manner. This is directly contrary to the facts observed by Sapin-Trouffy eight years ago, who in all cases found that the aecidiospores were borne on a mycelium with single nuclei, and that the nuclei only became paired at the time of aecidium-formation.

[1] Whether the two promycelial divisions are regularly different in other forms as in *G. clavariaeforme* remains for future work to show. Holden and Harper (23), in their recent paper, are of the opinion that in the species of *Coleosporium* which they examined the two divisions are of like nature, though they were not fortunate enough to have such a complete series of stages as were obtained for the form here investigated.

[2] In the divisions of the single nuclei, as observed in the spermatial hyphae, the spindle structure is not so clear. A precocious division of the nucleus of one of the four 'promycelial' cells has been observed, however (while this paper was in the press), in *G. clavariaeforme*, which shows a distinct spindle, though without centrosomes or polar radiations (Fig. 100). This shows that the achromatic part of the nucleus may not always be as reduced as in most of the divisions observed.

conceivable that in such a highly developed group as the Uredineae we should witness the evolution of mitotic division. The second division with its very complex achromatic mechanism but simple method of chromatin segregation points clearly to the process being a reduced one.

A comparative study of nuclear division in the Uredineae with that to be observed in the Basidiomycetes would be of great interest, and, considering the obvious relationship of the two groups, might throw some light on the question as to whether the reduction in nuclear mechanism in the former group is to be connected with the marked parasitic habit. The two chromosomes described by Maire (30) as universal for the Basidiomycetes are doubtless merely chromatin masses, comparable to those described above. Wager (55, 56), Ruhland (47), and Harper (21) have all shown that in the forms they investigated the chromosomes in the basidium were much more numerous. Maire seems to have observed such structures in certain cases, but he applies to them the term 'protochromosomes,' and states that they fuse later to form the two structures which he believes to be the real chromosomes. This is exactly what happens in some cases in the promycelium in *G. clavariaeforme* (Fig. 30), but there can be no doubt, as the figures show, that the first-formed structures are the true chromosomes.

With reference to the question of the function of the nucleus and the relation of direct to indirect division, the existence of a group of Fungi, which in the whole life-cycle show but one, or perhaps two, divisions in which there is a distinct formation of chromosomes, is certainly a very interesting fact. The view that direct nuclear division is confined to cells which are in a senile state has of late years been largely modified. It has been shown that a number of Protozoa and certain animal cells divide normally in an amitotic manner, and Nathansohn (39) has shown for *Spirogyra*, and Shibata (50) for *Podocarpus*, that cells which have divided amitotically can later divide in the ordinary mitotic manner. Similarly Häcker (19), Wasielewski (58), and Němec (40) have shown that nuclei which have divided in an abnormal[1] way under the influence of narcotics can again divide in the normal indirect manner when placed under natural conditions.

These observations are generally considered to show that the nucleus has remained entirely unaffected by the intercalation of one or more direct divisions in the normal series of mitoses, but they really prove no more than that the effect (if any) has not been sufficiently profound to destroy their power of normal division or to prevent them carrying on their normal development within the limits of the experiment. Němec has pointed out

[1] There seems considerable doubt as to how far these divisions are abnormal. Wasielewski considers them to be of the nature of amitoses, while Němec brings forward very considerable evidence to show that they are merely modified indirect divisions in which, however, there is still an exact halving of the chromatin.

that in the case of the lower organisms, the effect of a division, presumably unequal, of the nature of amitosis might be long delayed; and in the case of the higher plants might not be visible immediately. It seems quite possible, however, that even in the case of the higher plants a change might be produced which would not be observable at all in the ordinary restricted life-history of cells other than the germ-cells. We have no knowledge as to how unequal an indirect division may be in relation to the chromatin; no doubt the degree of inequality must be very variable (it may probably be sometimes as equal in effect as indirect division), but it is quite conceivable that the change produced by a direct division in the 'hereditary properties' of, say, a root-cell may be confined to those concerned with flower or leaf development, and so never be visible in the normal course of development of a root-cell.

The beautiful experimental researches of Boveri (9) on double fertilized eggs of the Sea-urchin (in which he practically proves the existence of physiological individuality in the chromosomes) lend strong support to such a view, for certain blastomeres of the three- or four-celled stage, though capable of normal division and apparently similar to the other blastomeres, were found later to be deficient in the power of producing certain organs (such as the skeleton, pigmentation, &c.), owing to the unequal sorting of the chromosomes in the first division.

It is clear however that in the case of the intercalation of an amitotic division in the series of divisions leading directly up to the germ-cells, such as that described by Meeves (32) and Macgregor (31) for the spermatozoa of Amphibians, the effect of any inequality of division would produce an effect, sooner or later, in the offspring. If such a division be confirmed it seems hardly possible to consider it as other than a true equational one of equal value with mitosis; at least if the ordinary view be accepted as to the meaning of chromosomes and their splitting. There seems to be some doubt, however, as to whether this form of division is regularly present. Sutton (52) has also suggested that, as the amitotic division in these cases does not apparently lead to cell-division, it may be that the two nuclei meet again on the spindle of the next mitosis, and so the direct division is without effect.

The view may perhaps be hazarded that the sufficiency for the cell needs of the Uredineae of such a simple method of division may be connected with the simple organization of such forms as compared with the higher plants; the 'idioplasm' must be far less complex.

Alternation of Generations.

If the view be accepted that the spermogonia and aecidia represent male and female organs respectively (and the observations in *Phragmidium* appear to leave no escape from such a view) it is clear that the Uredineae

with the aecidium in their life-cycle exhibit an alternation of generations as sharply marked as that of the higher plants. For not only are the two generations to be distinguished as sexual and asexual—bearing in the one case the sexual organs, spermogonia and aecidia, and in the other case only asexual spores, aecidiospores, uredospores, and teleutospores—but they are also to be cytologically differentiated, the sexual generation being characterized by the presence of single nuclei, the asexual by the presence of paired (conjugate) nuclei; the special nuclear condition of the sporophyte being the result, as in the case of the 2 n chromosomes of the higher plants, of a process of fertilization (at least in *Phrag. violaceum*).

Owing to the extreme reduction in the process of nuclear division in the Uredineae, so that the process of chromosome-formation is in abeyance in nearly all the divisions, one cannot distinguish the two generations by their number of chromosomes; yet one can distinguish in the nuclei of the oophyte *two* chromatin masses, while the two paired nuclei, which together correspond with the single nucleus of the ordinary sporophyte, show *four* chromatin masses. There is thus a near approach to the distinction of chromosome number which is to be found in the higher plants, and also in the interesting alternation of generations which Williams (59, 60) has lately observed in the Dictyotaceae.

The teleutospore clearly corresponds to the spore-mother-cell, for it is there that the return to the nuclear condition characteristic of the oophyte is brought about. In the teleutospore, although no actual reduction of chromosomes can be observed, there is a reduction from the four chromatin masses of the two nuclei to the two chromatin masses of the single nucleus, each mass, as shown above, appearing to correspond to a group of chromosomes. This reduction in number of chromatin masses is also associated with peculiar changes in the nucleus concerned which correspond to the process of *synapsis*.

As however the two nuclei remain individualized from the time of fertilization up to the time of reduction in number of chromatin masses we have associated with this process a *nuclear reduction*, i.e. a reduction in the number of the nuclei from two to one. Since this process of nuclear reduction takes place in most organisms at the time of fertilization, or soon after, it has been confused with that process itself, and so has led to the belief that the fusion of nuclei in the teleutospore was itself a sexual process. This view, which was hardly credible even on the facts hitherto known, has been rendered quite untenable by the discovery of an association of nuclei in the aecidium, which must itself be considered as the fertilization process.

After the process of synapsis and chromatin-reduction, the cell of the teleutospore undergoes a process of tetrad division to form the four cells of the 'promycelium' (=spores). The teleutospore (or its cell) is thus exactly

comparable to the spore-mother-cell of the higher plants and to the tetraspore-mother-cell in the Dictyotaceae. The gametophyte generation starts again in the teleutospore and is continued up to the 'fertile cell' (the cell bearing the mother-cells of the aecidiospores) in the aecidium; while the sporophyte continues throughout the rest of the life-history, through the aecidiospores, the uredospores (if present), and up to the teleutospores again.

Credit must be given to Maire (29 and 30) for having pointed out the resemblance between the nuclear history of the higher plants and that of the Uredineae. He believed, however, that the 'synkaryon' was a special condition always brought about by the association of two daughter-nuclei. He considered the spermogonia and aecidia to be non-sexual in nature, and was ignorant of any process of fertilization such as has just been described for the aecidium of *Phragmidium*.

The existence of actual histological differences between gametophyte and sporophyte is in full agreement with the usual rigid distinction which is to be observed between the two generations, both in heteroecious and autoecious forms. As is well known, the aecidiospore by infection gives rise only to a mycelium bearing uredospores or teleutospores, the uredospore gives rise to a mycelium bearing only uredospores again, or teleutospores. Again, the sporidium arising from the teleutospore gives rise by infection to a mycelium bearing only aecidiospores, if those are present in the life-history (i.e. in the *eu-* and *-opsis* forms) [1].

Apogamy. In the forms in which the aecidium is absent (*brachy-*, *lepto-*, *micro-*, and *hemi-* forms) the sporidium produces a mycelium which gives rise directly to uredospores or teleutospores with paired nuclei. In such a case we have a transition from the sexual to the asexual generation without the intervention of the female reproductive organ, and thus it is clearly comparable to *apogamy* among the higher plants. Sapin-Trouffy has shown that the change from single to paired nuclei takes place in these cases in connexion with the formation of the uredospores, or of the teleutospores, if the former are absent. The exact cytological details have yet to be worked out. It may be that the binucleate condition is brought about by the *interaction of two vegetative cells*, as in the case of apogamy in Ferns investigated by Farmer, Moore, and Digby (14), where they found the nuclei of neighbouring prothallial cells fusing to form sporophytic tissue. A form like *Puccinia Liliacearum*, DC., seems, like some of the Ferns, to be only imperfectly apogamous, for though the aecidia have been described they do not seem to be usually present.

Apospory. The peculiar cycle of development observed regularly in

[1] The very few observed cases of aberrations from the normal life-history, such as aecidiospores giving rise to a mycelium bearing aecidiospores again (A. P. Dietel, Zeit. für Pflanzenkrankheiten, iii, 1893, p. 25; Flora, lxxxi, 1895, p. 394), require further investigation; such cases may possibly be due to a separation of the nuclei of the pair, as in *Endophyllum*.

Endophyllum, in which binucleate aecidiospores, produced in a normal way, behave on germination like teleutospores, the two nuclei separating and uninucleate sporidia being produced (see Maire, 29), is just the opposite to the case discussed above. It is the transition from the asexual to the sexual generation without the intervention of the teleutospore (spore-mother-cell). It can therefore be compared with cases of *apospory* among the higher plants. Maire has described a case in *Endophyllum* in which the aecidiospore germinated in a normal way, but the life-history of the form is not known.

There can be little doubt that the *eu-* and *-opsis* forms which possess the aecidium must be considered as more primitive and the other (apogamous) forms as reduced. The view of Dietel, that the *lepto-* and *micro-* forms which possess only teleutospores are the most primitive, and that the other forms are derived from them, can hardly be accepted in the light of our present knowledge.

Heteroecism. As the heteroecious forms are confined to those possessing the aecidium, i.e. to the more primitive, it seems probable that *heteroecism* may not be, as generally conceived (see Klebahn, 27), a later adaptation, but may actually be the primitive condition in the group. Although we are ignorant of the origin of the group it is possible to conceive that the sporophyte was first developed in connexion with life on another host, just as the sporophyte in the higher plants seems to have been developed in connexion with a new terrestrial existence. The autoecious *eu-* forms would then be the first step in reduction—a purely environmental one ; later a morphological reduction of the number of spore-forms would appear to have taken place.

Fusion in the Basidium.

The fusion of nuclei in the basidium of the *Basidiomycetes* is exactly comparable to that in the teleutospore, for it has been shown by Maire (30) in the case of a large number of forms, and also by Harper (21) in two cases, that in this group, also, the two nuclei which fuse are conjugate nuclei, the ancestors of which have been associated together for a number of generations. It is clear then that the view of Dangeard and others, which considers this fusion as a process of fertilization, is quite untenable. The stage at which the nuclei first become conjugate is unknown, but it is there that one must look for a process of fertilization, and not to the fusion in the basidium, which, like that in the teleutospore, is a process of mere nuclear reduction, and (like chromosome-reduction) the necessary sequence of fertilization if the sexual cycle is to be completed.

As the Basidiomycetes are apparently without anything which could be considered even as a reduced form of sexual organ, it is suggested that the conjugate nuclear condition, the transition from gametophyte to

sporophyte, is brought about in a purely *apogamous* way (as in the case of the Uredineae without an aecidium), perhaps by the interaction of two vegetative cells, as in the union of vegetative cells of the prothallium mentioned above. Maire (30) has stated that the basidiospore gives origin to a mycelium with single nuclei (thus agreeing with the sporidium, which has always been found to behave in this way), and that later in the mycelium the nuclei become paired, but in what manner is not known. The condition of paired nuclei seems in some cases to arise very early, for Harper (21) was unable to observe in *Hypochnus* any but binucleate cells; the germination of the basidiospore, however, was not followed.

The Basidiomycetes would thus seem to resemble very closely the *lepto-* forms among the Uredineae, where the mycelium has at first single nuclei but later develops paired nuclei in connexion with the formation of teleutospores, and like them would appear to have *an alternation of generations obscured, however, by the apogamous transition from one to the other.*

The fusion of nuclei in the basidium, like that in the teleutospore, is followed by a tetrad division, and there seems little doubt that it is also followed by a process of chromosome-reduction, or one corresponding to it; the basidium, like the teleutospore, seems also of the nature of a spore-mother-cell, and is to be compared to that of the higher plants. The reduction in the basidium from four to two chromosomes described by Maire (30) as general for the Basidiomycetes can hardly be accepted, since these structures appear to represent *groups* of chromosomes, but the observations show clearly the analogy with *Gymnosporangium.* Whether the nuclear divisions in the Basidiomycetes will be found sufficiently typical to allow of the observation of an actual numerical reduction remains for future inquiry.

Maire has also described a process of synapsis in the fusion-nucleus of the basidium, comparable to that here described for the teleutospore.

Relationships of the Uredineae.

It is obvious that the Uredineae and Basidiomycetes are very closely related, for both possess single nuclei during the early part of their life-history (starting from the teleutospore and basidium respectively), which later become paired. In both cases, also, the paired nuclei fuse in special reproductive cells, the fusion being followed by a process of synapsis, and then by a tetrad division[1]. The Basidiomycetes seem wanting in all trace of definite sexual organs, so that the Uredineae must be considered as by far the more primitive and cannot be treated as a mere class of the Basidiomycetes, as in the well-known classification of Brefeld. As pointed out in the previous section the latter group appear rather to be reduced, apogamous forms of the Uredineae.

[1] As is well known the transversely divided teleutospore of *Coleosporium* is practically indistinguishable from a transversely divided basidium.

The existence of immotile male cells (spermatia) and of a sterile cell (in *Phragmidium*) which may possibly have formerly had the function of a trichogyne certainly points to a relationship of the Uredineae with the Florideae, a relationship which has been suggested by Meyer (35), chiefly on anatomical grounds, for all the higher Fungi. It is possible that an alternation of generations may yet be found in this algal group, for tetraspore-formation suggests a process of chromosome-reduction; the fact that in the majority of forms there is a distinction of sexual and asexual plants is also very striking in this connexion. Such a discovery—and it must be remembered that Williams (59 and 60) has already observed this alternation in the Dictyotaceae with chromosome reduction in the tetraspore-mother-cell—would certainly lend strong support to a view of a relationship between the Florideae, Uredineae, and Basidiomycetes.

Summary.

The peculiar nuclear cycle described by Sapin-Trouffy for the Uredineae was confirmed in the case of *Phragmidium violaceum*, Wint., and of *Gymnosporangium clavariaeforme*, Rees. In this cycle, the mature teleutospore is uninucleate and gives rise to four uninucleate sporidia, from which a mycelium is developed with the nuclei arranged singly, usually in separate cells. The spermatia produced on this mycelium are uninucleate, but in the young aecidium the nuclei become paired (forming binucleate cells) and divide together in very close association. This paired condition is then persistent throughout the rest of the life-cycle (aecidiospores, uredospores, and mycelia produced from them) up to the formation of the teleutospores, which in the young state are binucleate, but when mature become uninucleate by the fusion of the two paired nuclei. This cycle of development seems common to all the Uredineae (except *Endophyllum*) which have an aecidial stage in their life-history.

A study of the structure of the spermatia of the Uredineae shows that they have the characters not of conidia but of *male cells*, for they exhibit a large dense nucleus, very little cytoplasm, no reserve material, and a very thin cell-wall. These characters, together with their usual association with the aecidia, their absence of function, and the peculiar, apparently reduced, form of fertilization to be observed in the aecidium of *Phragmidium violaceum*, point clearly to the view that the spermatia are male cells which formerly took part in a process of fertilization in connexion with the aecidium, but have now become functionless.

The aecidium of *Phrag. violaceum* is developed immediately beneath the epidermis of the leaf and has a very simple structure. It consists of a layer of special cells, each of which cuts off a *sterile cell* above, which soon disorganizes, while the lower, the *fertile cell*, increases in size and shows abundant protoplasm, and after a pause in its development is *fertilized by*

the migration into it of the nucleus of one of the undifferentiated mycelial cells at its base. It then undergoes a series of rapid divisions, cutting off a series of aecidiospore-mother-cells. The 'fertile cell' has thus the characters of a *female cell.*

The aecidium of *Phrag. violaceum* is to be considered as *a sorus of female reproductive organs*, each of which consists of a *sterile* cell above and a *female* cell below, the nucleus of an ordinary vegetative cell bringing about fertilization and performing the part which was apparently formerly taken by the nucleus of the spermatium. The female cell thus develops by means of a *reduced form of fertilization.*

It is suggested that the sterile cell is reduced, and that it formerly pushed its way to the surface (as it can sometimes now be observed to do) and acted as a *trichogyne* to bring the spermatium into relation with the female cell below.

In the process of fertilization the two nuclei do not fuse, but merely remain very closely associated (as in the egg of *Cyclops*); there is thus started in the female cell the condition of paired nuclei which continues up to the teleutospore.

The aecidium throughout the group must be considered as *a sorus of reduced female reproductive organs*, for it appears to be always the fertile cell which becomes binucleate. Whether in all cases there is a nuclear migration, or whether in some there is a still further reduction and the process consists of an association of two daughter-nuclei, has yet to be determined, as has also the question of the presence or absence of a sterile cell.

As the spermogonia and aecidia are to be considered as male and female reproductive organs, respectively, it is evident that the Uredineae which possess an aecidial stage in their life-history (*eu-* and *-opsis* forms) exhibit a well-marked *alternation of generations* which are not only to be distinguished as sexual and asexual, but are also to be sharply differentiated cytologically, the *sexual* generation being characterized by single nuclei (with two chromatin masses on division), the *asexual* by paired nuclei (with four chromatin masses on division). The transition from the gametophyte to the sporophyte takes place in the aecidium and the transition from the sporophyte to the gametophyte in the teleutospore. The alternation of generations is thus as clearly marked as that of the higher plants or of the Dictyotaceae.

The fusion in the teleutospore of the two nuclei—the direct descendants of those which first became associated in the *fertile* (*female*) cell of the aecidium—is clearly not in itself a process of fertilization (nor the teleutospore an egg-cell), as Dangeard and Sapin-Trouffy supposed, but a mere secondary process, the result of fertilization and the preliminary to reduction. It is pointed out that three nuclear stages are to be observed in the sexual cycle of plants and animals—*nuclear association*, *nuclear reduction* (*so-called fusion*), *and chromosome-reduction.* Of these three stages, only the first

is the essential part of fertilization (as various plants and animal eggs and that of *Cyclops* show) ; the second may take place at the same time as the first, or it may be delayed for a time, or, as in the Uredineae, it may be delayed until the stage corresponding to chromosome-reduction.

The fusion-nucleus in the teleutospore undergoes changes which correspond to *synapsis*, and when it divides there is seen to be a reduction from the four chromatin masses of the paired nuclei to two chromatin masses; owing to the absence of chromosome-formation in most of the nuclear divisions an actual reduction in number of chromosomes cannot be observed. The process of fusion and reduction in the teleutospore is followed by a definite tetrad division in the 'promycelium,' so that the teleutospore corresponds exactly with the spore-mother-cell of the higher plants, the 'promycelial' cells being really of the nature of spores.

As the fusion of the two nuclei is delayed throughout the whole of the sporophyte there is associated with the reduction from four to two chromatin masses a process of nuclear reduction from two nuclei to one.

Nuclear division in most of the cells of the Uredineae is of an exceedingly simple type. The nuclei (whether single or paired) lose their membrane, the nucleolus becomes extruded, and the chromatin condensed into one, or sometimes two, masses. A rudimentary spindle can sometimes be observed, and upon this the chromatin becomes spread and is drawn apart into two or four pear-shaped masses which separate and form the daughter-nuclei. In the paired (conjugate) state the nuclei divide side by side in close juxtaposition, passing *pari passu* through the various stages of division, as described by earlier observers.

In the promycelium the two divisions are much more typical. In *G. clavariaeforme* a well-marked spindle with centrosomes and polar radiations was observed. The first division showed a formation of numerous chromosomes, though their behaviour on the spindle is not typical and there is doubt as to whether a definite splitting actually occurs. In the second division the chromatin forms no chromosomes, but merely a network which covers the spindle, and is later drawn apart into two portions.

The spindle in the case of the second division in the promycelium of *G. clavariaeforme*, and apparently also in the case of the first, is formed free in the cytoplasm between the two portions of a divided centrosome (like the 'Centralspindel' of Hermann), and afterwards comes into close relation with the nucleus.

The simple form of division found in the Uredineae is to be considered as reduced from the typical method of karyokinesis.

The two structures observed constantly during nuclear division by Sapin-Trouffy and Maire, on which they base their views of chromosome-reduction, cannot be considered of the nature of chromosomes, but are merely chromatin masses. They probably represent the chromatin derived

respectively from the two nuclei of the teleutospore, but are not always present. In the first division of the promycelium of *G. clavariaeforme* they can be seen to be formed by the aggregation of a number of chromosomes; in the other divisions they are formed directly, by the condensation of the chromatin of the nucleus.

In those Uredineae which have a reduced life-cycle without an aecidium (*brachy-*, *hemi-*, *micro-*, and *lepto-* forms) the transition from single to paired nuclei, and from gametophyte to sporophyte, apparently takes place in connexion with the uredospores, or if these are absent, in connexion with the teleutospores. Such a shortening of the life-cycle is comparable with the case of *apogamy* among the higher plants; the cytological details are not yet known, but it is possible that it is brought about by the association of the nuclei of two vegetative cells (cf. apogamy in ferns).

The peculiar shortening of the life-cycle to be observed in *Endophyllum*, where the binucleate aecidiospore germinates like a teleutospore and the uninucleate condition is brought about by the simple separation of the two nuclei, is clearly a transition from the sporophyte to the gametophyte without the intervention of the teleutospore (spore-mother-cell), and should accordingly be considered as comparable to *apospory* in the higher plants.

The fusion of paired nuclei in the *basidium* of the Basidiomycetes is exactly comparable with that in the teleutospore, and should also be considered, not as a process of fertilization, but as a purely secondary process of nuclear reduction preliminary to chromosome reduction.

How the condition of paired nuclei is brought about in the Basidiomycetes is not yet clear. The life-history of these forms seems comparable with that of the *lepto-* forms among the Uredineae, and it is suggested that, in the absence of sexual organs, the transition from single to paired nuclei, i.e. the transition from gametophyte to sporophyte, is brought about *apogamously* in a similar way.

The Uredineae and Basidiomycetes show an obvious relationship, for both have paired nuclei at some stage of their life-history, and in both groups the individuals of certain pairs fuse together in specialized reproductive cells, a fusion to be followed by synapsis and a tetrad division. The Uredineae with their sexual organs would certainly seem to be more primitive and can hardly be classed as a mere subdivision of the Basidiomycetes.

The Uredineae appear to show a relationship with the Florideae among the Algae.

The aecidium of *Phragmidium* with superficial position and very simple structure without a pseudoperidium is really not a definite organ, but a mere ill-defined sorus of reduced, female reproductive organs; it is no doubt *primitive*, while the typical aecidium with its pseudoperidium and deeper point of origin is a definite organ, and is probably a later development.

The binucleate condition of the sporidium exhibited by *Phrag.*

violaceum and some other forms is without particular significance, and is apparently a mere precocious division in which wall-formation is delayed. The view which has been put forward, that the sporidium starts in the life-history the condition with paired nuclei, is without foundation, for in all cases observed the mycelium arising from the sporidium has single nuclei, even in such cases as *Phrag. violaceum*, where the sporidium is known to be usually binucleate.

The teleutospore is really of the nature of a spore-mother-cell, the contents of which, usually after germination (but directly in *Coleosporium*), break up by a tetrad division into a series of four primary spores (the so-called promycelial cells). These can separate and put out germ-tubes, and no doubt cause infection; normally, however, when growing in free air, they remain connected and their germ-tubes become arrested, so that four secondary spores—the sporidia—are formed. The term promycelium is thus a misnomer.

NOTE.

It is obvious that the method of development of the fertile (female) cells in the aecidium of *Phragmidium* may be considered as a new type of so-called *parthenogenesis*; for, just as in *Artemia* there is a fertilization of 'parthenogenetic' eggs by the second polar body (nucleus) instead of by a spermatozoon, so in this case the nucleus of a vegetative cell has apparently replaced in function that of the spermatium. The term parthenogenesis is, however, an unsatisfactory one, as it is not very aptly applied to these cases in which there is a process of nuclear fusion; it would be much more satisfactory to confine the term to cases in which the sexual cells actually develop with the reduced number of chromosomes, without any form of fertilization. There is the same difficulty with *apogamy* now that we know that in apogamous Ferns the nuclei of prothallial cells may fuse together, for the application of this term suggests a denial of the existence of any process of fusion of cells or nuclei which could be considered of the nature of gametic fusion.

It becomes clear that cytological investigations of recent times have practically broken down the distinction between fertilization, parthenogenesis (in the wide, but not in the narrow, sense suggested above), and apogamy. Between the fusion of an egg-cell with a differentiated male cell, the fusion (association) of an egg-cell with a vegetative cell (or nucleus), the fusion of an egg-cell and its polar body, and the fusion of two vegetative cells (or nuclei) no sharp line can be drawn, and they must all be considered as terms in a series of *fertilizations*. Just as on one side of normal oogamous fertilization with its differentiated male and female cells there are such primitive types of fertilization as isogamy, and that, the most primitive, in which there is no distinction of sexual and vegetative cells; so, on the other side, there is a series of *reduced* processes of gradually increasing simplicity, the most simple being practically a return to the most primitive, where the sexual and vegetative cells are alike. Apart from the presence of the functionless spermata and the peculiar migration of the nucleus through a cell-wall, which show that it is reduced in evolution, there is nothing to clearly distinguish the process observed in *Phragmidium* from such accepted fertilizations as the conjugation of adjacent cells in a *Spirogyra* filament, the fusion of gametes (which are of the relationship of cousins) in *Actinosphaerium*, or from such a process as is to be observed in *Basidiobolus*, or even other Fungi, in which the sexual organs arise on neighbouring cells of a hypha.

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EXPLANATION OF PLATES

Illustrating Mr. V. H. Blackman's paper on the *Uredineae*.

PLATE XXI.

Figs. 1–13. *Phragmidium violaceum*, Wint.

Fig. 1. Teleutospore, lying in water, showing general form and stalk with swollen base; the two upper cells have just begun to germinate. × 450.

Fig. 2. Mature teleutospore, taken in autumn, in longitudinal section, showing the single nuclei and the structure of the wall and pits; the uppermost cell is not cut exactly through the middle, so the nucleus and the apical peg of thickening are not shown. × 800.

Fig. 3. Nucleus of the cell of a teleutospore, gathered in spring, which had been lying in water twelve hours. × 1900.

Fig. 3 *a*. Similar nucleus, but showing a later stage with single spireme thread. × 1900.

Fig. 4. Transverse section of cell of teleutospore, showing vacuolate nucleus; wall structure not shown. × 1150.

Fig. 4 *a*. Similar section, but showing abnormal state with *two* nuclei in the cell. × 1150.

Fig. 5. Teleutospore in optical longitudinal section, showing development of long germ-tube; the apical cell has already germinated and is empty; the nuclei in the two lower cells appear as clear areas. Fresh preparation. × 450.

Fig. 6. Germinating cell of teleutospore in transverse section, showing passage of tube through pit. × 970.

Fig. 7. End of germ-tube (promycelium) with nucleus. × 600.

Fig. 8. Promycelium divided into four cells, each of which has put out a germ-tube; one nucleus has migrated into the tube. × 1150.

Fig. 8 *a*. Promycelium with three sporidia and one cell not yet germinated. × 1150.

Fig. 9. Mature promycelium from which two of the sporidia have fallen; one of the two remaining is germinating *in situ*. × 950.

Fig. 10. Primary sporidium in section with single nucleus. × 1350.

Fig. 11. Secondary sporidium in section with two nuclei. × 1350.

Fig. 12. Primary sporidium, still attached to sterigma, showing two nuclei. × 1350.

Fig. 13. Secondary sporidium developed from primary and showing, apparently, four nuclei. × 1350.

Figs. 14–35. *Gymnosporangium clavariaeforme*, Rees.

Fig. 14. Mature two-celled teleutospore about to germinate. × 650.

Fig. 15. Cell of similar teleutospore in transverse section. × 1350.

Fig. 16. Germinated teleutospore, showing nucleus moving into tube on left, and nucleus dividing in middle of tube on right. × 620.

Fig. 17. Early prophase of division of teleutospore nucleus in germ-tube (promycelium), showing centrosome and kinoplasm. × 1900.

Fig. 18. Prophase showing chromatin collected, apparently as a single thread, towards one end of nucleus. × 1900.

Fig. 19 *a* and *b*. Two consecutive sections through a germ-tube with dividing nucleus; the majority of the chromosomes are to be found in *a*, while *b* shows the very young spindle with centrosomes. × 1900.

Fig. 20. Chromosomes collected irregularly round spindle; one pole shows apparently two centrosomes. × 1900.

Fig. 21. Chromosomes gathered towards centre of spindle. × 1900.

Fig. 22. Similar stage to above, but spindle oblique in position. × 1900.

Fig. 23. Chromosomes beginning to spread out on spindle; distinct radiations from upper pole. × 1900.

Fig. 24. Chromosomes are shorter and show a tendency to arrange themselves in two rows and apparently to fuse together. × 1900.

Fig. 25. Metaphase with shortened chromosomes stretched out on spindle. × 1900.

Fig. 25 *a*. Polar view of chromosomes on spindle. × 1900.

Fig. 26. Anaphase showing chromosomes separating into two groups. × 1900.

Fig. 27. Similar stage to above, but each group forms apparently a chromatin network. × 1900.

Fig. 28. Anaphase showing *two* groups of chromosomes moving towards each pole. × 1900.

Fig. 29. Later stage, showing chromosomes continuous from pole to pole and partially fused. × 1900.

Fig. 30. Later stage, showing two distinct chromatin masses continuous from pole to pole. × 1900.

Fig. 31. Chromatin forms an irregular mass at each pole with remains of elongated spindle between them; the radiations from the upper centrosome were about twice as long as shown and extended to the free end of the germ-tube. × 1900.

Fig. 32. Similar stage to above, but two distinct chromatin masses at one pole. × 1900.

Fig. 33. Daughter-nuclei formed; each shows kinoplasmic material (remains of spindle) and a centrosome with radiations. × 1050.

Fig. 34. First stage of second division; nucleus irregular and shows two centrosomes. × 1900.

Fig. 34 *a*. Early stage of second division in promycelium, showing spindle in young state, lying on one side of nucleus. × 1900.

Fig. 35. Later stage, in which spindle is now arranged axially in the tube and is partly surrounded by the chromatin mass. × 1900.

PLATE XXII.

Figs. 36–45. *G. clavariaeforme.*

Fig. 36. End of promycelial tube, showing second division with spindle now lying in the centre of the chromatin mass. × 1900.

Fig. 37. Section through spindle, showing chromatin in the form of granules. × 1900.

Fig. 38. Tube showing the two nuclei in different stages of division. At *a* the chromatin forms a distinct network and covers three-fourths of the spindle; at *b* a later stage is visible, in which the spindle is completely hidden by the elongated chromatin network, and only the centrosomes with their radiations are to be seen. × 1900.

Fig. 39. The two main portions of the chromatin network have drawn apart towards the poles, but are still connected by a number of threads. × 1900.

Fig. 40. Chromatin portions completely separated at the poles; the projecting arms are the remains of the threads which connected the portions in an earlier stage, as in Fig. 39. × 1900.

Fig. 41. A somewhat later stage than above; the projecting arms have been drawn in, and the chromatin forms a single mass at each pole.

Fig. 41 *a*. A similar stage to above, but chromatin forms *two* distinct masses at one pole. × 1900.

Fig. 42. Almost complete germ-tube (promycelium), showing the nuclei just formed, and a transverse wall in middle. In three of the nuclei the centrosome and the small portion of kinoplasm which connects it with the nucleus are clearly visible. × 1900.

Fig. 43. The four cells of the promycelium are formed. Three of the nuclei show a chromatin network, the other is in a younger state and still remains almost homogeneous. × 1350.

Fig. 44. Promycelium with outgrowths from each cell. The outgrowths are of the nature of germ-tubes rather than of sterigmata. × 880.

Fig. 45. Sporidium. × 1350.

Fig. 46. Portion of spermogonium of *Phrag. violaceum* on leaf of Rubus, showing general characters and thickened ruptured cuticle. × 660.

Fig. 47. Group of spermatia of same. × 1900.

Figs. 48–59. *G. clavariaeforme.*

Fig. 48. Section through spermogonium on leaf of *Crataegus*. The spermatial hyphae, spermatia, paraphyses, basal tissue (plectenchyme), and host tissue are all clearly visible. × 520.

Fig. 49–54. Stages in the development of a spermatium on a spermatial hypha. × 1350.

Fig. 55. Dividing nucleus of spermatial hypha, showing chromatin collected into a solid mass. × 1900.

Fig. 55 *a*. Stage showing nucleolus being expelled as chromatin shrinks to a homogeneous mass. × 1900.

Fig. 56. Later stage than above, showing chromatin separating into two daughter-masses. × 1900.

Fig. 57. Division in spermatial hypha in which chromatin shows a longitudinal division into two masses. × 1900.

Fig. 58. Very young spermatia with chromatin still an almost solid mass. × 1900.

Fig. 59. Group of mature spermatia. × 1900.

Figs. 60–65. Aecidium-development in *Phrag. violaceum*.

Fig. 60. Peripheral portion of young aecidium, showing the simplicity of structure. On the extreme left the special cells developed beneath the epidermis of the leaf are still undivided, while towards the middle a distinction into *fertile* and *sterile* cells can be observed and some of the fertile cells have already become binucleate. On the right the division of the fertile cells and the formation of aecidiospores and intercalary cells can be seen; the epidermis at this part has been ruptured by the development of the aecidiospores. × 340.

Fig. 61. Small portion of young aecidium, showing epidermal cells of leaf, sterile cells and fertile cells. Two of the fertile cells have become binucleate and one is still uninucleate; the one on the right has begun to grow up and push through the sterile cell. × 1350.

Fig. 62. Fertile cell with two nuclei of dissimilar structure. × 1350.

Fig. 63. Portion of aecidium showing fertile cell with nuclei of unequal size and different structure; the nuclei of the sterile cells have become partly disorganized. × 1350.

Fig. 64. Similar to above. × 1350.

Fig. 65. Fertile cell showing small densely staining nucleus in contact with larger nucleus with well-marked nucleolus. × 1350.

PLATE XXIII.

Figs. 66–77 *a*. Aecidium-development of *Phrag. violaceum*, continued.

Fig. 66. Portion of young aecidium, showing nucleus of cell (basal cell) below one fertile cell migrating into another fertile cell. Epidermis of leaf seen above and mesophyll below. × 1350.

Fig. 67. Similar stage to above, but sterile cells are empty. × 1350.

Fig. 68. Nucleus of basal cell migrating into fertile cell immediately above. × 1350.

Fig. 69. Very early stage of migration. × 1350.

Fig. 70. Late stage of migration; the distinction between the two nuclei very clear. × 1350.

Fig. 71. Fertile cell which has cut off the first aecidiospore-mother-cell. × 1050.

Fig. 72. Division of paired nuclei (conjugate division) in the first cell cut off from the fertile cell. × 1050.

Fig. 73. Later stage, in which the nuclei of the one aecidiospore and the intercalary cell are constituted, but no wall is yet formed between them. × 1050.

Fig. 74. Cell-row of aecidium showing, from above downwards, young aecidiospore, intercalary cell, aecidiospore-mother-cell, and fertile cell. × 1050.

Fig. 75. Mature aecidiospore. × 1050.

Fig. 76. Abnormal trinucleate fertile cell which has cut off a trinucleate aecidiospore-mother-cell. × 1050.

Fig. 77. Fertile cell with three nuclei undergoing triple conjugate division; the three solid masses of chromatin and the three nucleoli are to be seen. × 1050.

Fig. 77 *a*. Sterile cell which has grown up between two epidermal cells. × 1350.

Figs. 78–84. Uredospore-development of *Phrag. violaceum*.

Fig. 78. Group of developing uredospores on perennial mycelium, from stem of *Rubus*; four basal cells are marked ×. × 600.

Fig. 79. Group of basal cells from which the stalked uredospores will be developed. × 1000.

Fig. 80. Basal cell with young uredospore-mother-cell. × 1000.

Fig. 81. Young uredospore-mother-cell. × 1350.

Fig. 82. Uredospore-mother-cell dividing to form uredospore and stalk-cell. × 1350.

Fig. 83. Young uredospore with stalk-cell. × 1350.

Fig. 83 *a*. Portion of uredospore and stalk-cell to show protoplasmic continuity between the two. × 1350.

Fig. 84. Mature uredospore, showing the two nuclei of an irregular shape. × 800.

Figs. 85–87. Teleutospore development in *Phrag. violaceum*.

Fig. 85. Portion of leaf of *Rubus*, showing young teleutospore-sorus and mycelium; teleutospores in various stages and a few paraphyses are to be seen. Two basal cells of the teleutospores are marked ×, and two binucleate haustoria marked *. × 500.

Fig. 86. Three-celled teleutospore with stalk, in very young state. × 1350.

Fig. 86 *a*. Slightly older stage than above; the lowest cell is dividing and separating the fourth cell from the stalk. The wall shows the beginning of the characteristic thickening; the second nucleus in each of the two upper cells has been cut away. × 1350.

Fig. 86 *b*. Uppermost cell of young teleutospore, showing the apical projection only partially obliterated by thickening. × 800.

Fig. 86 *c*. Base of stalk of mature teleutospore, showing its attachment to the basal cell. × 1050.

Fig. 87 *a–c*. Stages in the fusion of the two nuclei in the teleutospore. *a* the nuclei in contact but not yet fused; *b* the nuclei have fused but not the nucleoli; *c* the two nucleoli have fused. × 1900.

PLATE XXIV.

Fig. 88 *a*, *b*. Further stages in the development of the teleutospore nucleus of *Phrag. violaceum*. *a* a very distinct twisted chromatin thread in the nucleus; *b* nucleus larger and returning to the condition of a simple network. × 1900.

Figs. 89–92. *G. clavariaeforme.*

Fig. 89. Two basal cells showing the development of the outgrowths which give origin to the stalked teleutospores. On the right the basal cell has just put out a second outgrowth, the first having developed into a teleutospore of which only the lower part of the stalk is seen. × 1050.

Fig. 90. Young outgrowth from basal cell, not yet divided. × 1050.

Fig. 91 *a*, *b*, *c*. Stages in the fusion of the nuclei in the teleutospore. At *a* the nuclei in contact but not yet fused; at *b* the two nuclei partly fused; at *c* the nuclei fused, but not the nucleoli. × 1350.

Fig. 92. A later stage than above, in which the two nucleoli have fused to a single nucleolus and the chromatin is in the form of a single twisted thread. × 1050.

Fig. 93 *a–e*. Stages of division of paired nuclei from the young dividing teleutospore of *Phrag. violaceum*. × 1900.

Fig. 94. Dividing cell of teleutospore in which each nucleus shows *two* chromatin masses. × 1900.

Fig. 95 *a–d*. Stages of 'conjugate division' from the uredospore-mother-cell of *Phrag. violaceum*. × 1900.

Fig. 96. Similar stage to that of 95 *b*, but the chromatin of each nucleus forms *two* masses. × 1900.

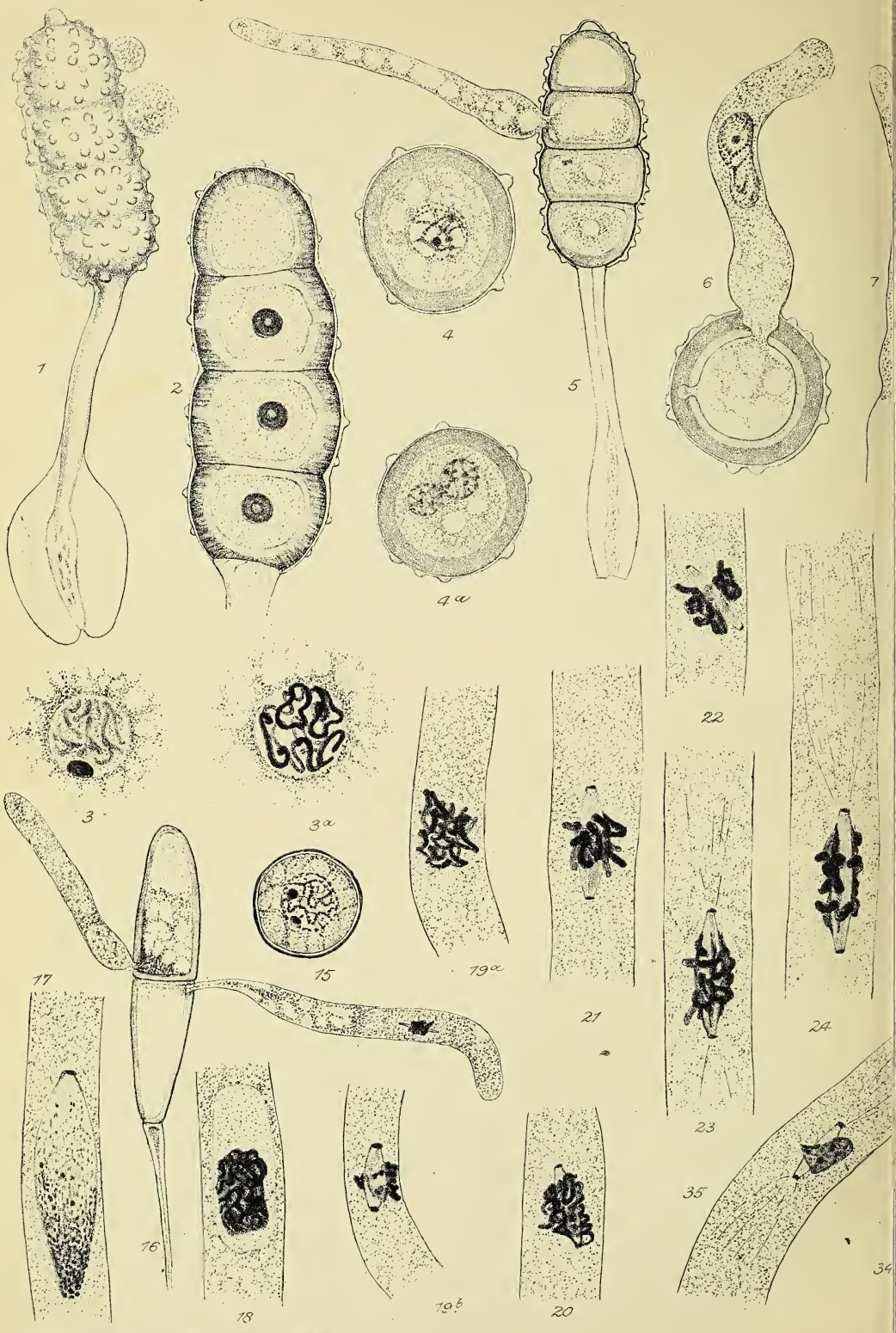
Fig. 97. Uredospore-mother-cell of *Phrag. violaceum* dividing; each of the daughter-nuclei shows *two* chromatin masses. × 1900.

Fig. 98. Conjugate nuclear division, showing four chromatin masses, from developing teleutospore of *G. clavariaeforme*. × 1900.

Fig. 98 *a*. First stage of division; the nuclear membrane has disappeared and the chromatin become condensed: from teleutospore as above. × 1900.

Fig. 99. Haustorium, showing two nuclei, from uredospore-bearing mycelium of *Phrag. violaceum*. × 1050.

Fig. 100. Nuclear division in one of the four promycelial cells of *G. clavariaeforme*. A distinct spindle is visible but no centrosomes; the arrangement of the chromatin is not clear. × 1900.



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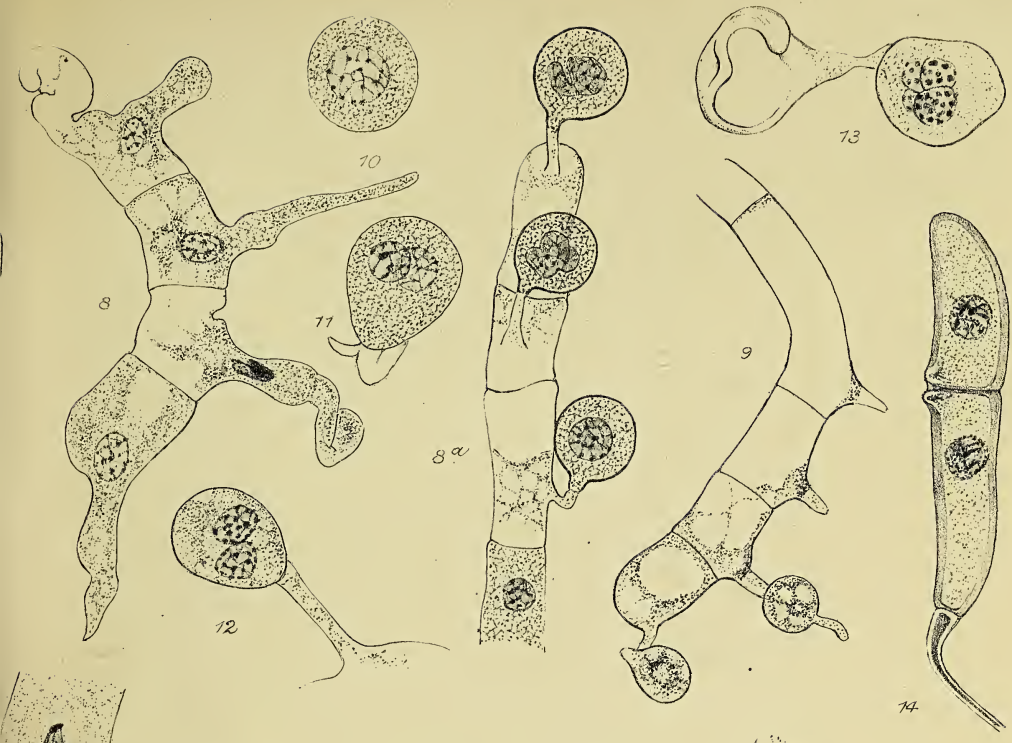

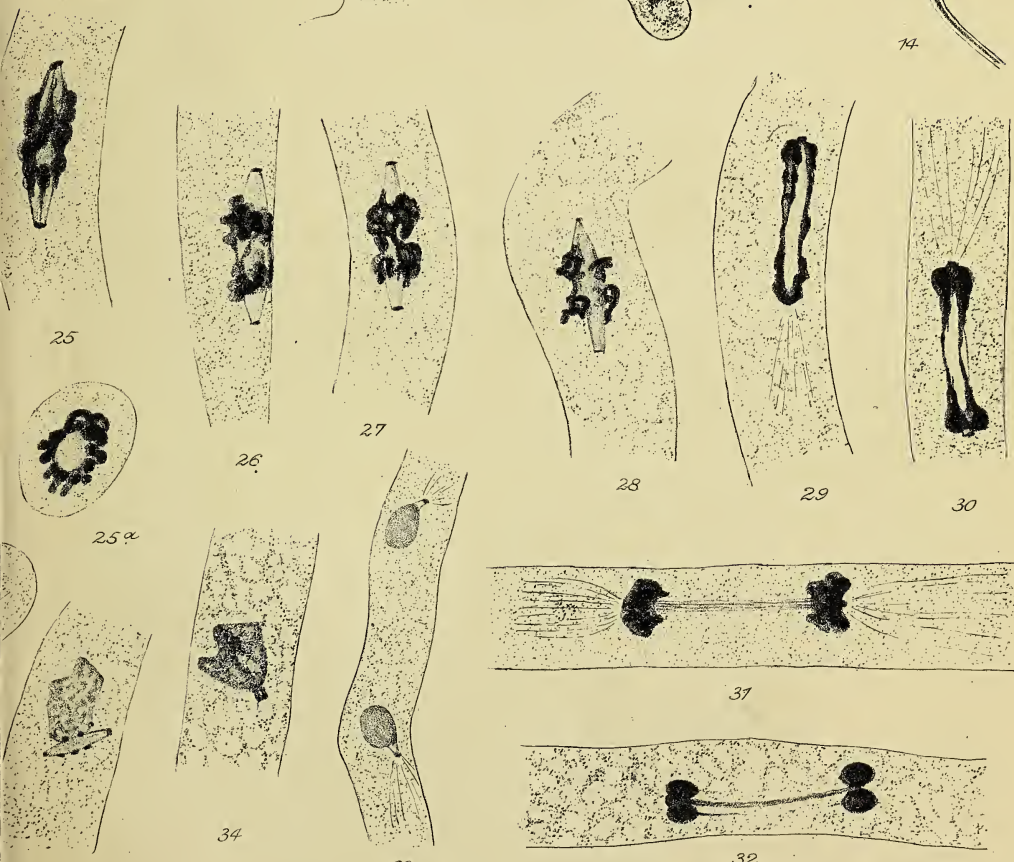

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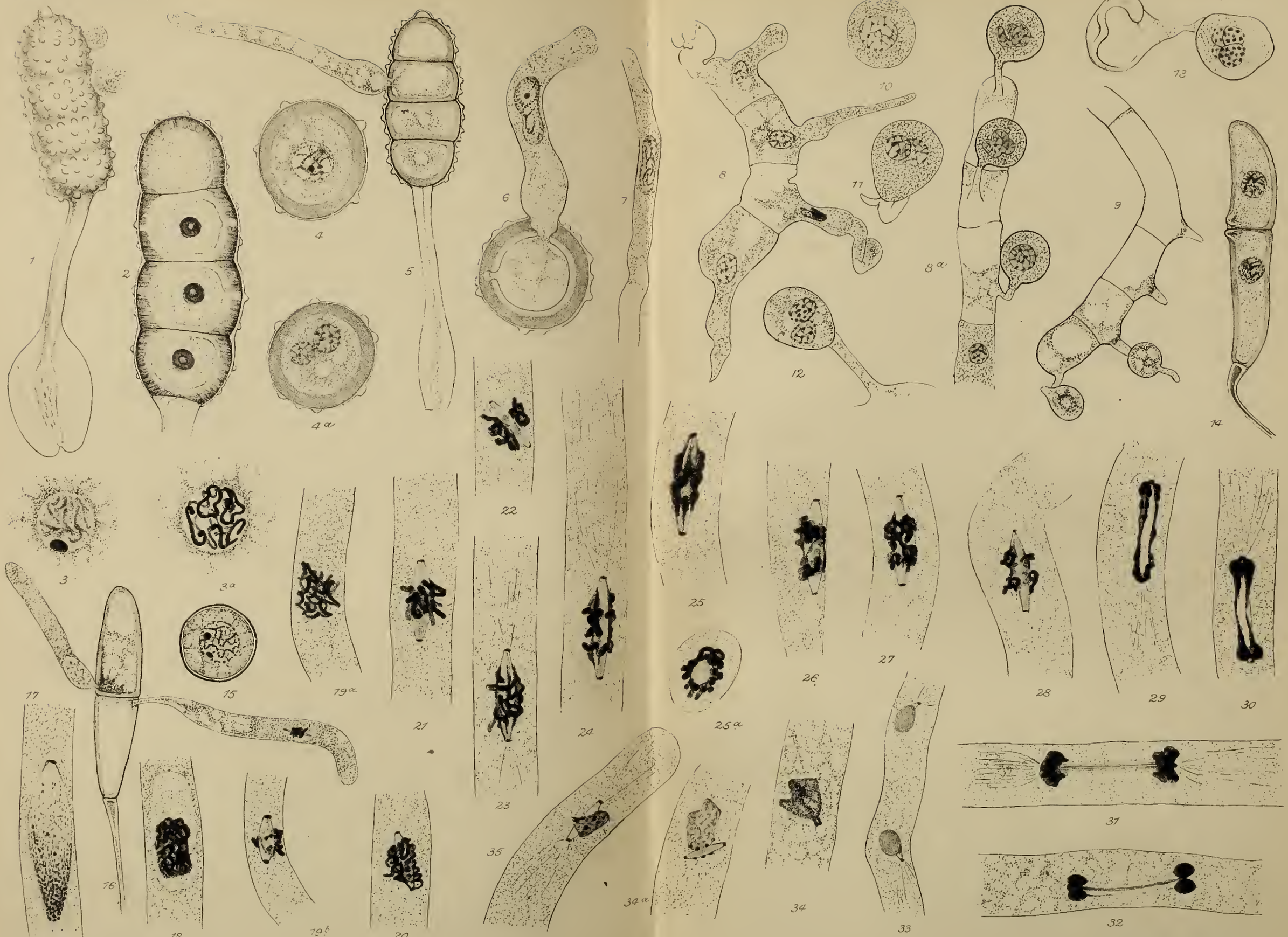
Uredineae



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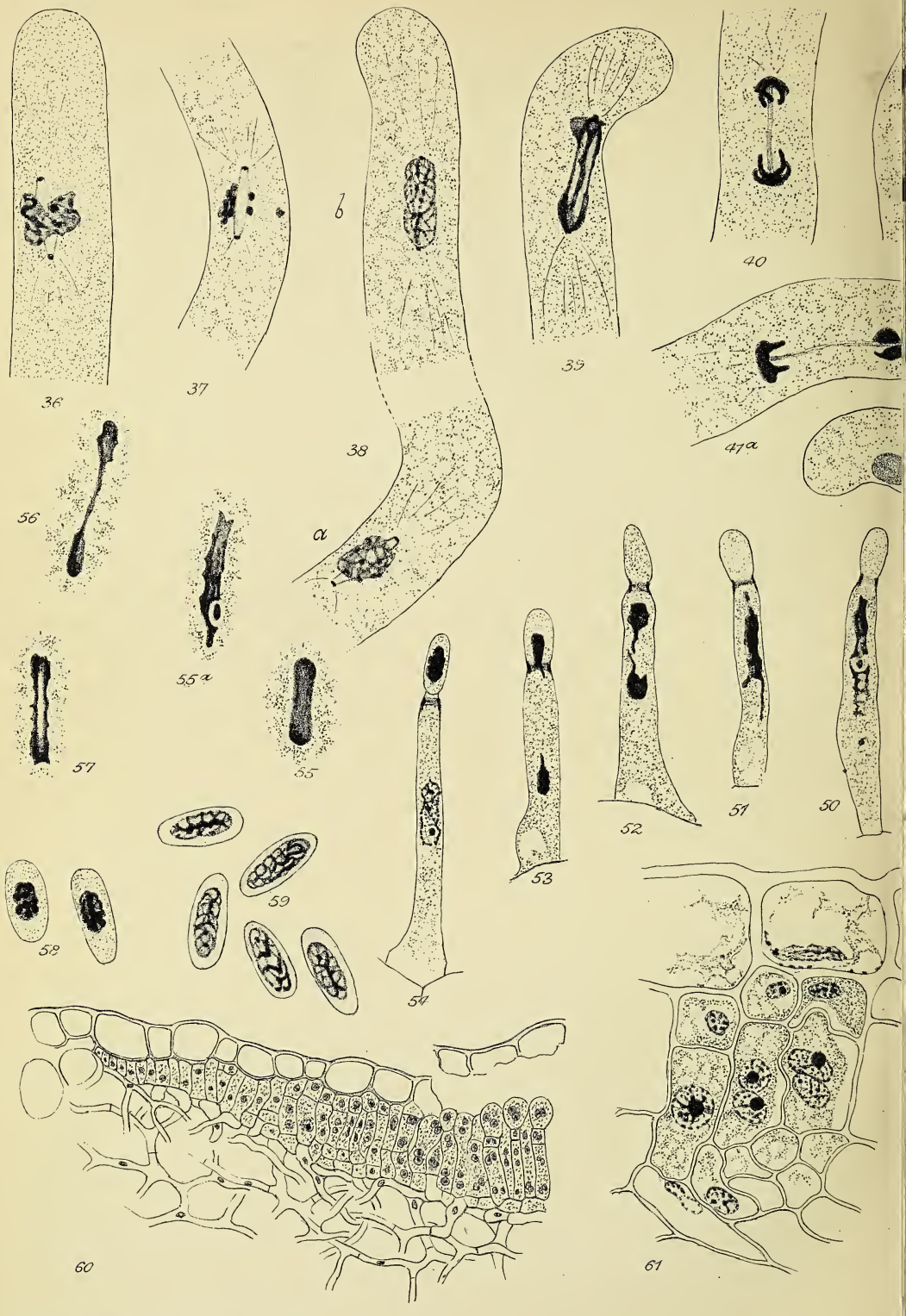

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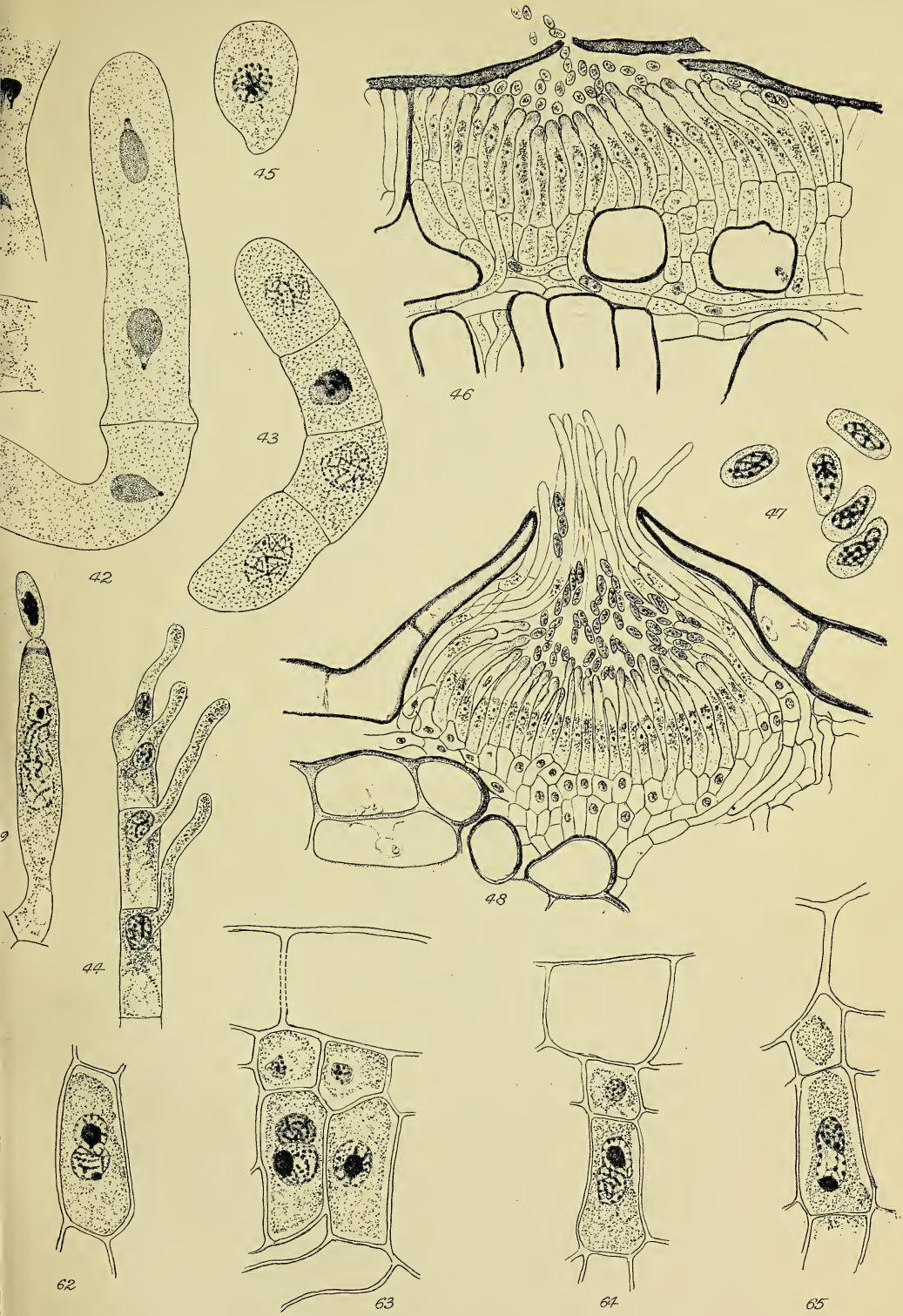

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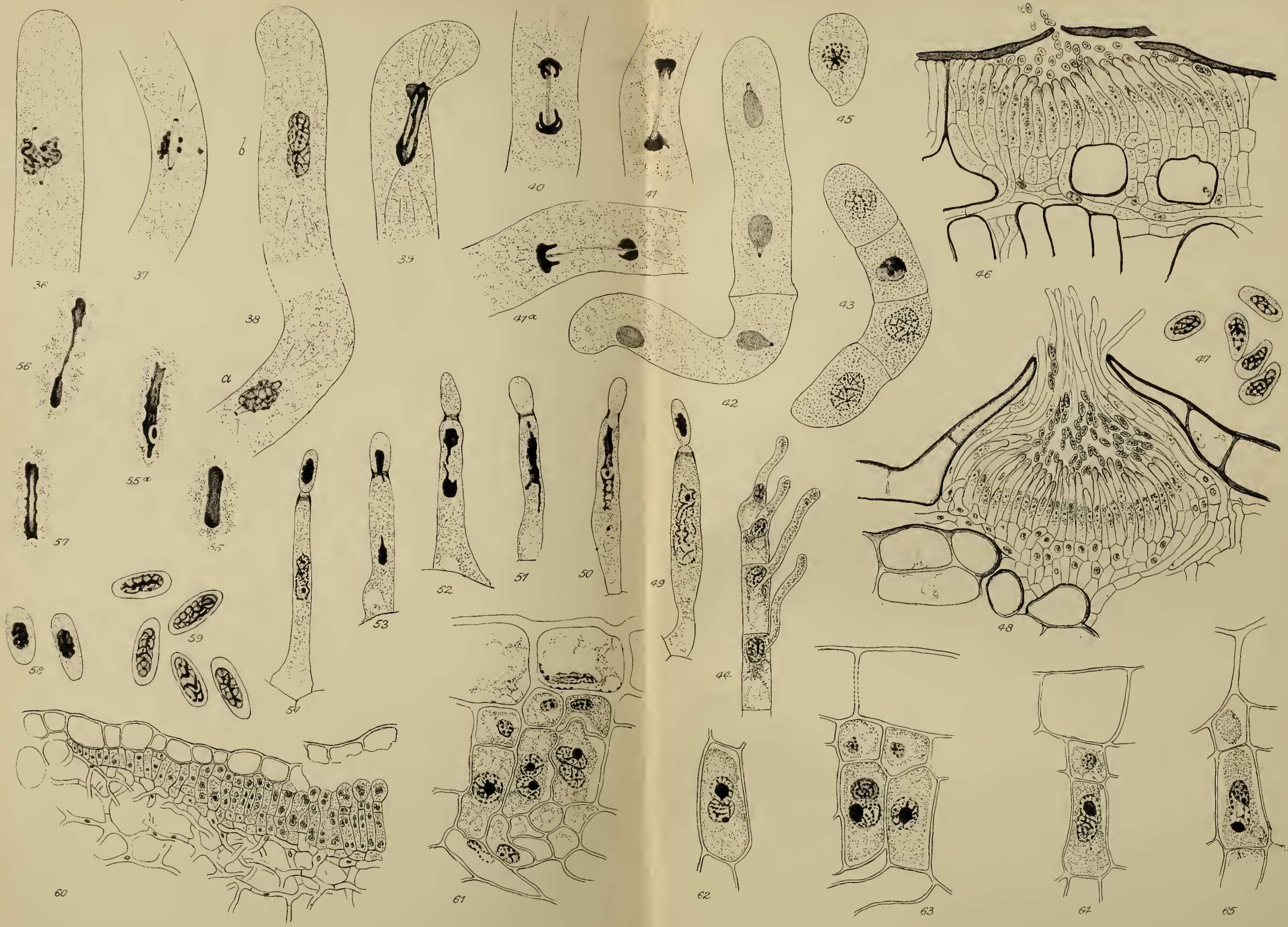

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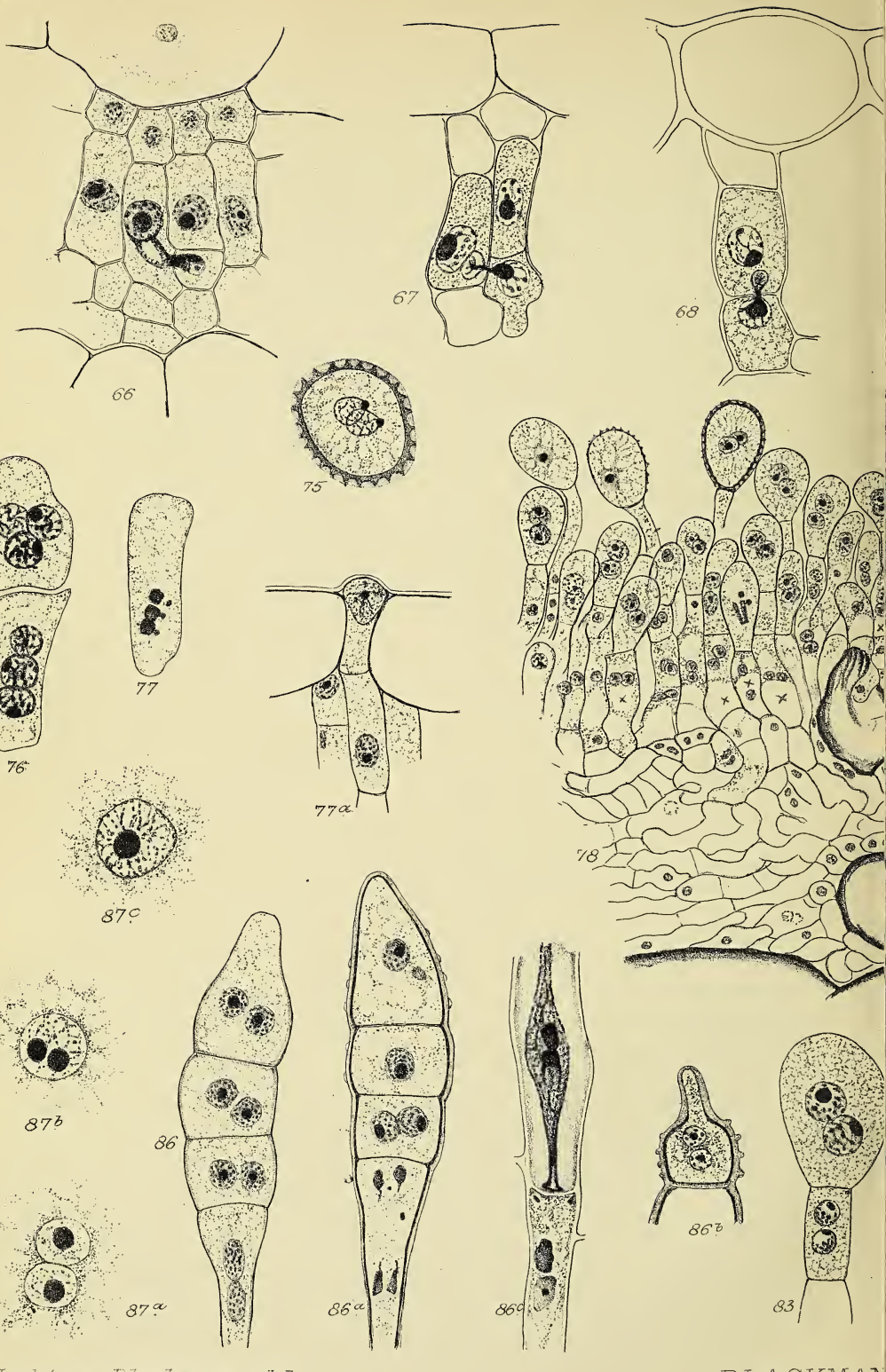

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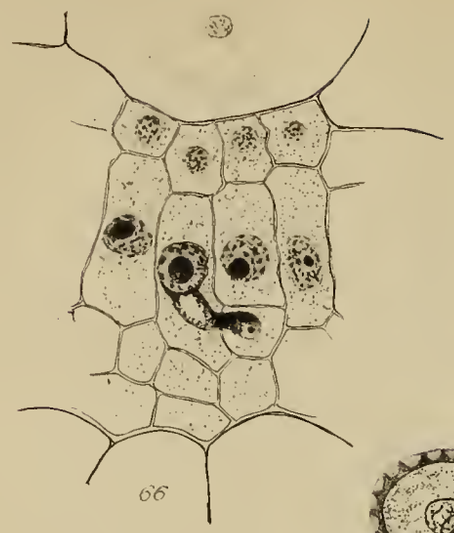
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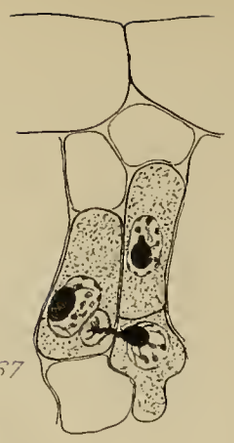
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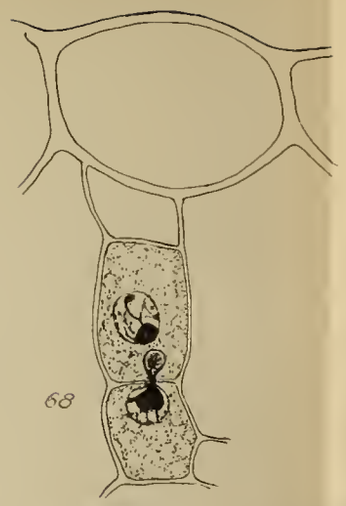
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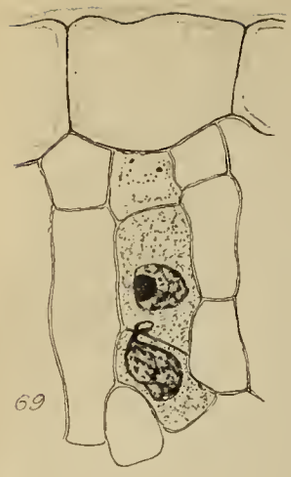
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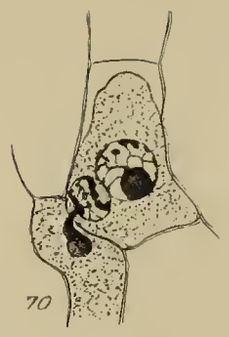
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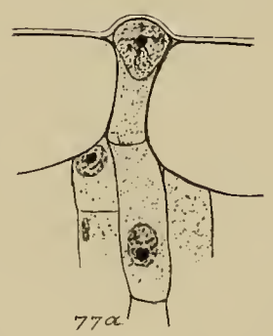
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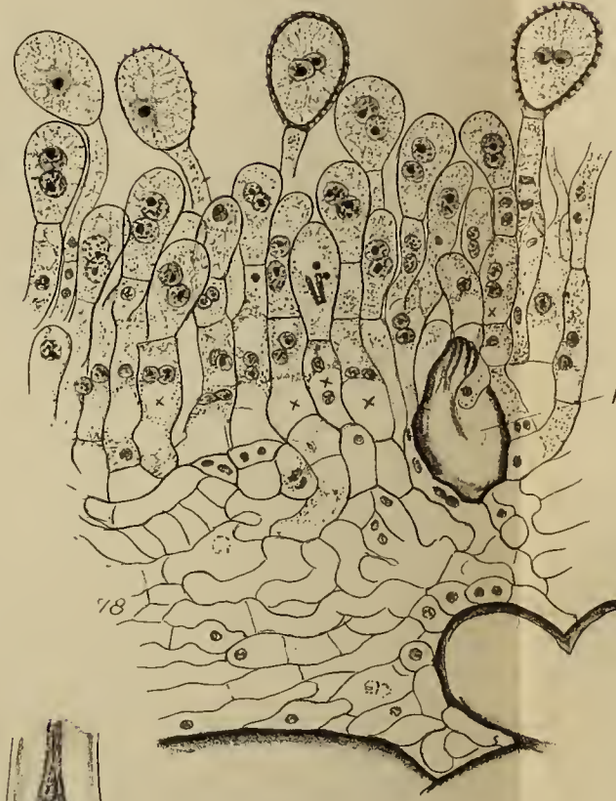
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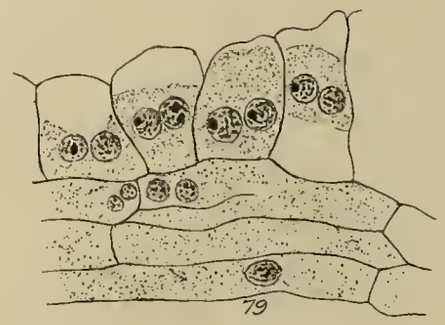
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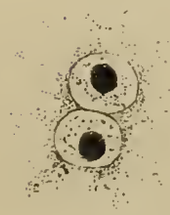
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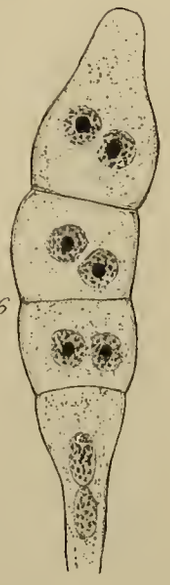
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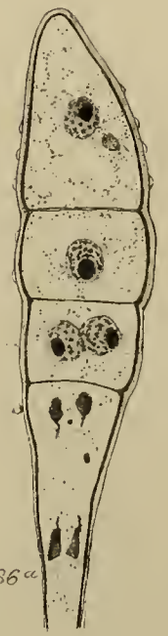
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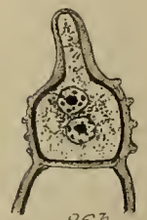
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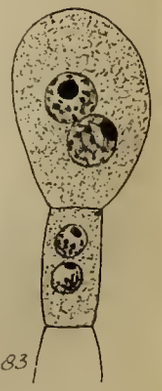
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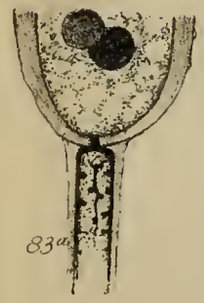
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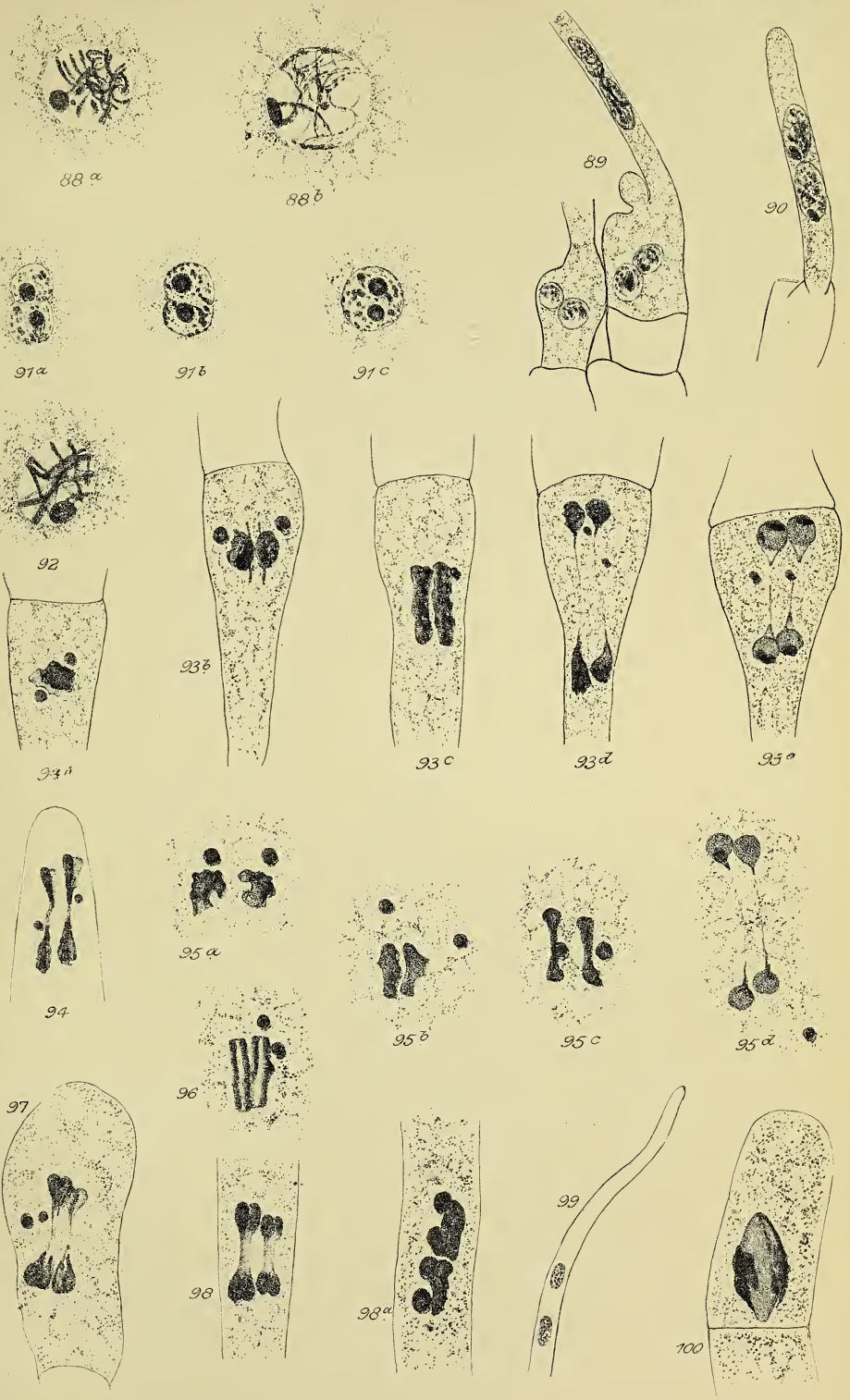
Highley et Blackman del.

BLACKMAN. *Uredineae.*

Highley ad nat. imp.



Highley et Blackman del. Highley lith. et imp.

Observations on Mamillaria elongata.

BY

OTTO V. DARBISHIRE.

With Plates XXV and XXVI.

CONTENTS

A. INTRODUCTION.

THE species of the natural order Cactaceae show a remarkable uniformity in the plant-form they represent. From this point of view the genus *Mamillaria* may be taken as typical for its natural order.

The Cactaceae occur in desert regions, and are thus subject to the influence generally of adverse conditions. The deviation which they represent, from what we may call the normal dicotyledonous type, makes a study of this order, as representing a definite plant-form, very interesting. This interest is heightened when we find the *Mamillaria* type occurring in another desert genus, belonging to a different natural order, namely in *Mesembryanthemum*, a genus of the Aizoaceae. *Mesembryanthemum stellatum* represents a plant-form of the same type as *Mamillaria elongata*.

In the literature of the subject very little information can be obtained concerning the physiology of *Mamillaria*, as a definite ecological type.

[Annals of Botany, Vol. XVIII. No. LXXI. July, 1904.]

Goebel and Ganong refer to the general biology of the Cactaceae, but we get only a superficial idea of the biological significance of the various organs and structures which characterize this plant-form.

The tubercles, crowned by a set of spines, form a very conspicuous feature in the various species of *Mamillaria*, but the spines are generally very briefly referred to in the literature. Their function is usually, if not always, put down, without further discussion, as that of affording protection for the living plant-body against grazing animals. Ganong, Goebel, Delbrouck, and others have put forward this view.

This explanation of the spines, which form such highly developed structures in many species of the Cactaceae, has never appeared to me to be quite satisfactory. A careful external study of the succulent plants at the Royal Gardens, Kew, still more increased my dissatisfaction with this explanation of their function.

The following paper is an attempt on my part to get nearer a better understanding of that plant-form of which I have taken *Mamillaria elongata* as a typical representative. The problem I put before myself, when I began these observations in 1901, was this: *what is the explanation of the plant-form represented by Mamillaria elongata, and what is, more especially, the meaning of the spines which form so characteristic a feature of this plant?*

A problem which has occupied numerous botanists is only referred to briefly in the following paper. This concerns the homology of the tubercles and spines of the Cactaceae. This question seems to have aroused far more general interest among botanists than their biological significance.

Of course no detailed account of the biology or ecology of any plant-group can be quite satisfactory, which is not based on field-work in the native haunts of the plants concerned.

For this reason great interest attaches to the establishment of a Desert Botanical Laboratory on which D. T. MacDougal reports in the Journal of the New York Botanical Garden of 1903 (18, p. 11). It is hoped that continuous observations will be made of conditions and plants, and with the material thus collected we should finally gain a clearer insight into the life of desert plants with which Volkens has already made us familiar to a certain extent. I have no doubt that many general physiological problems will be brought nearer a solution by being thus carefully studied in localities where the functions of the plant are carried out under adverse conditions.

I have, however, attempted to give some physiological explanation of some of the structures met with in *Mamillaria elongata*, although I have never myself visited any of the tropical American deserts.

It was first my intention to examine in detail thirty or forty different species of the Cactaceae, but unfortunately the time at my disposal would

not allow of this. For this reason I selected *Mamillaria elongata* as a good type. Of a few other Cactaceae I was able only to make a more or less superficial examination. The observations made on these latter, however, generally confirmed the opinions which I arrived at concerning *Mamillaria elongata.*

In order to make out the structure of the plants I cut numerous sections, a large number of which were obtained by the aid of the microtome.

The spines unfortunately cut very badly. This is not due to their hardness so much as to the air which they contain. All efforts to remove the air by an air-pump failed entirely. The resulting sections were generally therefore much torn, but did nevertheless help very much in making out the structures met with.

The sections were stained with Kleinenberg's haematoxylin or with brasilin, and mounted in Canada balsam, or they were mounted unstained in glycerine jelly to which some Fuchsin in a watery solution had been added. This last treatment gradually shows up very clearly all lignified, cuticularized, and suberized cell-walls which are well stained, the other walls being but faintly stained.

I would like to refer here to a method I adopt for marking the glass slides on which microtome-sections have been mounted. The old method of writing on the glass with a diamond, or the not quite safe plan of labelling the slide, both appear to me to be too cumbrous. I now merely write with a pen, dipped into the white-of-egg mounting solution, on one end of the glass slide, which must, however, be quite clean, a few distinguishing numbers or marks. Almost any stain will colour this albumen, and when dried it shows at once what the slide is. It is not easily removed during staining or even after. The writing can of course be replaced by a proper label later on when the slide is quite finished.

B. Observations on Mamillaria elongata, P.DC.

1. Morphology.

Mamillaria elongata, P.DC., according to Schumann (26, p. 518, Fig. 83) occurs in dense clumps, and is commonly met with in the Mexican state of Hidalgo. It forms patches which may be 1 m. in diameter. Each separate upright shoot is cylindrical in form. Its height may be as much as 30 cm., its diameter 1·5–8 cm. The plant-body, always unbranched, is usually however about 7–8 cm. high (Pl. XXV, Fig. 1).

As with all other species of this genus the surface of *Mamillaria elongata* is studded with numerous and very regularly arranged outgrowths which have been called mammae, warts, or tubercles (Pl. XXV, Figs. 2, 3). They projec about 2-4 mm. from the main body of the plant and are

surmounted by a set of spines. A single central spine may be separated from the marginal spines, which are so numerous as to obscure almost entirely from view the body of the plant. A dense mass of hairs is found in between the spines.

The plant is attached to the soil by a rather short and stout root (Pl. XXV, Fig. 3).

2. Anatomy.

I propose now to describe the structure of the main body of the plant-shoot, then to discuss the structure of the root, and finally in greater detail that of the tubercles and their spines.

(a) *Anatomy of the Stem.*

The specimens which I had the opportunity of examining anatomically were not more than about 4–5 cm. in height, and 1·5 cm. in diameter. They were obtained from Mr. F. A. Haage, junr., Erfurt, Germany, and appeared to be seedlings.

Disregarding for the present the structure of the projecting warts or tubercles, the main body of the shoot-part of the plant consists of a mass of fairly uniform parenchymatous ground-tissue, traversed by vascular bundles (Pl. XXV, Fig. 3).

The parenchymatous cells measure about 80–120 μ in diameter and in cross-section they appear roundish. Of this size they are found near and around the vascular bundle, in the cortex, the pith, and the broad medullary rays. They may be 380 μ long. Further away from the vascular bundles and nearer the epidermis the measurements for the cortical cells would be 60 to 70 by 180 to 250 μ. They all contain little cytoplasm, but a large nucleus. Intercellular spaces are found very extensively bordering on these cells in the cortex. They are, however, very shallow.

The epidermis, even in older parts of the plants I examined, which were themselves however not very old, was not replaced by cork, except in cases of injury or in that region where the root joins on to the stem. That part of the plant which is exposed to the air and to the light is almost entirely covered with the projecting tubercles. The remaining portion lower down is in the soil. Stomata are present here and there on this buried part, but very possibly they no longer function. Protoplasmic contents were not discernible. The radial walls of the epidermal cells are wavy and strongly cuticularized.

As just mentioned, the lower end of the plant frequently develops cork. The lower rounded end of the shoot in the plants I examined was covered by 6–10 layers of cork. The passage of any adventitious root through the ground-tissue of the stem is lined completely with a similar mass of cork. Those parts of the lower end of the plant which are actually in contact

with the soil are generally protected by cork. The epidermal layer does not, however, seem to be thrown off till fairly late, if it is got rid of at all. It can in fact generally be distinguished even outside the deepest masses of cork by the sinuate radial walls of its cells. The phellogen takes its origin in the layer of cells immediately inside the epidermis. The cork-cells are frequently very much flattened and stretched. They may thus attain a length of 100 μ, being at the same time 20 μ and less in radial diameter (Pl. XXVI, Fig. 27).

The main axis of the plant is traversed by a ring of bundles, which in my plants at least remained separate, fairly large medullary rays connecting the pith with the cortex (Pl. XXV, Fig. 2). At any given point we can find between eight and twelve or fourteen bundles in transverse section.

An examination of their structure shows the arrangement already described for the Cactaceae by several authors since the time of Schleiden (25, pp. 20–36). Solereder gives a brief summary of the literature which refers to the structure of the Cactaceae (28, pp. 459–468).

I will, however, briefly recapitulate the structure of our little plant (Pl. XXVI, Figs. 21–27).

The bast forms a small and inconspicuous part of the whole collateral bundle (Pl. XXVI, Figs. 21, 22). Following the nomenclature of Vöchting (32, p. 409) we can distinguish between large clear cambiform cells (*p*), and smaller and darker protophloem elements (*o*). The former measure 35 to 40 by 15 to 20 μ, in transverse view, being generally somewhat compressed radially; the latter are more isodiametrical, measuring about 3·5 to 7 μ across. In length both kinds of bast-cells measure about 90–100 μ. In the same way as we shall be able to notice in the xylem later on, the transverse walls of the bast elements are all found to be almost at the same height. Both kinds of cells finish longitudinally at the same level, their ends being but slightly drawn out. We might almost distinguish nodes and internodes in the bast, as later on in the wood. The protophloem-cells form groups of two to three to twelve or more cells, which lie embedded in the large cambiform cells. Both contain protoplasm, the protophloem-cells, however, more abundantly.

The bundles possess but little cambium, which very slowly adds on new tissue (*r*). This consists only to a very small extent of bast, being chiefly wood.

We now come to the wood, the elements of which occur in four different forms, three of which only are represented in every central bundle of the main stem.

The protoxylem is made up of the long and narrow spiral tracheids (Pl. XXVI, Fig. 21, *l*) so generally met with in this part of the bundle. They vary in diameter from 10 to about 20 μ, at which size, however, they are already passing into the metaxylem. The thickening forms a fairly

close spiral, but does not project very far into the cavity of the tracheid. In a tracheid measuring 10 μ across, the spiral thickening projected barely 2 μ into the cavity, and even in a 20 μ tracheid the spiral ledge was but a fraction thicker. Between these lignified tracheids we get a few thin-walled parenchymatous cells. These are of about the same size as the smaller spiral tracheids.

The metaxylem is made up of a different form of spiral tracheid, but the same kind of parenchymatous cell met with in the protoxylem. The spiral tracheids form a very characteristic constituent of cactaceous wood (Pl. XXVI, Figs. 23, 25) and have already been carefully figured by Schleiden (25, Pl. VII, Fig. 1, &c.). Subsequent authors have generally been satisfied with drawing them in a purely diagrammatic way or referring to Schleiden's paper only (28, p. 463, Fig. 91).

These tracheids have angular walls where they adjoin other tracheids, but rounded convex walls where parenchymatous cells are their neighbours. 50 μ probably represents the greatest diameter to which they may attain, the average lying between 30 and 40 μ for the larger ones, 20–30 μ for the smaller ones. In length they show a very uniform measurement, this being about 100 μ. The ends of adjoining tracheids meet at about the same level as was noticed already for the bast-cells (Pl. XXVI, Fig. 23). The ends are but slightly pointed or drawn out. Each tracheid has a cellulose wall, which is quite continuous, but from this projects a lignified spiral thickening of very large dimensions. The cellulose wall can plainly be made out and is probably about 1 μ thick. The spiral band of thickening projects into the cell-cavity a distance of 10–12 μ in the large tracheids, but never less than about 6 μ in the smaller ones. The spiral band is thinner at its point of attachment to the cell-wall and gets slightly thicker towards the centre. In longitudinal section we again usually see the cellulose wall bulging in towards the cavity of the tracheid when the latter is neighbour to a parenchymatous cell. The spiral thickening, however, always protrudes into the parenchymatous cell (Pl. XXVI, Figs. 23, 25).

The tracheids contain no protoplasm, as soon as they have become properly lignified. Frequently we can however see just inside the cambium tracheids which already show a large spiral thickening, which does not however respond to the ordinary wood-stains. Such cells contain cytoplasm and nucleus.

Van Tieghem seems to maintain that the spiral tracheids of the Cactaceae invariably contain protoplasm and should therefore be called parenchymatous cells (28, p. 462). It seems very likely, however, that his observations were made on some such younger tracheids as I have just referred to. These peculiar, broad and short, tracheids with their very well developed single spiral band form the bulk of the bundle of the main axis.

The parenchymatous cells found in between the spiral tracheids are

living cells, and they are practically of the same breadth and length as the tracheids. In breadth they are not unfrequently compressed, but their length may be said to be identical with that of the lignified cells.

Another kind of cell is found in the small plants of *M. elongata* towards the lower end of the main stem. In the later additions to the metaxylem we get in the latter, apart from the large spiral tracheids and parenchymatous cells, some libriform cells, which have very much thickened walls. In length they are about equal to the spiral tracheids, but in the region where they occur, the position of the tracheids with regard to one another has become rather irregular and they may be longer than at other points. The libriform cells measure up to about 90 and 120 μ in length (Pl. XXVI, Fig. 25, *q*). The transverse walls of the neighbouring cells still remain at about the same level. The libriform cells contain cytoplasm and nucleus, both of which can be made out clearly in transverse and longitudinal sections (Pl. XXVI, Fig. 26). The cells are angular in outline, and measure about 15 to 30 μ across (Pl. XXVI, Fig. 24). The thickening, which is continuous except for a number of pits, is not more than 4–5 μ in depth. The walls of these cells are flat and not rounded even when abutting on a parenchymatous cell. They have very distinct crossed pits. The opening of the pit towards the cell-cavity is a long and narrow slit, which widens out towards the middle lamella. At the latter point, the pit may be 2 μ across. The slit-like opening to the cell-cavity is about 3–4 μ long. The slit in any given cell lies at right angles to the corresponding slit of a neighbouring cell. The pits are found on all the walls (Pl. XXVI, Fig. 26).

The cells of the ground-tissue, which immediately surround the bundle, are very much compressed (Pl. XXVI, Fig. 21), so that their cavity is often very small as compared with their whole bulk.

(b) *Anatomy of the Root.*

The few small plants of *M. elongata* which I have examined were evidently grown from seeds. The whole root-system in these consisted of a distinct tap-root, with a small number of lateral roots. The former extends into the soil for a distance which is about equal to the height of the shoot above the soil.

The tap-root is usually very much thicker than the lateral roots, more particularly near its junction with the shoot (Pl. XXV, Fig. 3).

The structure of the root-tissues is very simple, but shows several important differences as compared with the shoot, apart from its general arrangement as a normal dicotyledonous root.

The growth of the root-tip and the origin at this point of the various tissue systems has been described by v. Breda de Haan for *Melocactus* (4, pp. 9–11). Pretty much the same condition of things, I imagine, will obtain

here, but my examination has been very cursory with regard to this question.

I will here merely describe the structure of a younger, and then that of an older root.

We can take first a young lateral root with a diameter of about 1 mm. The roots which I examined were all pentarch, with the exception of one, which was tetrarch (Pl. XXVI, Figs. 33, 34).

A few parenchymatous cells in the centre of the root, about eight to twelve in number, form the pith, in the outer part of which lie the small groups of protoxylem, consisting of 5–8 elements (Pl. XXVI, Figs. 31, 32, *l*). The cells of the pith are more or less round in transverse section, but rather elongate in longitudinal view. They measure about 12–18 by 100–150 μ. Their ends fit on to one another square, i.e. at right angles to the longitudinal direction of the cell, and the cells themselves contain a fair amount of cytoplasm and a distinct and fairly large nucleus (Pl. XXVI, Fig. 32).

The protoxylem-elements appear to be tracheids and not vessels. They are annular throughout. They measure about 10–15 μ across, but I have been unable to ascertain their length. The thickened ring is 1·5 to 2 μ thick, and projects about 1·5 μ into the cell-cavity. The distance from one ring to the next is very regularly about 8 μ. The very thin unthickened part of the tracheidal wall consists of cellulose.

Radiating outwards from the protoxylem-bundles may be seen the primary medullary rays. The cells of the primary medullary rays are usually much compressed tangentially, measuring occasionally as much as 50 μ in radial direction, and 19 μ in tangential direction. They become less compressed towards the periphery of the transverse section. The medullary ray as a whole naturally becomes slightly broader towards the outside, the rows of living cells radiating outwards very regularly. Finally they pass into the cortical tissue (Pl. XXVI, Fig. 31).

The large wedge-shaped masses of metaxylem fill up the space between the medullary rays. The elements of the metaxylem are tracheids and wood-parenchyma. Both are arranged in regular rows, but the distribution of the two kinds of wood-elements varies. Some of the radiating rows will consist almost entirely either of tracheids or parenchymatous cells, or both may be equally represented. The parenchymatous cells, whether merely xylem-parenchyma or belonging to the secondary medullary rays, are smaller than the tracheids. Their diameter varies between 10 and 20 μ; their length, like that of the cells of the primary medullary rays, may reach as much as 200 μ. They are of course living cells. The tracheids exhibit annular thickening throughout, although in a few cases two neighbouring rings may be connected by a band of thickening—thus forming a short spiral. They measure 10–26 μ in diameter, and seem to attain a length of 200 μ, but only in a few cases could the transverse walls be made out. The

narrower ones are generally found nearest the centre of the root (Pl. XXVI, Figs. 31, 32).

The projecting ring of thickened wall-substance is a very conspicuous feature. It may project as much as 8 μ all round, but generally only as much as 5–7 μ. The separate rings are regularly about 3 μ high and separated from one another by a distance of about 18–20 μ. In cases where an annular tracheid adjoins a parenchymatous cell the tracheidal wall collapses slightly, thus becoming concave towards the cavity of the former (Pl. XXVI, Fig. 32).

A layer of cambial cells, slightly compressed radially, surrounds the metaxylem, but does not seem to be very actively dividing opposite the primary medullary rays (Pl. XXVI, Fig. 31, *r*).

The elements of the bast are of the same structure as already described for the main axis of the shoot. Small groups of protophloem (*o*) are dispersed in between the large cambiform cells (*p*).

Then follow a few layers of large cells, which form the cortex. Their diameter is 18–30 μ in a radial direction, and 40–50 μ in a tangential direction. Their length seems to be about 150 μ. They are of course living cells.

The outer portion of the root is made up of numerous layers of cork-cells. Most of these have collapsed in a radial direction and can only be roughly counted (Pl. XXVI, Figs. 31, 32). The cork is about 10–20 layers deep at the most. The size of the cork-cells is very uniform. They are 70–80 μ long, and about 50–60 μ broad in the tangential direction of the whole root. Their radial diameter is often almost nil, owing to compression. It does not exceed 24 μ (Pl. XXVI, Figs. 31, 32).

In the young root of about 1000 μ in diameter just described the different tissues make up on the average the following proportions of any given radius:—Cork 40 μ, cortex 100 μ, bast 40 μ, wood 270 μ, the remaining tissues to the centre 60 μ.

An older root higher up, just before it passes into the shoot, does not differ much from the thinner root just described. But certain structures are found, which we have not yet met with, at least in the root.

At the point where the root passes into the stem there occur in the wood-part of the bundle small masses of libriform cells, already mentioned as being found near the older and lower end of the vascular bundle of the stem. The presence of these libriform cells seems to be characteristic of the lower end of the stem and upper portion of the root. The roots showing these libriform cells seemed to be 1·5 to 2 mm. in thickness. Quite large quantities are found at these points in the stem and main root. In smaller adventitious roots I have never, however, seen them pass into the root-tissue except for a distance of 10–15 μ, though they may be found plentifully in the continuation of the latter into the stem. In the larger main roots I

have seen them penetrate as far as 3 mm., but at this point they are much reduced in quantity. The libriform cells of the root agree in every way with those of the stem in structure and form.

We find that the tracheids of the root with their characteristic annular thickening are gradually replaced by the spiral tracheids of the stem. In some cases intermediate stages are found, giving the appearance of reticulate tracheids. Cork is extensively formed in this region. It lines, as already mentioned, the passages through the cortex made by the adventitious roots, and also covers the roots for a very long distance.

Root-hairs may occur in large quantities on the root, often obscuring very largely the growing-points of the young lateral roots. They very soon apparently lose their absorptive function and then may resemble fungal hyphae. But I was unable to detect the presence of any mycorhiza at all.

In the older roots the proportion of wood to parenchyma in the metaxylem is different to what it is in the younger root. There is in the former more lignified tissue, and it is therefore harder and tougher.

In *Melocactus*, v. Breda de Haan notes that the protoxylem of the root consists of spiral vessels (4, pp. 9, 10) and the metaxylem of scalariform vessels (4, p. 12).

(c) *Anatomy of the Tubercle.*

The arrangement of the tissues in the tubercles or warts which form such a prominent feature of the Mamillariae is extremely characteristic and interesting. Each tubercle is roughly of the shape of a stunted cone, the blunt apex of the latter being crowned by a marginal ring of spines with one central one (Pl. XXV, Figs. 1, 2, 3).

We can distinguish first an epidermis, which covers the whole tubercle (Pl. XXV, Fig. 15, *e*). The cells are flattened and have wavy outlines. The cuticle is not very thick, namely about 1·5 μ. The epidermis contains no chloroplastids. It is interrupted by fairly numerous stomata (Pl. XXVI, Figs. 9, 15). These are of the typical cactaceous type (28, p. 459). Several subsidiary cells are found running parallel to the guard-cells. In a transverse median section the guard-cells are seen to be at the same level as the other epidermal cells and are not depressed. A small ledge of cuticular wall projects in such a way as to produce a small antechamber which leads to the actual passage between the guard-cells. This leads to the internal air-chamber with which the whole very extensive system of intercellular air-spaces inside the tubercle communicates.

Disregarding the spines and the cushion on which they are inserted, the remaining tissues of the tubercle are ground-tissue and vascular tissue (Pl. XXVI, Fig. 15).

The cells of the ground-tissue are parenchymatous throughout. Immediately inside the epidermis is found an hypoderma (*d*), consisting of flat, short cells, which really differ only in form from the next inner cells. They usually contain a few chloroplastids. Except near the stomata, they leave no intercellular spaces between themselves and the epidermis.

These cells are succeeded by rows of palisade-cells, which run parallel to one another and make a definite angle with the epidermal layer; each row consists of 2–6 cells. Very extensive and continuous air-spaces are found in connexion with these rows of cells. They seem often to completely surround the palisade-cells, so that these appear to be like the assimilating filaments in *Marchantia*, namely, loose threads. This is, however, not actually the case. They represent the chief assimilating cells of the plant and contain therefore very many chloroplastids. These are round in form and measure 5–7 μ across. I have nearly always found the chloroplastids applied to the two walls which run parallel to the longitudinal axis of the filaments, at other times they closely surround the nucleus. Most of the remaining inner part of the ground-tissue consists of large roundish cells, with but few chloroplastids. Some of these cells, more particularly nearer the apex of the wart, contain large crystals of calcium oxalate. The ordinary round ground-tissue cells pass into the filamentous palisade-cells very abruptly.

A number of parenchymatous cells are enclosed in the cup-like ending of the vascular tissue. They are almost colourless, clear cells, and also frequently contain large quantities of calcium oxalate.

Of very great interest is the ending in the tubercle of the vascular system. This is a very highly developed structure, and it alone would show that *M. elongata* represents a very high degree of adaptation to external conditions.

The bundle-system of the tubercles is of course connected with that of the main stem. The arrangement met with is that described by Ganong for those Mamillariae which have no furrows on the upper side of the tubercles (10, p. 35, Fig. 14). From Ganong's observations and from my own it appears that one strand of vascular tissue leaves a bundle of the main stem for every tubercle (Pl. XXV, Fig. 3). This bundle is about 120–150 μ thick and it originates in the inner end of one of the stem-bundles. It consists in fact of part of the protoxylem and part of the metaxylem and the phloem. On leaving its parent bundle it passes between the two bundles to the cortex, rising slowly in a direction towards the tubercle. From this one lateral bundle are derived the bundles of the cortex of the main body of the plant, the bundles of the tubercle and those of the lateral bud, which is found in the axil of each tubercle. According to Ganong the bundles of the assimilating tubercle consist of a leaf and a cushion system of bundles fused. It is quite immaterial here what their morphological

nature is. It is important, however, to notice that the one branch from the main bundle divides, but that its branches in the tubercle and also in the cortex anastomose freely, and in the tubercle itself finally end in a large cup-like mass of big tracheids (Pl. XXV, Fig. 15).

On leaving the main bundle the lateral branch leading to the tubercle-system consists of extremely minute spiral tracheids. They are 12 to 16 μ in diameter. In the protoxylem the spiral bands are about 2 μ thick and as much as 8 μ apart. In the younger metaxylem the figures would be 2·5 and 3–4 μ respectively. I was unable to make out the length of the tracheids at this point in the bundles. But they are evidently fairly long, at any rate in proportion to their diameter. In this they differ very much from the spiral tracheids of the metaxylem of the main bundle. They are in fact more of the type of spiral vessel or tracheid met with in most protoxylems of the normal Angiosperm. Here and there we do, however, get one of the tracheids in the cortical bundles suddenly passing into a large spiral tracheid, measuring as much as 80 by 36 μ, which is of the typical cactaceous form. It will be found to be in contact with some two or three large parenchymatous cells of the cortex.

The bundles branch fairly frequently and anastomose again freely. A certain number of branches pass towards the growing-point, which is situated in the axil of the tubercle, but a greater number of bundles bend out and grow towards the outer end of the latter. Of these a certain number pass towards the rows of palisade-cells of the wart, and here they end blindly (Pl. XXV, Fig. 15).

All the bundles are accompanied by bast, the position of which, however, changes in such a way that finally it always lies outside the wood. The bundles passing to the tubercles therefore contain the following structures: bast, small and narrow tracheids with very close spirals, and larger tracheids with very loose spirals. This is what one might expect, but owing to the short distance between protoxylem and bast the contrast between the two forms of spiral tracheids is very marked (Pl. XXV, Fig. 19). The bast may extend to as great a depth as the wood when entering the tubercle, but later on it becomes very much reduced. The very last endings of the central mass of vascular tissue just underneath the cushion of spines are quite free of bast, being surrounded by parenchymatous cells only.

In a transverse section of a tubercle we would be able to notice, when cut half-way down, about 6–7 bundles which form an outer cortical ring (Pl. XXV, Fig. 10). These bundles end just inside the palisade-tissue. At the first point the cortical bundle would generally be about 70–90 μ deep in a radial direction, and about half that in a tangential direction with regard to the periphery of the whole tubercle (Pl. XXV, Fig. 7). In bulk the wood (*b* and *c*) is slightly in excess of the bast, although the

bast-cells (*a*), being smaller, exceed the wood-cells in number. The components of the wood are tracheids, which in the smaller cases are spiral, but in the larger ones are spiral to reticulate. The tracheids of the cortical bundles do not show any such remarkable development as we shall meet with in the case of the central bundles. But we may here and there get a large tracheid developed centripetally from the inner end of the wood-portion of the bundle (*c*). But these large tracheids, which may be 16 μ in diameter, are large only as compared with the smaller ordinary tracheids of this bundle. These vary between 8 and 12 μ in their largest diameter.

The last tracheids of a cortical bundle may be as much as 20 μ in diameter, the thickening spiral being about 5 μ deep (Pl. XXV, Fig. 8). They are unaccompanied by any bast-tissue.

The cortical bundles are closely surrounded by a number of large parenchymatous cells, which possess an internal cytoplasmic lining of 4–5 μ thickness in which are embedded the very numerous chloroplastids.

Of great interest is the structure of the central mass of vascular bundles.

If we follow out the course of the more centrally placed or medullary bundles, we see that they are quite separate from the outer ones. The cortical bundles run just inside the cell-rows of the palisade-tissue, the medullary bundles are found further inside. At first they form a disconnected ring in transverse view, although they are actually anastomosing freely (Pl. XXV, Figs. 10, 11, 12 and 15). This refers to the lower end of the tubercle, as soon as the medullary and cortical bundles have become separate. The former appear gradually to move closer together, but as a matter of fact they are merely increasing in circumference at the expense of the surrounding ground-tissues. The diameter of the whole wart decreases, but the diameter of the bundle-ring may even increase slightly as we near the top of the tubercle.

Each medullary bundle consists primarily of a number of spiral tracheids, which are fairly narrow in diameter and have already been described. External to this xylem is the bast, which in the beginning may be in extent nearly equal to the wood-portion (Pl. XXV, Fig. 4). But gradually, as in the cortical bundles, large spiral tracheids are developed centripetally from the xylem of each bundle (Pl. XXV, Figs. 4, 5, *c*). These spiral tracheids soon extend from bundle to bundle, and may even completely surround all the wood of the original bundle (Pl. XXV, Fig. 6, *c*). But by this time the bast has practically disappeared. The completed ring of lignified cells consists finally then of groups of a few small spiral tracheids, which groups correspond in number to the original wood-bundles or branches of the latter (Pl. XXVI, Figs. 6 and 14). There may be as many as twelve such groups. Not unfrequently some of the bundles send branches into that part of the tissue enclosed by the cylinder of bundles

(Pl. XXVI, Figs. 13 and 14). These groups are joined together by the large tracheids.

The larger tracheids, which are formed here centripetally at first and later on all round, are very large indeed and show very well developed spiral or reticulate thickenings, which are usually very close together (Pl. XXV, Fig. 19). The tracheids may be as much as 60 μ across in a transverse direction, and 160 μ long. One particular tracheid measured 200 by 40 μ, another 100 by 20 μ. Next to these large cells we find the small, narrow spiral tracheids of the old bundles, measuring 10 μ in diameter (Pl. XXV, Fig. 19).

The spiral thickenings of the larger spiral tracheids are very massive. Seen in surface view from the outside they are about 3 to 6 μ broad, their actual point of attachment being about $\frac{1}{2}$ or $\frac{1}{3}$ this measurement in each case. They project into the cell-cavity a distance of 3–6 μ. The central point of the attachment of any spiral thickening at any given height is very generally about 10 μ distant from the same point on the spiral above or below. The spirals do not in fact move further apart, they remain stationary, but the thickenings increase in size and so they appear to get nearer together. The form of these larger cells appears to be more or less barrel-shaped. The ends are slightly narrower than the middle. The difference, however, is less marked when the tracheids are long and narrow. The last tracheids at the top of the bundle-ending measure fairly regularly about 20–30 μ across and are 100 to 120 μ long (Pl. XXV, Fig. 15).

The fusion of the medullary bundles and the formation of the large spiral tracheids results in the production of a cup-like ending to the vascular system just underneath the top of the tubercle. The cup consists of the small groups of smaller tracheids united laterally by the larger ones.

On the outside the tracheids are surrounded by large active parenchymatous cells, about two or three of which intervene between the tracheids and the palisade-cells. The cup is filled with parenchymatous cells likewise, which again are large and active. The tissue inside the cup and immediately outside it appears lighter in section than the palisade-cells because its cells contain very few chloroplastids. No air-spaces are found inside the cup.

The tissues mentioned so far as being found in the tubercles are not the only essential ones. The apex of the whole tubercle is occupied by a cushion of tissue in which are inserted a number of curiously complex spines, the lower ends of which are furthermore surrounded by a mass of hairs. There are on the average about twenty marginal spines in each set. In the centre is found a single spine, larger than the others. The marginal spines are 4 to 4·5 mm., the solitary central one 5 mm. long. The whole set of spines covers an area with a diameter of 7 to 8 mm. The distance from the centre of one set of spines to the centre of the nearest neigh-

bouring set is 4 to 5·5 mm. The various sets of spines therefore overlap considerably (Pl. XXV, Fig. 2).

I will now describe the full-grown spines and the cushion of tissue they are inserted on.

The cushion and its spines are quite definitely separated from the underlying tissue of the tubercle. The latter, although it includes the vascular tissue, may be considered the living and active part of the whole lateral organ; the former may be called the dead or passive portion (Pl. XXV, Fig. 15). These two parts are separated by a layer of cambium, which is continually adding to the cells of the outer dead tissues. It is not at all unlikely that a few cells of the living tissue of the tubercle are also derived from this cambial layer. The last few small cells of this tissue at least seem to run in regular rows, which end in one of the cambial cells (Pl. XXV, Fig. 15).

The cambial layer runs right across the whole tubercle, but it does not appear as a straight line in section. It is practically continuous with the epidermis of the tubercle, and it joins the latter just inside the small rim, with which the tissue of the tubercle surrounds the set of spines.

The cushion in which the lower ends of the spines are inserted is entirely made up of cork-tissue (*k*). The separate cells are arranged in the typical way, namely regular rows, the walls being often much contorted owing to unequal pressure. The cork-cells of the cushion at first show a clear cell-cavity, but as they get further away from the phellogen they become so much twisted about that it is almost impossible to recognize any cavity or even the thickness of the cell-wall. The whole cushion may reach a thickness of about 600–700 μ, being at the most about 800 μ broad. It is entirely cut off from the living cells of the tubercle, even portions of the epidermis giving rise to cork-cells (Pl. XXV, Fig. 15, *g*, *k*).

On the cushion are inserted the spines and a number of multicellular hairs. Inside the rim formed by the upper end of the tubercle, and growing from the corky tissue of the cushion, we meet with a ring of numerous dried hairs. These hairs may be no more than 4 μ thick, but about 20 to 40 μ broad (*i*). They have collapsed almost entirely in one plane and the transverse walls project like prominent ridges (Pl. XXV, Fig. 20, *i*). They are about 800–900 μ long, but often are much crumpled and twisted. These hairs consist almost entirely of cellulose, except a very thin outer wall of cuticle. This circle of hairs is followed by a ring of spines, then follows another circle of hairs, and finally a solitary central spine.

The spines are all of practically the same structure, but the solitary central one is larger than the others. It will, therefore, be sufficient to describe either one or the other (Pl. XXV, Figs. 15, 16).

In each spine three different tissues can be well distinguished, at least at the lower or basal end. At this point the core of the spine, whether it

be a central or a marginal one, is seen to consist of a conical mass of thick-walled fibres which are always filled with air (Pl. XXV, Fig. 15, 16, 17, *h*). The broad end of the cone somewhat abruptly passes into the corky layers of the cushion. It may become slightly narrower at its lower end. In a long central spine the mass of air-filled fibres would be about 700 to 800 μ long from the base to the top, and about 250 μ broad, the breadth of the spine being about 400 μ. For a shorter marginal spine the measurements would be about 700 μ, 160 μ, and 250 μ respectively. The separate fibres are not in the least crushed, but they have rather angular walls, not rounded off at the corners (Pl. XXVI, Fig. 28). Their length varies very much, but does not exceed 200 μ. They have tapering ends. In diameter they measure as much as 25 μ, and of this only about 2 μ all round must be taken as thickened wall. But, of course, smaller cavities are met with as the fibres taper off. The fibres form a compact mass at the lower end of the spine, but higher up they separate, and in transverse section appear to be quite separate (Pl. XXVI, Fig. 30, *h*), though they are not actually separated in a longitudinal direction. The last tapering ends may be no more than 3–4 μ in diameter, their walls, to a large extent at least, consisting of cellulose. In a spine which has not been sectioned, but which has been mounted whole, the conical mass of fibres with their cavities filled with air looks very striking. It is impossible to remove the air except by exposing the cavity of each single fibre, so firmly is it held.

These central fibres lie embedded in a mass of cortical fibres, which differ very much from the central ones (Pl. XXVI, Figs. 28 and 29, *t*). They appear round in transverse section, but it is almost impossible to make out their length, so closely do they fit together and so much reduced are their cavities. But they appear to have long tapering ends, and one I measured was 200 μ long. This was one of the inner ones closely surrounding the central mass of air-containing fibres. The inner cortical ones appear fairly round in transverse section, whereas the outer ones are rather flattened and also have more contents than the inner ones. The outer rather irregular fibres preponderate in the lower part of the spine, but higher up they are almost entirely replaced by the regular round ones, which low down are at first only found sparingly. But they gradually make their appearance between the central fibres, and finally they make up the bulk of the tissue of the spine. The outer and lower ones are compressed in a radial direction. They are about 12–15 μ in diameter, half of which may be wall-substance which seems to show no trace of any cellulose. The fibres higher up are generally isodiametrical and measure at the most about 20–25 μ, with a minute cell-cavity of generally about 1 μ diameter (Pl. XXVI, Fig. 29). These fibres are more rounded off than the lower central air-containing fibres. The thick walls show very clearly concentric striation.

It now remains to briefly describe the outer layer, which covers the whole spine and is a single plate of cells continuous with the epidermis of the tubercle. The cells are squarish and elongated in a direction parallel to the longitudinal axis of the spine. They are usually about 100 μ long and in external view about 25 μ broad. Their radial walls are 3–4 μ thick, but are not quite even.

At the lower end of the spine these cells are rather flattened radially, being then about 7 μ deep, of which 3–4 μ form the outer wall, and 2 μ the cell-cavity, which is filled with a brownish substance (Pl. XXVI, Fig. 28, *e*). Higher up they appear quite uncrushed, being sometimes as much as 15 μ deep, 6 μ of which fall to the outer wall, and a similar number to the cavity. The inner wall is very thin (Pl. XXVI, Fig. 29, *e*). The outer wall of these cells has peculiar projections into the cavity, which give the wall a transversely striated appearance in surface view. In the upper half of the spine, and even lower down, the epidermal cells are remarkable on account of a peculiar knob, which grows out from the upper end of each cell, and is an outgrowth of the wall only (Pl. XXV, Fig. 16). These knobs project as much as 25 μ, and in diameter measure about 12 μ. The thick walls of the epidermal cells consist almost entirely of cellulose, a fine cuticle only covering them on the outside.

Caspari briefly describes the structure of the spines in the Cactaceae generally. He finds, however, that the central portion of the spine consists of thick-walled sclerenchymatous cells, the outer portion of thin-walled cells (6, pp. 6, 7). This does certainly not agree with the observations on *Mamillaria elongata* just referred to above.

It has already been mentioned that the corky tissues of the cushion pass rather abruptly into the hard fibrous cells of the spines. The central spine ends square and is not very firmly secured in the tissue of the cushion. It therefore is very easily broken off. This we very frequently find to be the case. The marginal spines are much more rigidly connected with the underlying tissues. On their lower or inner side—nearest the living tissues of the wart—the central hard fibrous cells of the spine are continued some distance into the cushion. They are smaller here than higher up and are very much crushed and contorted (Pl. XXV, Fig. 20). They are here in very close contact with the corky cells of the cushion, which at this point, also very much contorted, dip down slightly. This internal projection from the spines into the cork-cushion is found just above, but outside the margin of the vascular cup (Pl. XXV, Fig. 15).

For the sake of completeness I must mention here that I have occasionally found fungal hyphae between the hairs on the cushion and also on the spines. These hyphae even penetrate into the spines, and some sections reveal the fact that the central air-filled fibres contain numerous fungal hyphae. I have not attempted to make pure cultures of these

Fungi, as I think they are in no way connected with the life of the plant. The parts which they infest are cut off completely from all cytoplasmic connexion with the living part of the plant. An investigation of their life-history would be of no interest except for mycologists. I therefore leave it to the latter to investigate the Fungus in question.

3. Homology of the Tubercle.

I have not considered it necessary or even important to investigate very fully the development of the different members and organs of our plant. But as I was not able to fully agree with some results obtained by other authors I did follow up the formation of the new organs at the growing-point of the shoot more in detail.

The actual growing-point of *Mamillaria elongata* can best be examined by cutting a series of microtome sections in directions parallel and transverse to the longitudinal axis of the plant. The vegetative point of the main axis, however, is often very difficult to cut except with the hand-razor, owing to the hardness of the spines and the impossibility of removing the air from the lower ends of the older and larger spines. But the small lateral detachable shoots can be embedded in paraffin *in toto*, and they give fairly satisfactory serial sections. Their spines are not yet very hard, and by prolonged lying in absolute alcohol the air can be almost entirely removed, but the older parenchymatous tissues shrink to a great extent. The cells near the apex, being full of protoplasm, seem to remain fairly well preserved.

The actual organic apex of the shoot is seen to be a very flat cone (Pl. XXV, Fig. 18 ; Pl. XXVI, Figs. 39 and 40). It is covered by a very distinct epidermis of fairly large cells. Further inside we get undifferentiated periblem, which is succeeded by a clear indication of the differentiation of the vascular strands of the plerome cylinder. A large-celled pith is soon marked off.

Laterally on the apical cone of the main apex smaller conical protuberances are being formed at very close intervals (*w*). They first appear as small outgrowths in the formation of which both dermatogen and periblem take an active part. We will follow out their development without discussing at present their homologies. Each of these new growing-points in its turn grows out, and at first slightly overgrows one of the next young growing-points. At this stage the most actively growing part of the outgrowth is on that side of the hump furthest from the central growing-point (Pl. XXVI, Fig. 40). The whole hump, which is simply a young tubercle, gradually becomes more differentiated (Pl. XXVI, Figs. 40–43). The body of the tubercle consists of fairly large cells which do not look as if they were very actively growing (Pl. XXVI, Fig. 45, *w*). The meristematically most active portion is the future cushion and its

spines. This part has gradually been carried up away from the growing-point (Pl. XXVI, Figs. 39–42, *v*). The active cushion is marked off by a rim which runs right round the upper end of the tubercle (Pl. XXVI, Fig. 43). Finally, the meristematic cushion-tissue is carried up to a position transverse to the longitudinal axis of the plant, through the inner side of the tubercle growing more rapidly than the outer one. The embryonic tissues of the cushion gradually show a number of well-developed conical projections, which represent the future spines (Pl. XXVI, Figs. 41–43, *v*). They are made up of dermatogen and periblem layers at first (Pl. XXVI, Fig. 45, *v*). In the centre of the tissue of the tubercle procambium-elements are visible, which branch and obviously lead to the embryonic spines (Pl. XXVI, Fig. 45, *v*). One of the small conical outgrowths of the tubercles may here be described more in detail. It is covered by an epidermis, which is continuous with the epidermis of the neighbouring embryonic spines and the epidermis of the whole tubercle (Pl. XXVI, Fig. 45, *e*). The tissue inside the young spine may be seen to be connected with the procambial strands forming lower down in the tubercle. From the epidermis which covers the tissues of the cushion lying between the spines, arise hairs, which form an important part of the spine-cushion in some of the later stages of its development (Pl. XXVI, Fig. 45, *i*). At this stage the outer cells of the sides of the cylindrical body of the tubercle are beginning to show the regular arrangement into palisade-rows. The depression immediately outside the spines now gradually becomes more accentuated by a ring-like outgrowth, which grows up all round the cushion and finally surrounds the spines so that the spines come to stand in a cuplike depression (Pl. XXVI, Fig. 43).

The spaces between the spines and between the younger tubercles in different stages of development are filled with hairs developed from the cushion of spines (Pl. XXVI, Fig. 39). Gradually the spines elongate, but they still at first stand erect on the tubercle and appear quite colourless. Later on they assume a reddish colour.

The procambium in the wart gradually becomes vascular tissue, but the lower cells of the spines, although they may for some time be connected with the procambial tissue, do not become vascular (Pl. XXVI, Fig. 44, *h*). Later on, the tissues at the lower end of the spine give rise to the hard, air-containing fibres. The epidermis of the spine is never thrown off, and the mature spine therefore remains covered with its original epidermis. The hairs found so plentifully between the mature spines also are epidermal structures.

The spines develop in such a way that in the earlier stages the larger ones are on that part of the cushion which is nearest the organic centre of the main axis. They may thus at one end be 0·4 mm. long and at the other end 1·2 mm. At a later stage they may be 1·5 mm. at one, and

3·5 mm. at the other. At this stage they would be red in colour for about ½ or ⅔ the way from their upper end downwards. The central spine may now be distinguished by being slightly broader and redder in colour than the others. A few small protuberances from the epidermis of the spines are to be noticed now. But these develop more later on. The spines are subsequently, through the activity of the cork cambium, unfolded in the typical spreading fashion. This change in position is accompanied by the appearance of air in the lower hard fibrous cells of the spines (Pl. XXV, Fig. 18).

The points of the spines still remain reddish brown for a time, but later this colour also disappears. The small outgrowths from the epidermis of the spines are found almost exclusively on the inner and middle portion of the marginal spines, but all round on the large central one. The whole set of spines remains permanently in a slight depression; at any rate the circular mound of tubercle tissue keeps pace with the growth of the whole spine-cushion (Pl. XXV, Fig. 15).

What then is to be said about the morphological nature of the tubercle and the spines?

The first small conical projections near the growing apex arise just in the way leaves would arise. To my mind there is no doubt that they represent leaves, that is leaf-primordia ('Blattanlagen'). The leaf-primordium grows, and at the top of it there appear a number of small protuberances, new actively growing portions (Pl. XXVI, Figs. 40, 41, 42 *v*). What do they represent? I have no doubt that the body of the primordium at this stage represents the leaf-base and the small protuberances represent the leaf-blade. The leaf-blade does not develop except to form the spines, and the leaf-stalk does not develop at all. The leaf-base develops most, but in the mature plant the tubercle may possibly represent in addition to the leaf-base, but to a limited extent, certain portions of the shoot which have become fused with it.

The leaf-nature of the tubercle-primordium becomes clearer still, when we notice how in its axil, a short distance behind the growing-point of the shoot, a small lateral bud is formed (Pl. XXVI, Fig. 46, *y*). This lateral bud, however, is formed more on the lowest portion of the base of the leaf-primordium than on the main shoot itself.

In *Mamillaria elongata*, at any rate, I can therefore see in the mature tubercle only the highly developed leaf-base. The spines together represent the leaf-blade, the leaf-stalk being absent. This view does not agree with that expressed by most other authors who have examined the morphology of the tubercle of the Cactaceae. Ganong briefly summarizes these views (10, p. 45). Kauffmann considers the spines to be leaves, Vöchting and Delbrouck look on them as emergences, in which point they agree with Schumann. Goebel makes out the whole tubercle at first

to be a leaf, and in its axil a lateral bud arises. Leaf and lateral bud grow up together and develop so as to form the cushion of tissue which gives rise to the spines or metamorphosed leaves. In the axil of a mature leaf-tubercle a lateral bud may be found. This, according to Goebel, is merely the result of a division into two of the original lateral growing-point which grew up with the leaf-base (12, pp. 77–84).

Quite recently Rudolph has published some observations on the spines of *Opuntia Missouriensis* (23, p. 103–109). He considers that in this species at any rate the spines are trichomes which have arisen in the axil of the leaf. He does not, however, wish to express any opinion concerning the other Cactaceae which he has not examined.

It will be seen therefore that I do not quite agree with any views held by those who have examined the growing-points of Cactaceae. Referring to a figure by Goebel (12, p. 81, Fig. 41) which corresponds roughly to my Figs. 39 and 40 (Pl. XXVI), I can only say that what Goebel calls the growing-point of the axillary shoot is to my mind merely the embryonic apical part of the leaf-primordium. The axillary bud is formed much later, and at least in *Mamillaria elongata* has never been connected with the embryonic tissue which later on gives rise to the spines and which is supposed by Goebel to represent the axillary bud.

The tubercle as a whole, in *Mamillaria elongata* at least, represents mainly the leaf-base, although its lower end may be partly derived from the stem-portion of the shoot. The spines represent the modified leaf-blade.

Wetterwald's observations on the Euphorbieae and Cacteae were interpreted by him in such a way as to confirm Goebel's results. In my opinion his figures of *Mamillaria coronaria* (Pl. XX, Figs. 33, 34) seem to agree quite well with my interpretation.

Caspari was unable to detect any vascular tissue in the spines, and therefore considers the spines to be anything but reduced leaves (6, p. 6). But an examination of the young spine-primordia does show that there is at first a rough indication of a continuity of the plerome of the tubercle with the inner tissue of the future spine, at least in *Mamillaria elongata* (Pl. XXVI, Figs. 44, 45). Ganong also figures a spine of *Opuntia coccinellifera* with a delicate spiral vessel at its lower end (10, p. 9, Fig. 3).

4. Physiology.

(a) *Introductory Remarks.*

As already mentioned in the introduction to this paper very little is known concerning the meteorological and other conditions under which the Cactaceae as a whole live.

Our plant *Mamillaria elongata* is mentioned by Schumann (26, p. 520) as occurring very extensively in the state of Hidalgo in Mexico. It has

been recorded from Limapan, and las Ajuntas on the river Moctezuma, near Ixmiquilpan, Meztitlan, between Zucualtepan and the river Toliman and the Rio Grande. It is supposed to occur also in Chihuahua, but from this latter locality Schumann has not seen any authentic specimens. Hidalgo is a state or province of Mexico right in the centre of the high plateau on the top of the Mexican mountain range.

Schimper gives the following as the conditions obtaining in the Mexican plateau (24, p. 675). The climate generally is dry, though moister than that of the North American deserts. The annual rainfall appears never to be less than 50 cm., which is considerably above that of the typical desert climate. Even with a high temperature prevailing one would not expect to find a very poor vegetation except on soil very permeable to water.

Karsten gives us a very useful glimpse of the conditions prevailing in these localities (24, p. 678). In the summer the days are warm and sunny, little rain falls, and the nights are relatively very cold. In winter snow falls, but it very soon melts away.

It is difficult indeed from the data which are given in the literature of the subject to really get an accurate idea of the Mexican climate. It seems to be very hot during the day and cold during the night, in the summer. The rainfall, though low, is not a desert rainfall. But, as Schimper remarks, the height of the Mexican deserts makes them subject to many of the desiccating influences of an alpine climate. Edaphic influences, as yet not at all properly understood or even known, also probably are at work in determining the nature of the plant-forms. Volkens's observations on the Arabian-Egyptian desert are very important, great stress being laid by him on the strength and clearness of the light (33, p. 15).

Walther briefly, but I think very well, summarizes the five conditions which in the desert are chiefly responsible for the poor development of the vegetation. They are the following (35, p. 79): (1) The scarcity of rain and dew; (2) the strength of the sun's rays; (3) the violence of the dry winds; (4) the looseness of the particles of soil; (5) the salinity of the soil.

Armed with these very few data I wish now to offer an explanation as far as possible of the structures met with in *Mamillaria elongata*. Of these some are not directly connected with the actual climatic conditions, but to make this paper more complete they will also be referred to.

The external and internal structure which a plant exhibits is mainly due to the way in which it has responded to the influence of external conditions. Ontogenetically or phylogenetically the form which a plant represents is an expression of those external conditions which in some way influence adversely or the reverse those functions which are carried out in the plant and which are of vital importance. Some structures met with in a plant may no longer be of use to the plant; they may in fact be merely

of morphological interest, indicating the persistent remains of an obsolete organ or member. The more adverse the conditions are, however, the more likely are we to find in a characteristic plant-form peculiar physiological structures which owe their presence to the adverse conditions directly, the less likely are we to find any useless members.

The conditions of the Mexican desert are very unfavourable, and we get there a very typical and characteristic plant-form, represented by almost the whole of the Cereoideae group of the Cactaceae. I think it very probable that the whole structure of these plants reflects almost in its entirety the influence of the prevailing adverse external conditions of the desert.

Of the vital processes which are being carried out in the plant, two, I think, may be considered as depending most on external conditions. The structure of the plant, as representing any particular plant-form, therefore, will be the more modified from what we can call the normal form, namely, a green land-plant with well-expanded foliage leaves, the more adversely external conditions affect the carrying out of these functions.

The first of these two functions includes all those processes which go to make up, or which take part in what is generally known as, the transpiration-stream: namely, the absorption of water with the raw material from the soil in solution, the carrying of the latter to the green leaves, their deposition in the green cells of the leaves, followed by the giving off of the greater part of the water thus brought up from the cell-surfaces in the intercellular spaces of the mesophyll. The second includes all the processes necessary for the carrying out of photosynthesis.

(b) *Physiology of Mamillaria elongata.*

Mamillaria elongata grows in dry and hot places and one might expect to find a fairly large root. Judging, however, from the plants I have been able to examine, the root is rather short and fat and but little branched (Pl. XXV, Fig. 3). It is impossible, however, for me to say what the root would be like if a plant were allowed to grow under natural conditions.

The root shows one very striking feature in the structure of the xylem. It consists of annular tracheids throughout, disregarding for the present the parenchymatous cells surrounding the wood tracheids. The spiral nature of the thickening in the protoxylem-elements of young plant-members of both root and stem has been thought to be of use in allowing the tracheids or vessels to elongate during the growth of the plant, without rupturing the whole tracheidal thickening (31, p. 469).

If this be the case, the annular tracheids of Mamillaria might be considered an adaptation to a possible and very likely shrinkage of the whole plant and especially the root during the dry season, and a subsequent swelling up again and elongation during the moist season. A shrinkage of

the root during the former would tend to fix the plant more firmly in the soil, as the lower tips of the roots would of course be firmly attached to the particles of soil by the root-hairs.

This fixing of the plant to the soil has been described by Michaelis for *Anhalonium fissuratum*, Lem. In this case, the whole plant during the dry season is bodily drawn into the soil (19, p. 22, Pl. III, Fig. 12). This plant appears also to exhibit annular thickening in the wood-portion of the root (19, pp. 17, 18).

It is not surprising that in the case of the root of *Mamillaria* we find not only the metaxylem but also the protoxylem developing annular to the complete exclusion of spiral elements (Pl. XXVI, Fig. 32). The annular tracheids can probably also store water.

As we ascend into the stem we find the annular tracheids of the root-metaxylem giving way to beautifully developed spiral tracheids. These are short and broad, and are not in any way of the type to which the protoxylem-tracheids of stem and root in other normal plants belong. In the large spiral tracheids of the succulent stem of our plant, I see as much water-storing as water-conducting organs. The spiral, again, is a structure which allows of an elongation of the cell to which it belongs, but probably not of a great subsequent contraction. The continuity of the lignified thickening is, as we can see by comparing the wood of the root and shoot, not necessary for the conduction of water. But of course the method by which the water passes along in the root may possibly differ from that by which it passes along in the shoot.

The libriform cells met with at the point of attachment of root to shoot may safely be put down as representing mechanical elements to strengthen the firmness of the plant at the point where it is fixed in the soil. For this reason we find these cells developed in the later-formed wood-portions of the bundle.

The large parenchymatous cells of the cortex of the main plant-body are no doubt cells which store water. I do not think, however, that they will exert a very strong osmotic pull on the wood-elements.

The ground-tissue cells immediately surrounding the bundles are flattened (Pl. XXVI, Fig. 21, *s*) and would possess a comparatively small vacuole. It is therefore probable that the transpiration stream is drawn osmotically to other parts of the plant where the water and its solutes are primarily more urgently needed, namely to the tubercles.

The large central vascular bundles send off branches, which consist, in their wood, almost entirely of spiral tracheids, narrow and apparently very long.

These spiral elements lead to the tubercles, and here they separate into two systems, namely a cortical and a medullary system—if I may use the terms cortical and medullary in this sense.

The cortical bundles pass along just inside the palisade-cells of the tubercle and then end blindly, before the tip of the latter is reached, without changing very much in structure with the exception of the bast, which is absent from the endings of the cortical bundles (Pl. XXV, Figs. 7, 8, 15).

The medullary bundles at first anastomose freely, and finally form a cup-like mass of wood-elements just beneath the tip of the tubercle (Pl. XXV, Figs. 10 to 15). At this point the bast again is seen to be absent, although it was present in the bundles lower down (Pl. XXV, Figs. 4 to 6). The wood-elements also have undergone a remarkable change. The long, thin spiral tracheids have gradually given way almost entirely to a mass of large, broad and stout cells with spiral and reticulate to annular thickening. The cup-like ending of the medullary bundle-system consists almost entirely of these cells, especially towards its outer margin, where it ends blindly (Pl. XXV, Figs. 15, 19, 20). These cells are surrounded immediately by large and clear cells, which by means of their large vacuole, no doubt, exert a strong osmotic suction on the water contained in the neighbouring tracheidal elements.

Not only are the surrounding parenchymatous cells large and more or less round in form, but the separate wood-cells also offer more than one flat surface to be acted upon by these cells. I have no doubt that the large wood-cells act also as storage-tracheids.

The water, with its solutes of raw food material, is drawn into the surrounding parenchymatous cells. In due time the water in the form of vapour passes out of those cells which are bordered by extensive intercellular spaces, into the latter, and finally makes good its escape through the stomata, by which the epidermis of the tubercle is interrupted.

We have thus far followed out the path of the transpiration-stream.

It might be of interest just to recall in a tabular form the measurements of the tracheids in the different parts of the plant, with reference to their varying function.

		Nature of thickening.	Breadth.	Length.
Root	protoxylem	Annular	10–15 μ	
	metaxylem	Annular	10–26 μ	200 μ
Stem	protoxylem	Spiral	10–20 μ	100 μ
	metaxylem	„	30–40 μ	100 μ
Bundles leading to tubercles		„	12–16 μ	
Tubercles	Medullary	„	8–12 μ	
	Endings			
	Cortical	„	16–20 μ	
	Medullary	„	20–60 μ	100–200 μ

The narrow tracheids in the root and stem, and those leading into the tubercles, are primarily conducting elements; all the other larger ones, besides conducting, will also store. This view held by Strasburger seems to fit in well here (31, p. 469). The tracheids in the tubercle are also giving off water. Water is being taken from them by the osmotic action of the surrounding cells. The cells surrounding the large tracheids of the stem, however, probably have no such function. Their cavities, as already pointed out, are small (Pl. XXVI, Fig. 21).

From the figures given above, the goal of the upward current of water is always a large tracheidal cell. Strasburger mentions that the current of water is always towards the smaller cavity (31, p. 873), an observation which does not accord with my observations on *Mamillaria elongata* recorded here.

Mamillaria elongata growing in a dry desert region would naturally show a number of xerophil structures. Before however referring to these, it will be necessary to describe the arrangements for carrying out photosynthesis.

We have a very well-developed palisade-tissue on all sides of the tubercle (Pl. XXV, Fig. 15). The cells of this are supplied with raw material from the soil directly by the cortical bundles, and, more indirectly, by the medullary bundles. It may at first sight appear remarkable that the rows of palisade-cells should run at such a definite oblique angle with the epidermal layer of cells. The strongest light that falls on the palisade-tissue very probably impinges on the plant at this same angle. Light coming vertically down from the sun would not reach the tubercles low down on the plant (Pl. XXV, Fig. 3), but would be caught by those higher up, which are nearer the growing-point. But these tubercles are placed at a slightly different angle, with regard to the axis of the plant, than the lower ones. Their palisade-tissue also has its cell-rows at a different and again probably correct angle, in order again to catch the rays end on (Pl. XXV, Fig. 18). The same explanation serves to make clear the meaning of the rows of palisade-cells on the underside of the tubercles. They catch the also very strong light which is reflected from the surface of the soil. Whether this is glistening sand or not I cannot say, but it very likely frequently is in the natural habitat of our plant. This certainly appears to be the case with *Cereus peruvianus* and other Cactaceae as depicted by Karsten and Schenck (16, Pls. XXXIX to XLVIII).

The plastids are generally found on the radial walls of the palisade-cells, except on occasions when they congregate around the nucleus, an occurrence noted and figured already by Schleiden (25, p. 6, Pl. VII, Fig. 3). To the inside of the cortical bundles the large parenchymatous cells are arranged more like the ordinary cortical tissue of the main body of the plant (Pl. XXV, Fig. 15).

The arrangement of the rows of palisade-cells offers a strong confirmation of the view held by Stahl, that the characteristic development of this tissue is influenced by the strength of the light (30, pp. 36–38).

Haberlandt sees in the cell-rows of the palisade-tissue an arrangement for rapidly conducting away the elaborated organic products of photosynthetical activity. Their position and direction is in no way influenced by the light directly (13, p. 250). Eberdt puts down the varying development, but not the presence of palisade-tissue, as an adaptation to give transpiration and photosynthesis equal rights (19, pp. 373, 374). It is in fact a compromise between the two. Personally I should consider the elongation of the cells to be a concession only to photosynthesis, but the reduction in depth of the air-spaces accompanying the palisade-tissue as a concession to transpiration.

Nordhausen has recently published some very interesting observations, which in one particular are, however, not yet quite complete. He shows that the buds of Beech shoots, which have been grown in the shade, give rise to shade-leaves even when grown exposed to the full light (21, pp. 30 to 45). The bud in fact takes on the character of the leaf, in the axil of which it is formed. The chances generally are of course that it will grow up surrounded by the same conditions as that leaf. This is an interesting fact. But, as Nordhausen himself points out, it is desirable that further experiments should be carried out. A bud is developed in the axil of the leaf, which though itself exposed to strong sunlight is derived from the bud of a shade-leaf, and shows shade-leaf characters. Does this last bud produce shade-leaves or sun-leaves at once, or only after one or two years? The removal of the branch with its leaves from shade to light before the leaves in the bud are quite differentiated might lead to interesting results, and show how direct the influence of shadow and light is.

Areschoug (1, pp. 1–18, 38–43) lays greatest stress on the influence which the external conditions exert on the plant in its desire to keep the transpiration-stream under control. The reduction of air-spaces causes a reduction in the rate of transpiration. Therefore the palisade-tissue with its not very extensive air-spaces is an adaptation to reduce transpiration. In the case of *Mamillaria elongata* and those Cactaceae which show similarly equipped tubercles the palisade-tissue is clearly in my opinion a protection against the influence of the strong light on the green plastids, and not against undue transpiration, for if the latter were the case the inner cells would also show a similar arrangement (Pl. XXV, Fig. 14). The air-spaces in the inner tissue of the tubercle are slightly bigger than those in the palisade-tissue, but the cells are also less exposed to strong sunlight.

The extensive air-spaces by which almost every one of the carbon-dioxide absorbing cells is bordered to a smaller or larger extent do not separate the cell-walls very much. They are, that is to say, long but

shallow. I need only refer to the work of Brown and Escombe (5) as showing that the small diameter of the air-spaces is rather favourable to the rapid introduction of the carbon dioxide of the air into the air-spaces of the green tissues than otherwise.

The shallow air-spaces, however, serve a double purpose. The water brought up from the soil is evaporating into them, and the narrow diameter of the air-spaces has the important effect of reducing the rate of transpiration. Plants in dry regions reduce the lateral diameter of their air-spaces, although they cannot reduce their length, without interfering with the photosynthetical functions of the green cells.

This then is the first instance, in our plant, of an adaptation to the dry locality, where the two vital processes, photosynthesis and transpiration, come into conflict. To suit the former they must be retained and must extend to as many cells as possible, to enable the actual absorption of the carbon-dioxide gas, which is always slow, to be carried out. To suit the latter they are reduced as much as possible in depth.

The hypoderma, with its very few chloroplastids, no doubt serves as an additional protection, together with the epidermis, for the chlorophyll of the palisade-cells (Pl. XXV, Fig. 15).

There are not many stomata. These, furthermore, show none of the many well-known xerophil characters (Pl. XXV, Fig. 9). The stomata are on a level with the epidermis. The latter has not developed a very thick cuticle, and the outer cell-wall of the guard-cells is only a little thicker than that of the ordinary epidermal cells.

In what way then does the plant protect itself against the strong and clear light, which forms such a feature of the Mexican desert? Its harmful effect lies in its destructive action on the chlorophyll, and in the fact that strong light increases very rapidly the rate of transpiration.

The whole cylindrical form of the plant is beautifully adapted to exposure to strong light. The sides of the tubercle get very little of the strongest light during the day. Their sides very rarely get the light falling directly at right angles on their surface (Pl. XXV, Figs. 2, 3). This is owing to the surface of the plant being elevated into tubercles. Strong light falls on to the plant generally at some angle, which will probably correspond on the average to the direction in which the rows of palisade-cells run. The light which falls on to the plant and actually reaches the body is therefore undoubtedly partly reflected in such a way as to be fairly evenly distributed over the whole surface. The result is important both from the point of view of the transpiration-stream and from that of photosynthesis.

I have not, however, referred to the most characteristic external feature of our plant, namely the spines, which crown every tubercle.

The rate at which transpiration goes on depends to a great extent on the rate at which the air in the air-spaces is renewed. The flat and narrow

air-spaces do not favour a very rapid movement of the air in the plant. But the air immediately outside the fleshy part of the plant is kept more or less stagnant by the passive action of the spines. The whole set of marginal spines are spread out and the spines of one tubercle overlap those of the next (Pl. XXV, Figs. 2, 3). The spines in fact form a fairly complete screen, separating the air which immediately surrounds the plant from the air outside. The former is to a certain extent stagnating.

I have been unable to make any experiments to show that this is the case with *Mamillaria elongata*, but I have done so with *Echinocactus cylindraceus*, Eng. Placing a plant in the sunlight the temperature inside the spines and outside was measured. The temperature inside at three different times was 23° C., 28° C. and 28·5° C. The temperature outside was 17·75° C., 18·75° C. and 19·75° C. respectively. This proves to me that the air inside was stagnating. It was close, and hotter than outside. The air inside the spaces will therefore not be rapidly renewed. The spines consequently have a very important function to perform: they reduce the rate of transpiration. Further observations were made, at the risk of losing a valuable specimen, in order to determine the temperature right inside the plant body. *Echinocactus cylindraceus* was again used, a thermometer being forced into a hole bored into the body of the plant. When the sunlight was falling directly on to the plant, the temperature inside the plant body was 15° C., in the space between the plant body and the screen of spines 19° C., and that of the air outside 16° C. The three thermometers had been in position 1½ hours, the temperature outside the greenhouse in which the particular specimen of *Echinocactus* was growing being very low at the time.

The air in the air-spaces is therefore evidently lower than that immediately outside the plant body, and we will clearly not get a rapid current of air outwards. The low temperature is clearly of use in reducing the rate of transpiration.

Peirce makes the statement that the rate and volume of transpiration is reduced, in plants like the Cactaceae, by the body temperature of the plant being lower than that of the air when the air could otherwise take up most moisture (22, pp. 137, 138). He refers for support of this view to Goebel, Schimper and Volkens. In the books of the two first authors mentioned I find reference made to a paper by Askenasy (12, p. 34, and 24, p. 49). Askenasy gives a higher temperature for the inside of the plant than for the outside. This author enumerates a large number of plants where this is the case (2, p. 441). Volkens records an observation on the body temperature of *Mesembryanthemum Forskalii*. During the hottest part of the day the temperature of the leaf is 5 to 8° C. above that of the air (33, p. 40). It is a question about which a large number of readings need to be taken—for the Cactaceae at least—in the desert.

Being not very closely set, the spines do not interfere with the light which the plant needs to be supplied with for the photosynthetical functions. But they will probably act as a useful sunshade also in this direction.

The whole set of spines again serves as a protection for the main ending of the medullary bundles in the tubercle. These with their storage-water are protected from the strong light by the broad lower ends of the spines, glistening with the imprisoned air. A mass of white glistening hairs between the bases of the spines helps in the same way. No water can escape by evaporation at this end, because a broad plate of corky tissue underlies the set of spines, and almost overlies the mass of storage tracheids. How effectually the spines do keep off the light may be seen from the light colour of the cells which lie inside the storage-tracheids. They contain hardly any chlorophyll.

The single central spine acts in the same way as the other spines, but is most effective when the sun shines directly on to the tip of the tubercle.

It may be mentioned here that the apical and more delicate portion of the whole plant is extremely well protected against the strong light by the spines and hairs arising from the young developing tubercles (Pl. XXV, Fig. 18). These are at first very closely set, and completely obscure the growing apex. The function which the whole set of spines performs for the benefit of the plant is, therefore, to sum up, that of a screen or sunshade.

I consider this function so important that I have thought it worth while calling such an organ as the whole set of spines represents a *paraheliode.*

Attention was already called in 1876 by Wiesner to the possibility of hairs being of use to the plant in acting as a protective screen between the strong sunlight and the chlorophyll of young developing and therefore rather delicate organs of the plant. Wiesner instances the case of *Tussilago Farfara.* The coat of white hairs on the upper surface remains on the leaf as long as the green colour has not fully developed. It is then thrown off. If removed prematurely the formation of the chlorophyll seems to be impeded (39, pp. 24, 42).

Warming also refers to this function of hairs, as damping the effect of the sun's rays (36, p. 18).

I do not intend in this paper to refer to the question of the function or meaning of the deposits of calcium oxalate, nor to the well-known strong acidity of the cactaceous cell-sap (vide 3 and 34, &c.).

(c) *Comparison with other plants.*

As already mentioned, the similarity in appearance of certain species of *Mamillaria* and certain species of *Mesembryanthemum* is very striking.

I have been able to examine the structure only of *Mesembryanthemum stellatum*, but there are quite a number of species which have the same external appearance.

The conditions under which *M. stellatum* lives are probably very much the same as those under which our *Mamillaria elongata* flourishes. An examination of its structure is therefore of particular interest.

Mesembryanthemum stellatum has fleshy leaves, which are roughly triangular in transverse section, but more or less cylindrical in longitudinal view.

The leaves are here the chief assimilating organs, and in their function and structure they correspond exactly to the tubercles described for *Mamillaria elongata*.

We can follow up the vascular bundles coming from the stem and see them branching and anastomosing freely in the leaf. They become closer and closer towards the tip of the leaf, where they end blindly, in a number of fairly large tracheids. Just underneath the apex of the leaf we get the largest mass of tracheidal tissue, the component cells of the latter being large reticulate storage-tracheids.

The assimilating tissue forming the outer layers of the leaf-organ consists of cells arranged in regular rows, and represents roughly the same type of palisade-tissue as that met with in *Mamillaria*. It makes roughly the same angle with the epidermis as we found in *Mamillaria elongata*.

The assimilating tissue of the leaf is traversed extensively by long but shallow air-spaces, which communicate with the outside air through the stomata.

The leaves of *M. stellatum* are protected against the effects of the strong sunlight, to which the plant is exposed in its native localities, by two means. The whole leaf is covered by large cells, which grow out from the epidermis and expand so as to screen the epidermis and the underlying tissue very effectively from too strong light.

These large cells possess a very thin lining of cytoplasm and a very large vacuole. The cell-wall is very thick and hard, and it seems to glisten in the sun, a sign that light is being reflected. In this way transpiration is reduced.

Volkens refers to the large epidermal cells of *Mesembryanthemum crystallinum* as water-storers (33, p. 123, Pl. XIII, Figs. 4, 5). I would not however like to call them water-storing cells, although they may possibly retain water for a long time. Generally we find water stored inside the plant and away from its chief enemy the sun. Rather should

we primarily look at these large cells as an arrangement for the reduction of transpiration. Before however any opinion could be expressed on this question, it would be necessary to determine more carefully the histology of these epidermal cells, and the exact nature, chemical composition and degrees of concentration of their contents. It might then be possible to explain their relation to transpiration more fully. As it is now, I consider that they represent paraheliode structures. The rate of transpiration is reduced by the large epidermal cells keeping down the circulation of air around the stomata, a fact which was proved experimentally by Hagen some thirty years ago (14, p. 24). The stomata are placed in between these peculiar cells and do not lie exposed to the air directly.

Each leaf is crowned by a set of hairs. Each hair is derived from a single epidermal cell. But despite its unicellular nature, it very much resembles in structure one of the multicellular spines of *Mamillaria elongata*. It has a pointed upper end, but its lower end is much swollen and contains a glistening mass of air. The swollen part is also coloured brick-red, and will absorb rays from the sun. These hairs are very soft and almost papery in texture, but I have no doubt that here, as in *Mamillaria elongata*, they form a useful sunscreen or paraheliode for the protection of the underlying leaf-structures, the endings of the vascular tissue being found most abundantly here.

Mesembryanthemum stellatum forms, I think, a very strong parallel case in support of my views on the function of the spines, as, though being a member of a natural order quite different to the Cactaceae, it has nevertheless developed the same plant-form as these.

A very great number of Cactaceae belong to the same plant-form as *Mamillaria elongata*. Schumann has pictured many of these in his Iconographia Cactacearum. His representation of *Echinocereus subinermis*, Salm-Dyck (27, Vol. I, Pl. III), is very interesting on account of the fact that the young flower-shoots are well protected by spiny paraheliodes, but in the older portions these almost disappear (26, p. 250). The tubercles, with the apical set of spines, gradually pass into the outer leaves of the flower. These leaves are foliaceous in form, and they also at first bear an apical set of spines. This confirms my view on the homology of the spines in *Mamillaria elongata*.

Paraheliode structures of the nature and kind described for *Mamillaria elongata* are not, however, met with in all Cactaceae. It would be very interesting and most instructive to examine a large number of species of this natural order, and determine the relation existing between the external conditions on the one hand and the development of the paraheliodes on the other. It would be of great interest to determine whether, when the paraheliode spines are smaller and evidently less efficient as

sunshades, some other structure in the plant takes on the paraheliode function.

I have been able to examine only a few species from this last point of view. The results obtained are not satisfactory, as long as it is impossible to take into very careful consideration the details of the natural conditions which surround the plants in their native habitat.

I am therefore simply quoting a few of the plants examined, in order to show on what lines such an inquiry could, I think, be carried out with advantage.

	Paraheliode effect of spines.	Depth at which chlorophyll begins.	Total wall-thickness in this depth.	Cuticle.
Leuchtenbergia principis	Very small	208·5 μ	75 μ	3·5 μ
Echinocactus cylindraceus	Very strong	185·5 μ	127 μ	17·5 μ
Anhalonium Williamsonii	Absent	141·2 μ	——	7 μ
Echinopsis Mülleri	Strong	122·5 μ	31·5 μ	2 μ
Echinocactus cornigerus	Weak	105 μ	70 μ	3·5 μ
„ *gibbosus*	Weak	101 μ	16 μ	2·5 μ
Cereus Baumanni	Weak	76·5 μ	10·5 μ	2 μ
Mamillaria Bocasana	Very strong	24·5 μ	2·5 μ	1·5 μ
„ *pusilla*	Very strong	21·5 μ	3·5 μ	1·5 μ
„ *elongata*	Very strong	19 μ	7·0 μ	1·5 μ

From this table, which refers only to very few species, we can see the degree to which the depth varies at which the chlorophyll begins. The cuticle is generally found to be thin. I have not been able to detect the relation which may exist between the spines and the layers overlying the chlorophyll. It is, I think, more likely that the paraheliode effect of the spines should be added to that due to the white protective layers of epidermis and hypoderma, and these two features together should be brought into relation with the surrounding conditions.

Michaelis refers to the thick cuticle of *Echinocactus* and the thin cuticle of *Mamillaria*, but he mentions the hypoderma of the latter as consisting of tall, slightly thickened cells (19, p. 26). In the few plants of this genus which I have examined they appear flat (Pl. XXV, Fig. 15).

The Cactaceae and the species of *Mesembryanthemum* are, of course, not the only plants which have paraheliode structures. Very few green land-plants are entirely devoid of such. A beautiful example of a plant living in dark caves, and therefore not provided with any paraheliode structure but rather with a light-collecting arrangement, is met with in the case of the protonema of *Schistostega osmundacea*. This plant is figured and described by Noll (20 and 24, p. 70).

The epidermis which covers the upper and under surface of most leaves of our zone can, I think, be taken as having a paraheliode function.

This function is accentuated by the presence of a colourless hypoderma in a plant like *Ilex aquifolium*. Paraheliode structures may have two or one of two functions. They may damp the strong light in order to protect the chlorophyll, or they may do so for the purpose of keeping down the rate of transpiration. In the latter case only would they be xerophil structures.

Examples of paraheliode structures could be added to in large numbers. I would like, however, without citing too many examples, only just to quote a few, in order to make it clear in which way the word paraheliode should be used, if it is adopted.

A paraheliode is an organ for damping the effect of the strong sunlight. It thus acts like a parasol or sunshade. A layer of cells, like the hypoderma, can well be called a paraheliode. The whole set of spines crowning the tubercles in *Mamillaria* form a paraheliode. I can well imagine that the masses of hard white bast-plates, which we so frequently meet with in many plants of dry and light regions, are really, in part at least, paraheliodes to protect the underlying tissues. But I am in this case merely making a suggestion as to a possible explanation. I am thinking here of some of the leaves, sections of which are figured by Volkens in his splendid book on the Egyptian desert-flora (33, Pls. XVI, XVII, XVIII). The green tissues not unfrequently have interposed between them and the direct sunlight thick plates of strong mechanical tissue, which absorb a very large amount of light. This tissue is no doubt of most importance when these plants are in a dry condition. The leaves then roll up, and the green cells would thus be in almost complete darkness. The leaves of *Aristida ciliata* would seem to be of this nature (33, Pl. XVI, Fig. 4). From the description by Volkens they appear to be permanently rolled up longitudinally (33, p. 150).

The red colour clearly offers in many cases a protection to the underlying chlorophyll or protoplasm against the undue strength of the sunlight (36, p. 18).

C. Concluding Remarks.

Before putting together in an abbreviated form the results of this investigation, I would like to make some general concluding remarks.

I have not so far referred to the function which is very generally assigned to many of the spines, thorns, and prickles of many plants.

Large and strong prickly structures are generally credited with being defensive organs for keeping off animals, which might otherwise graze on the plants concerned, and thus destroy or at least injure them.

With regard to the spines of the Cactaceae this is the view held by Goebel (12, p. 35), and, I think I may say, by botanists generally. Ganong

(11, p. 129), Kuntze (17, p. 30), and many others agree with Goebel in this particular.

The evidence in support of this view is not, I think, of a very satisfactory kind. We have no general direct evidence that these spines do even really keep off animals, which otherwise to a large extent might feed upon the plants in question with fatal results for the plant. Experiments will have to be made on an extensive scale on the Cactaceae in their native haunts before the question can be taken as settled.

Let us for a moment turn to the Hawthorn, *Crataegus*, which develops thorns. Delbrouck, in 1873, suggested that this plant through its thorns offered protection to certain birds which feed, or the young of which feed, on insects frequently found on buds and young twigs, thus proving injurious to these. This he finds to be the case with *Silvia curruca* and *cinerea*, which he calls 'Dornvögel.' The plant has developed thorns, which protect the birds and their nests against beasts of prey, and the birds by feeding on injurious insects protect the buds of the plant. Grain-feeding birds, or birds which feed on insects on the wing, or feed on grubs and caterpillars, are never 'Dornvögel,' but secure their nests by hiding them in the bushes (7, pp. 38 to 43). Delbrouck therefore imagines that these thorns have been developed, I presume, by natural selection as a protection against the raids of injurious insects. His views may rest on facts correctly observed, but even in that case his explanation is unsatisfactory. I do not think, for one thing, that the advantage and therefore importance of the thorns to the plant will be very great. This theory again does not account for the varying degree of development of the thorns in different localities and on different parts of the same plant.

Henslow is of opinion both from his own observations and those of other naturalists that the 'spinescent features of so many desert plants are simply the immediate results of the effect of the comparative waterless character of the environment' (15 *a*, p. 226).

Wiesner puts down the transformation of shoots into thorns to the light being either too intense or too weak (40, p. 87). This explanation at least rests on a physiological basis, the thorns being the expression of the effect and influence of the light on the growth of the plant-shoot.

Hansen mentions that the Cactaceae appear to him to be plants which are best protected against the drying influence of the wind (15, p. 84). But it does not appear from his remarks whether they owe their immunity from the evil effects of the wind to their internal structure or to the spines. The spines clearly prevent the too rapid renewal of the air inside and immediately around the plant-body, but inside the screen of spines, by the action of the wind.

I would like here to point out why I consider it unlikely on general

grounds, that plants develop in such a way as to offer armed resistance to animals, although they may as a matter of fact carry organs which may actually inflict injury and pain on animals.

Most green land-plants are during the vegetative phase of their development of sedentary habits. They are tied to the spot to which they have once affixed themselves. It is necessary therefore that under these conditions plants should possess the property of adapting themselves to a certain extent to external conditions. I am not referring here so much to ontogenetic as rather to phylogenetic adaptation. The former differs from the latter only in degree.

A race of plants incapable of adapting itself to altered climatic and edaphic conditions must succumb.

Only those external conditions influence the plant which in some way adversely or favourably affect the carrying out of the vital functions of the plant. Of these there are the two already referred to, through which the plant is most powerfully influenced by the external world, namely, transpiration and photosynthesis.

The green plant must carry out these two life-processes itself and under all conditions. It must provide its cytoplasm with organic food, representing matter and energy. It is dependent on its immediate neighbourhood for the supply of both. The main idea which therefore I consider underlies the adaptation of plants is that of building up organic food—that is, of carrying out at all costs the processes of transpiration and photosynthesis. The plant has inherited in an increasing degree this property of adapting itself in such a way as to carry out these functions most effectively. Thus we get the various plant-forms—which in each case represent the balance between the tendency of the plant to develop its organs of transpiration and photosynthesis, and the influence of the external conditions on the carrying out of these functions.

In every plant-form a struggle is going on between transpiration and photosynthesis, until a compromise is arrived at. In a very dry place the function of transpiration, influenced by the adverse external conditions, is withdrawing the plant away from exposure to light and air. Photosynthesis is drawing the organs to the light.

To put it briefly I might say that the main principle which underlies adaptation in plants has a physiological basis. It is on this basis mainly, if not entirely, that many of the remarkable, as yet little understood plant-forms will be explained. Schimper has thus rested his book on the geographical distribution of plants on a physiological basis. It will then be found, I think, that organs, which are considered as offering protection against attacks by animals, and therefore would not of course represent a physiological adaptation, are really very often, if not entirely, structures with a physiological meaning. This would not however prevent, in

individual cases, their being as a matter of fact of use in warding off the attacks of animals.

A green plant collects, elaborates, stores and assimilates its food without moving. How different the higher animals. The animal can collect its food on one spot, store it in another, and digest it in still another. The energy which I am now displaying in writing this paper may have been fixed by plants in New Zealand, being finally transferred by various indirect ways to my body. For their food-supply animals are in fact far more independent of their immediate neighbourhood than plants, and often entirely so.

The guiding principle which underlies the adaptation of the animal form is far more likely to be protection. Animals generally possess weapons of various kinds and degrees to protect what they have got and to protect their offspring, which they are able to do thanks to the intelligence which they possess.

The views put forward here are not mere speculation but are based on numerous observations. They certainly may help to explain many structures which otherwise it might be difficult to interpret.

Stahl has described in a classical paper how snails will not touch plants which contain certain substances. These substances, according to Stahl, have no physiological meaning, but are protective excretions (29, p. 126). I cannot agree with Stahl in this point. These excretions will, no doubt, be found to have some physiological meaning, even if they turn out to be nothing else but useless by-products of metabolism. At the same time I do not wish to imply that they do not as a matter of fact keep off snails.

In a chapter on the methods of defence which plants adopt in order to ward off the attacks of animals, Weismann enumerates a number of plants, which for various reasons are not liked by herbivorous animals and are therefore not touched by them. The question is this: are these structures, which keep off animals, primarily protective organs, or is their function primarily a physiological one? I consider their chief function to be the latter, and the former to be only of secondary importance. Weismann refers specially to the spiny Cactaceae (37, p. 141), pointing out that these plants are protected against drying up by a thick epidermis, the spines being developed solely for the purpose of animal protection. This statement is certainly not correct, many of the Cactaceae having remarkably thin epidermal layers.

I would like to refer here to two publications which I did not see till after the completion of this paper in December, 1903.

MacDougal, D. T.: Some aspects of Desert Vegetation. From 'The Plant World,' 1903, vol. vi, p. 249. The author puts a very significant question on p. 257: 'Are the spines, thorns, prickles and poisons of desert

plants really the results of efforts on the part of the plant for self-protection?'

Coville, F. V., and MacDougal, D. T.: Desert Botanical Laboratory of the Carnegie Institution, Washington, 1903. This first report of the Desert Laboratory is naturally of a preliminary character; but it contains some excellent photographs, a brief account of some valuable observations, and also a useful Bibliography.

The main results obtained during the preparation of this paper may be briefly summed up thus.

(1) The set of spines by which the tubercles of *Mamillaria elongata* are crowned, form a structure which acts as a screen, protecting the underlying tissues of the tubercle from the strong sunlight. Such an organ may be called a paraheliode.

The set of hairs found at the top of the leaf of *Mesembryanthemum stellatum* also form a paraheliode. The large cells of the epidermis of the same plant are also paraheliode structures.

(2) The development of palisade-tissue is governed by the influence of the light on the photosynthetical processes, the depth, but not the extension of the air-spaces, is dependent on the conditions favourable or otherwise to transpiration.

(3) The tubercle of *Mamillaria elongata* represents morphologically the leaf-basis, and possibly in addition a portion of the stem. The spines are modified portions of the leaf-blade. There is only one bud in connexion with each tubercle or leaf, and that is axillary to the leaf, i. e. the tubercle.

(4) The guiding principle which underlies the adaptation of plants, and the production of plant-forms, is physiological. There is no evidence to show that direct protection against attacks by animals influences the development of any plant-form.

The foregoing remarks on *Mamillaria elongata* may, as regards the physiological results, be made to include, though in a form varying with specific peculiarities, most of the other members of the natural order Cactaceae, the structure of which is remarkably uniform.

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DESCRIPTION OF PLATES XXV AND XXVI.

Illustrating Dr. Darbishire's paper on *Mamillaria*.

a = phloem.
b = spiral tracheid.
c = storage tracheid.
d = hypoderma.
e = epidermis.
f = palisade-tissue.
g = phellogen.
h = air-containing fibres.
i = hairs.
k = cork.
l = protoxylem.
m = metaxylem.
n = wood-parenchyma.
o = protophloem.
p = cambiform cells.
q = libriform cells.
r = cambium.
s = cortex.
t = thick-walled fibres.
u = medullary ray.
v = spine-primordium.
w = tubercle-primordium.
x = axillary bud.
y = cuticle.
z = intercellular space.

PLATE XXV.

Fig. 1. Photograph of clump of *Mamillaria elongata*, taken in the succulent House, Royal Botanic Gardens, Kew. Slightly reduced.

Fig. 2. Transverse section across main axis of young plant. The vascular bundles are seen to be cut across in the centre. Near the margin are the projecting tubercles and the paraheliode consisting of spines. × 3.

Fig. 3. A complete young plant in longitudinal section. The root and shoot are seen, and in the latter again the tubercles, spines, and vascular bundles. × 3.

Fig. 4. Central bundle of a tubercle, cut across low down in the latter. To the inside of the bast (*a*) is the wood (*b*), to which four storage tracheids (*c*) have been added centripetally; air-spaces (*z*) are also seen. × 160.

Fig. 5. Two central bundles cut across higher up. The bast (*a*) and the smaller wood-cells (*b*) are seen. To the latter numerous storage tracheids (*c*) have been added; air-spaces (*z*) are also seen. × 160.

Fig. 6. Three central bundles just underneath the paraheliode set of spines. The last has disappeared; the spiral tracheids (*b*) of the old wood and the new storage tracheids (*c*) can be distinguished. × 160.

Fig. 7. A cortical bundle cut transversely low down in the tubercle. The bast (*a*), the wood (*b*), and a large storage tracheid at the inner side. × 430.

Fig. 8. The ending of a cortical bundle higher up. The bast has disappeared and two storage tracheids (*c*) alone are visible. × 430.

Fig. 9. A stoma from the epidermis of the tubercle. Cuticle (*y*), epidermis (*e*) with the two guard-cells, hypoderma (*d*), and a few palisade-cells (*f*), with air-spaces (*z*) can be noticed × 430.

Fig. 10. Transverse section of a tubercle low down. The radiating lines mark the rows of palisade-cells. Five small cortical and seven larger central bundles can be distinguished. × 5.

Fig. 11. The same higher up. The inner central bundles are forming a circle. × 5.

Fig. 12. The same higher up. The central bundles have closed in still more. Of the cortical ones only two are left. × 5.

Fig. 13. The same higher up. The cortical bundles have come to an end. The central bundles have closed in still more, but fourteen bast-portions are still shown by dark dots in the bundle-ring. × 5.

Fig. 14. The same higher up. The bast has disappeared; the small crosses mark the old wood-portions, as distinguished from the storage tracheids. × 5.

Fig. 15. A tubercle in longitudinal and vertical section. The following structures can be made out: cuticle (*y*), epidermis (*e*) with stomata, hypoderma (*d*), palisade-cells (*f*), air-spaces (*z*), cortical bundles, central bundles ending in the storage tracheids (*c*), bast (*a*), the cork layer (*g* and *k*) separating off the spines with their core containing air (*h*), the hairs in between (*i*). × 30.

Fig. 16. A marginal spine, with air-containing core (*h*) at the lower end. × 30.

Fig. 17. Transverse section of a large central spine showing the air-containing core (*h*). × 30.

Fig. 18. The growing apex of a young plant. The developing tubercles with spines and hairs are seen. The upright position of the tubercles at first can be noticed and their gradual bending outwards. The actual growing point is well protected by the paraheliodes formed by the spine and hairs. × 6.

Fig. 19. A few spiral tracheids (*b*) and storage tracheids (*c*) of a central bundle of the tubercle seen in longitudinal view. × 160.

Fig. 20. Apical portion of tubercle, in longitudinal section, showing the multicellular hairs (*i*), the air-containing fibres (*h*) of the spine, the cork (*k*) underlying the spine, the cork cambium (*g*), and the storage tracheid endings (*c*) of the central bundles of the tubercle. × 150.

PLATE XXVI.

Fig. 21. Transverse section across a bundle of the main axis. Cortex (*s*), protophloem (*o*), bast-parenchyma (*p*), cambium (*r*), wood-parenchyma (*n*), annular tracheids of the metaxylem (*m*), and of the protoxylem (*l*) can be made out. × 60.

Fig. 22. Portion of the same. × 130.

Fig. 23. The wood-portion of a similar bundle in longitudinal section. Spiral tracheids (*m*) and wood-parenchyma can be made out. × 130.

Fig. 24. Transverse section of a bundle low down in the whole plant: wood-parenchyma (*n*), spiral tracheids (*m*), libriform cells (*q*). × 130.

Fig. 25. A similar portion in longitudinal section. × 130.

Fig. 26. Two adjoining libriform cells in longitudinal section. × 360.

Fig. 27. Portion of the cork layer low down on the plant: cork (*k*), cork cambium (*g*), cortex (*s*). × 130.

Fig. 28. Transverse section of a spine low down: epidermis (*e*), thick-walled fibres (*t*), air-containing fibres in the centre (*h*). × 360.

Fig. 29. The outer layers of a spine higher up: epidermis (*e*) and thick-walled fibres (*t*). × 360.

Fig. 30. The same further in: thick-walled fibres (*t*), and air-containing fibres (*h*). × 360.

Fig. 31. Transverse section across young root: cork (*k*), cortex (*s*), bast-parenchyma (*p*), protophloem (*o*), cambium (*r*), annular tracheids of metaxylem (*m*), wood-parenchyma (*n*), annular tracheids of protoxylem (*l*). × 130.

Fig. 32. The same in longitudinal section. × 130.

Fig. 33. Transverse section of young root: cork (*k*), bast (*a*), metaxylem (*m*), protoxylem (*l*), and medullary ray (*u*). × 15.

Fig. 34. The same but older. × 15.

Fig. 35. The same but older. × 15.

Fig. 36. The outer layer of the transverse section of old root: cork (*k*), cortex (*s*), bast-parenchyma (*p*), protophloem (*o*), cambium (*r*), medullary ray (*u*). × 130.

Fig. 37. The next inner layers of the same section: annular tracheids (*m*) and parenchyma (*n*) of the metaxylem, medullary ray (*u*). × 130.

Fig. 38. Innermost portions of the same section: annular tracheids (*m*) and parenchyma (*n*) of the metaxylem, annular tracheids of the protoxylem (*l*). × 130.

Fig. 39. Longitudinal (microtome) section of growing point of young plant. Several young tubercle-primordia (*w*) can be made out, in between which is a mass of hairs. The black object to the left near the top is a spine cut across. × 14.

Fig. 40. Portion of the same more highly magnified. Immediately on the left of the organic apex is a young tubercle-primordium (*w*), to the left of which is a further tubercle-primordium with a spine-primordium on its top (*v*). × 42.

Fig. 41. An older tubercle-primordium (*w*) to the right of the organic centre, with a number of spine-primordia (*v*). × 42.

Fig. 42. An older tubercle-primordium (*w*), with spine-primordia (*v*) and hairs (*i*). The palisade-cells are showing (*f*). × 40.

Fig. 43. An older tubercle-primordium (*w*) to the right of the organic centre. The palisade-cells (*f*) are marked off and the rim surrounding the spines (*v*) and hairs (*i*). × 40.

Fig. 44. Insertion of young spine-primordium on to tubercle. The epidermis (*e*) is clear, and the inner cells also, which will later on form air-containing fibres (*h*). × 330.

Fig. 45. Young spine-primordium (*v*), with its epidermis (*e*), surrounded by hairs (*i*), joined on to the provascular cells of the tubercle-primordium (*w*). × 330.

Fig. 46. Section through the axil of tubercle (*w*), from which springs a hair (*i*). The bud (*y*) is in the axil of the tubercle on the right; the other tubercle therefore is the one nearest the growing point. × 330.

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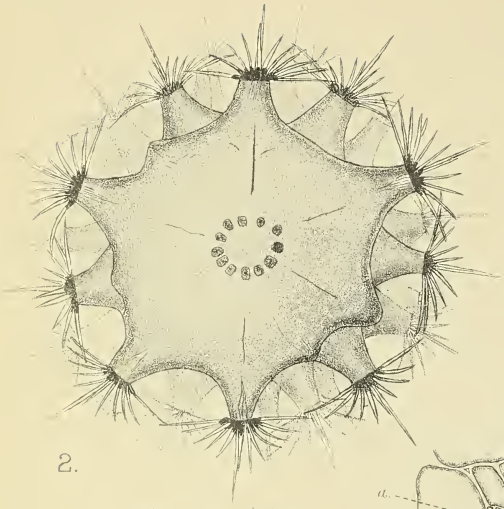

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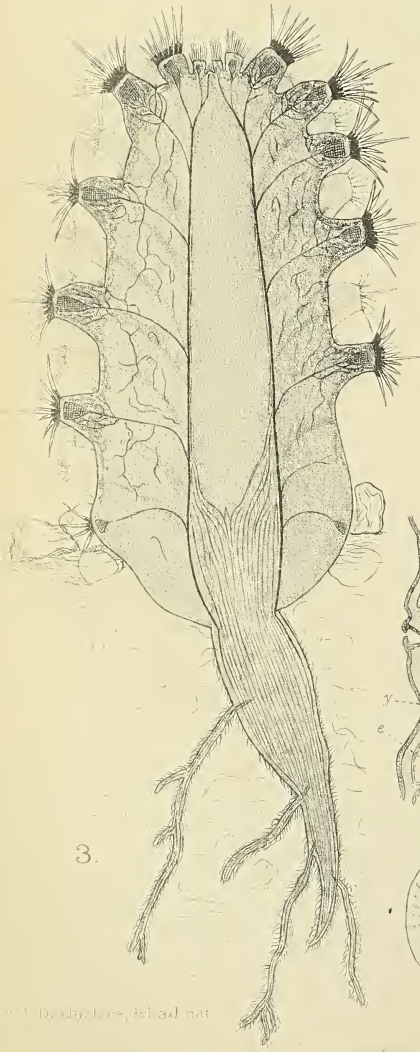

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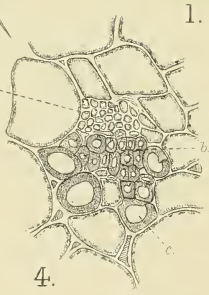

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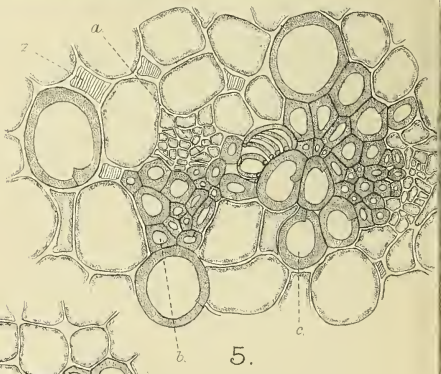

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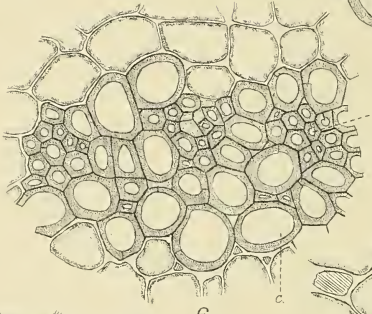

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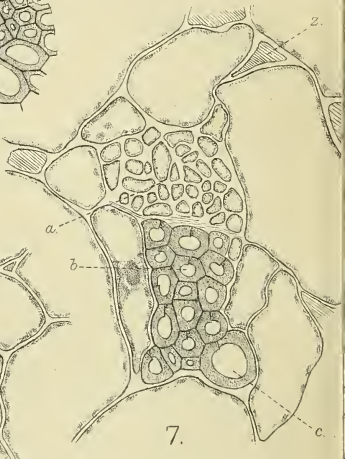

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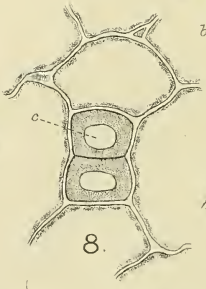

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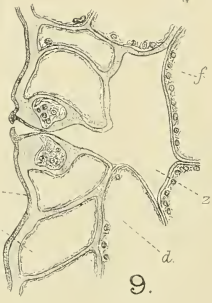

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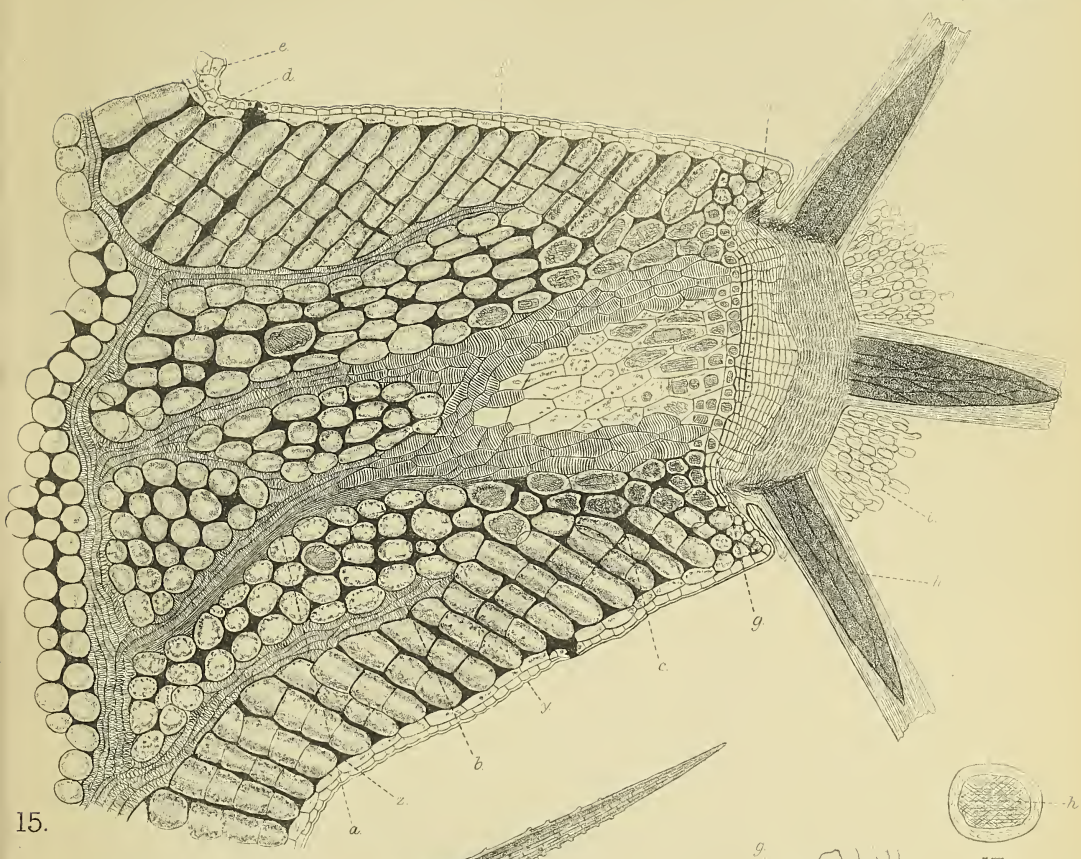

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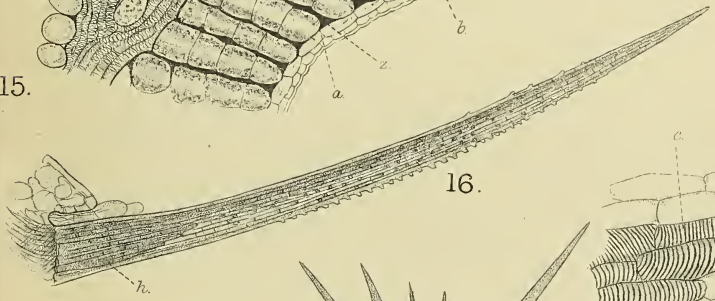

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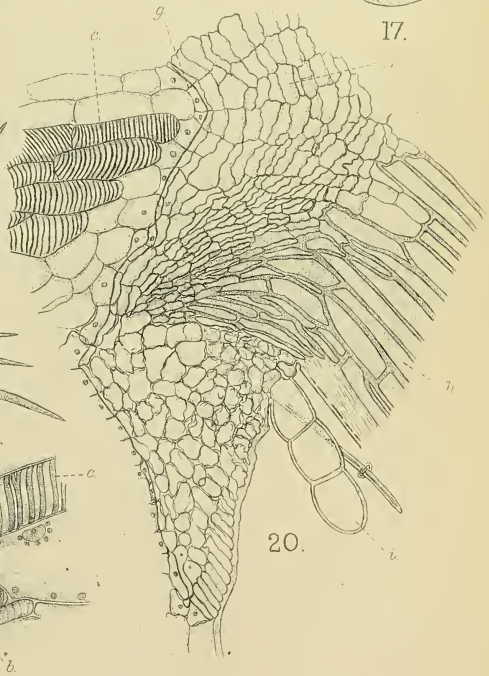

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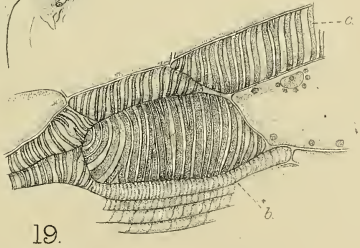

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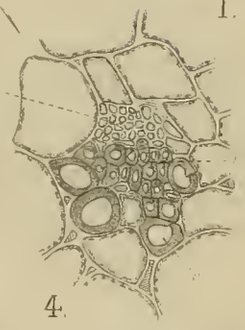

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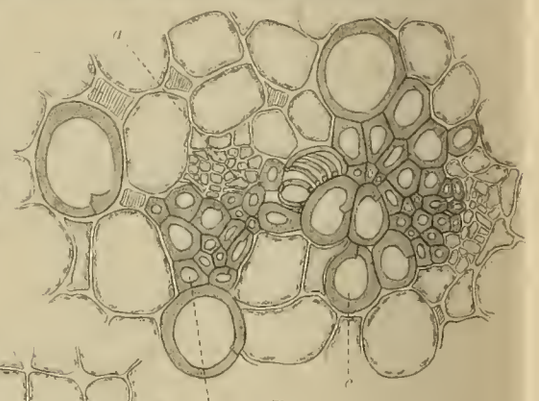

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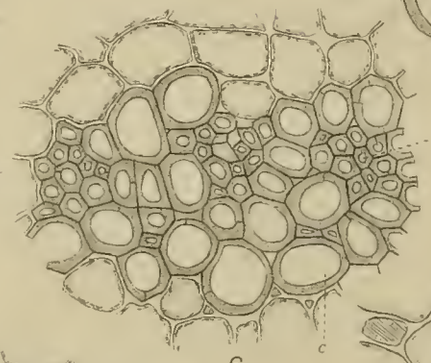

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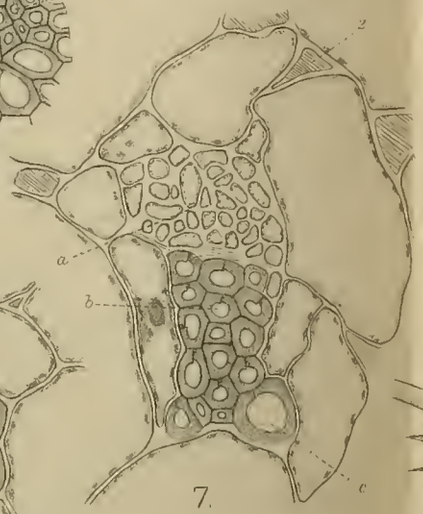

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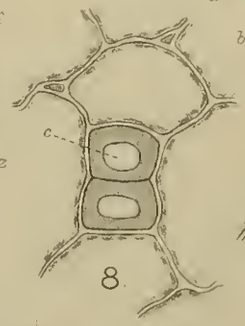

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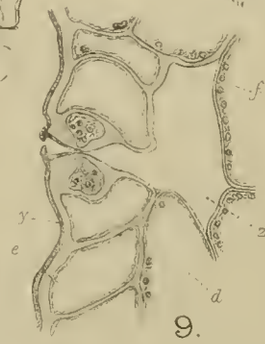

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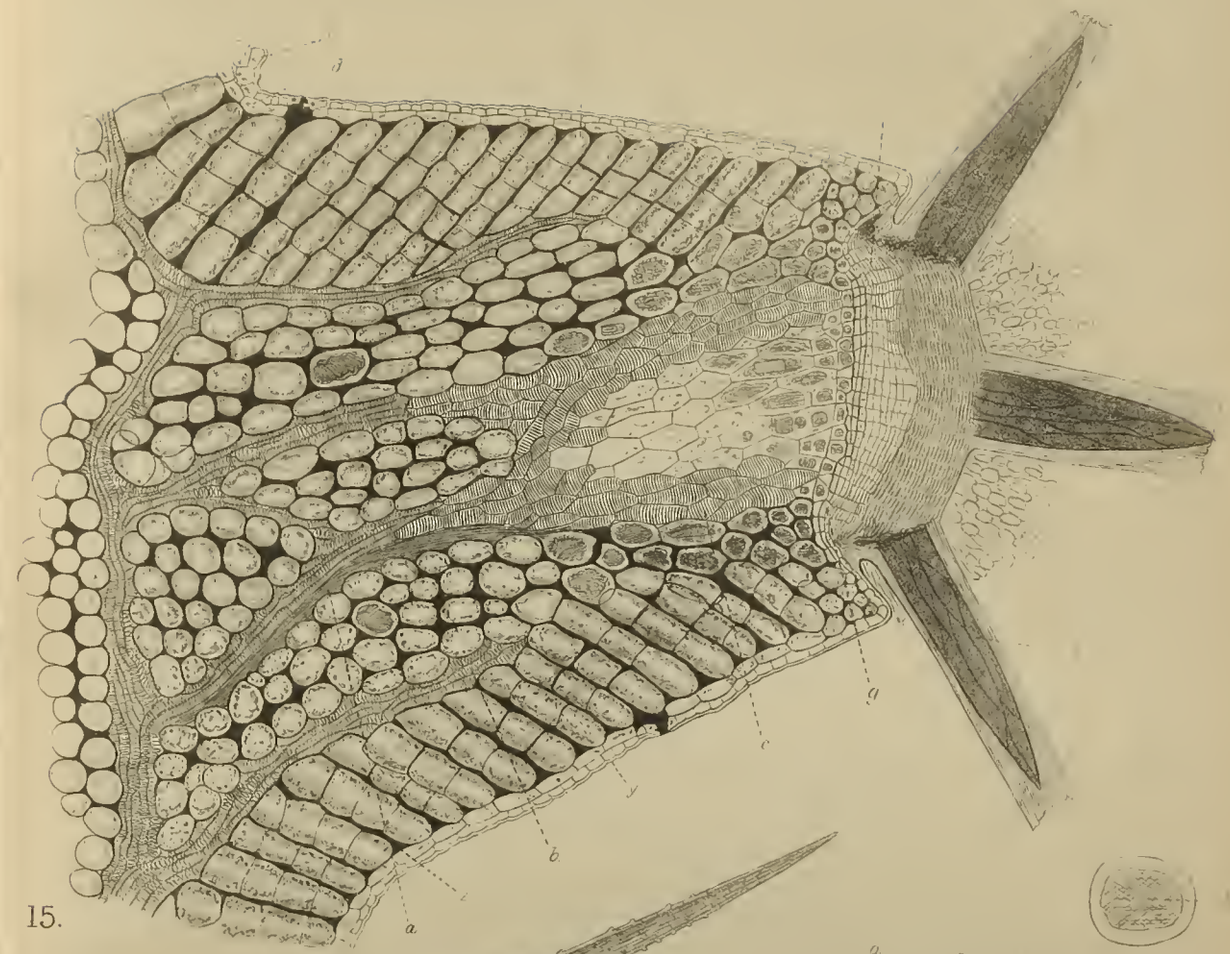

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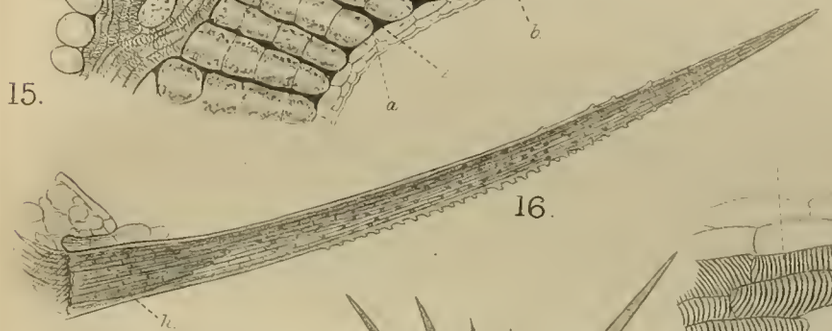

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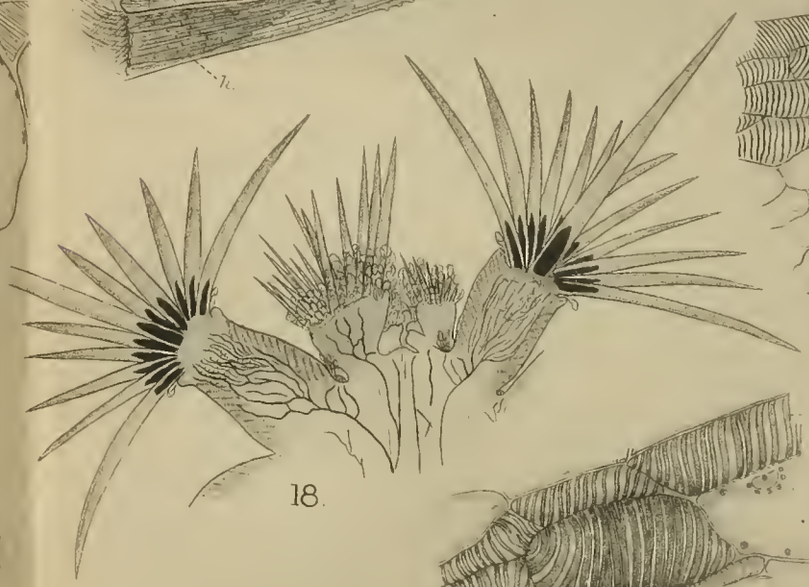

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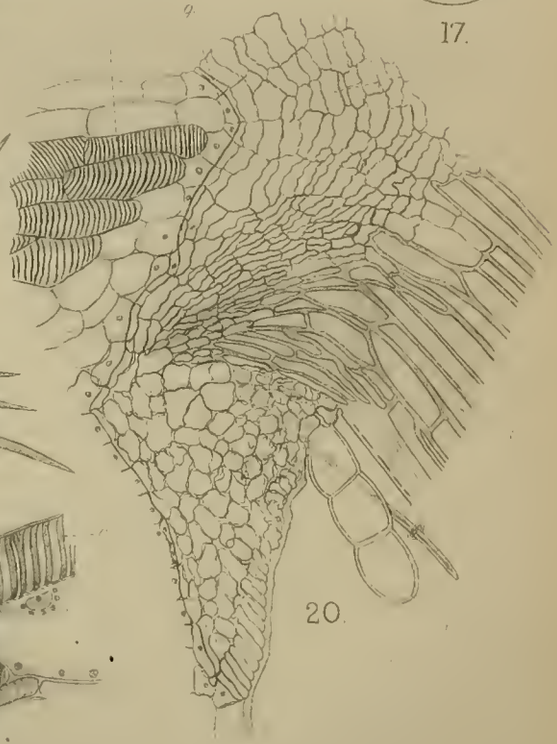

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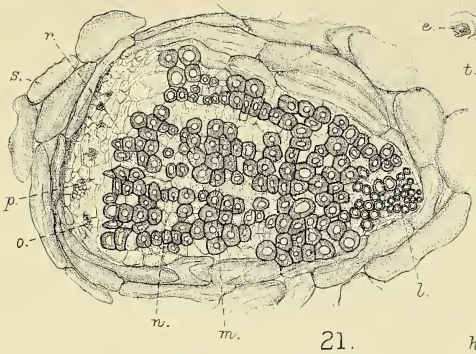

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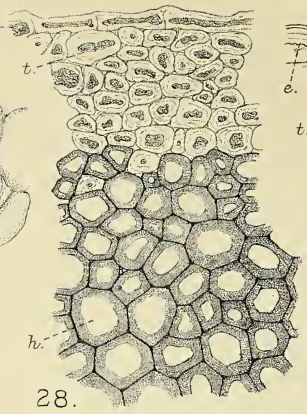

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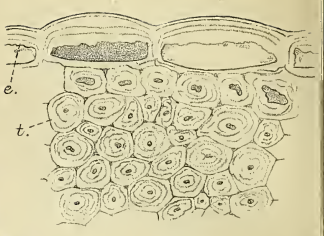

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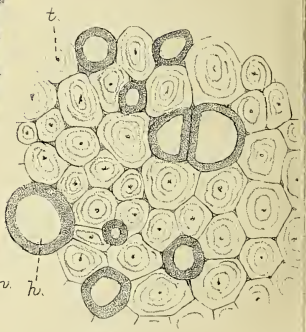

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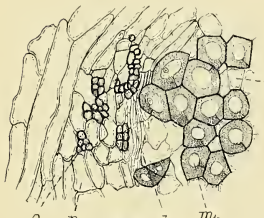

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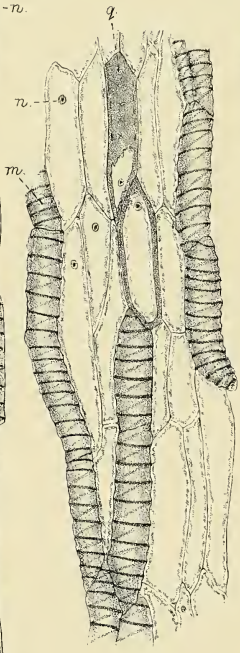

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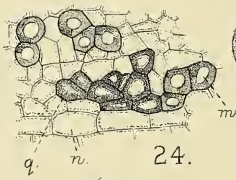

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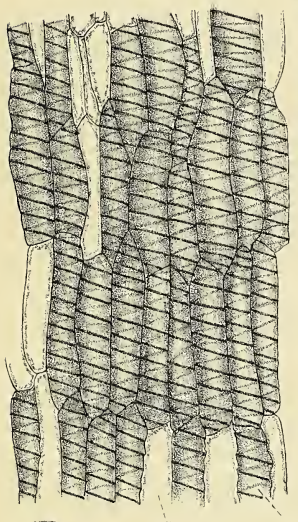

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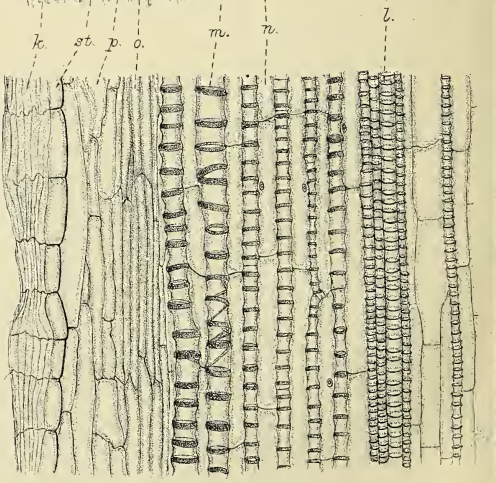

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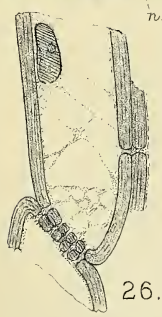

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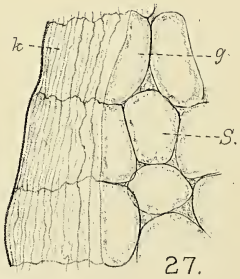

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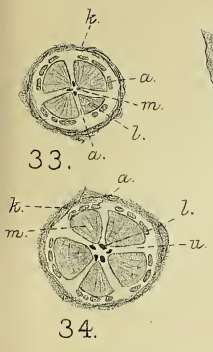

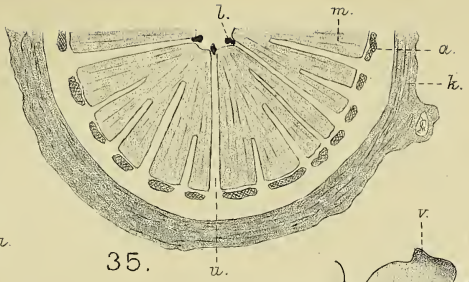

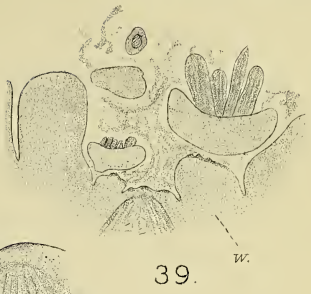

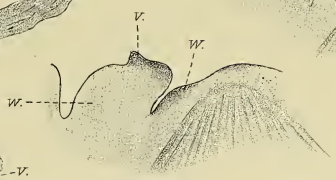

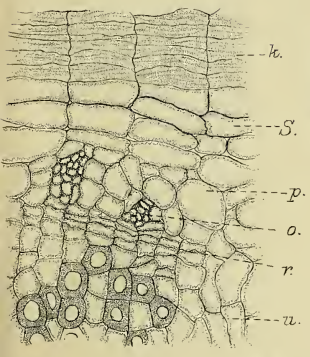

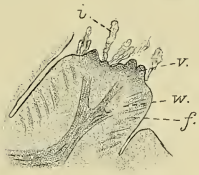

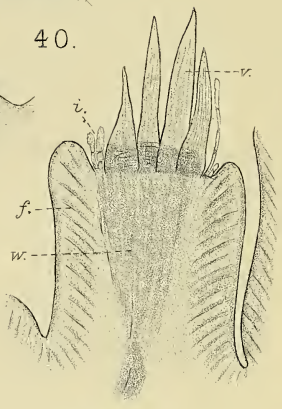

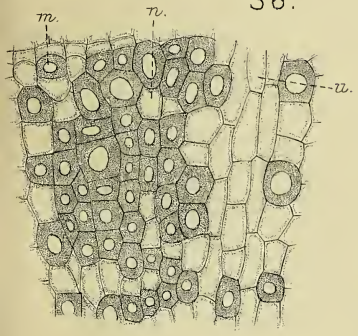

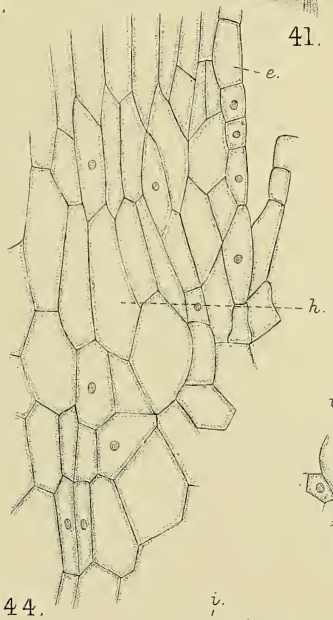

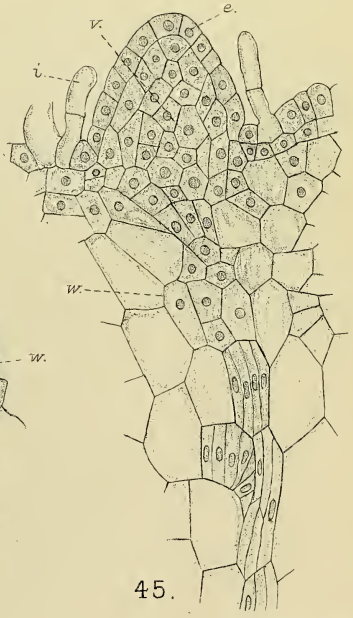

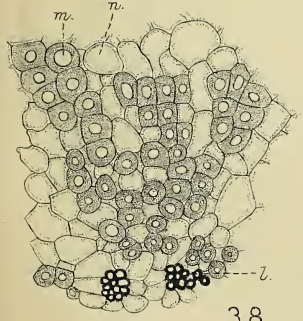

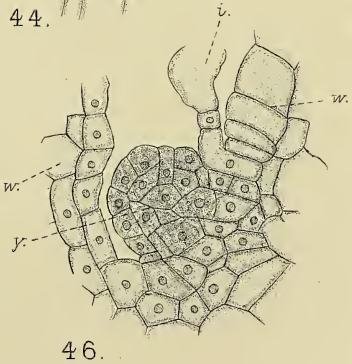

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The Gametophytes, Fertilization and Embryo of Cryptomeria Japonica.

BY

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With Plates XXVII–XXX.

INTRODUCTION.

INVESTIGATIONS of the last few years have added much to our knowledge of the morphology of the Coniferales. Some of the more recent, and probably the most important of these contributions, refer to the family Taxodieae. Arnoldi's observations on *Sequoia*, *Taxodium*, *Glyptostrobus*, *Arthrotaxis*, *Sciadopitys*, *Cunninghamia* and *Cryptomeria*, while more or less fragmentary in nature, have nevertheless thrown considerable light on the morphology of the gametophytes or embryos of these forms. Coker's work on *Taxodium* is probably the most complete account of the life-history of any Conifer that has yet been published by one investigator, and constitutes a valuable addition to our knowledge of this group. The present writer's account of the gametophytes of *Sequoia sempervirens*, which recently appeared in the pages of this Journal, completes the life-history of one of the most important and interesting of the Coniferales.

In addition to the broad phases of morphology that have been brought to light, these investigations have shown quite conclusively that the present family of the Taxodieae is an artificial one, and the genera representing the Taxodieae and Cupresseae should be rearranged. This Arnoldi has already suggested, and he believes that the genus *Sequoia* should constitute a family by itself, the Sequoiaceae; that *Cunninghamia*, *Taxodium*, and *Cryptomeria* should be placed with the Cupresseae, and that *Sciadopitys* should constitute a family by itself, the Sciadopitaceae.

While the results of the above-mentioned investigations would seem to warrant such a change, our knowledge of the gametophytes and embryo of the majority of these Conifers is still very meagre. Indeed *Taxodium* and *Sequoia* are the only two whose recorded life-histories are approximately complete. It has therefore seemed to me, that before making any

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definite rearrangement of these two families, it might be well to complete, as far as possible, the history of the gametophytes and embryo of some of the most important forms, and this is one of the objects of the present investigations on *Cryptomeria Japonica.*

Arnoldi ('01) is the only writer who has recorded any important observations on the gametophytes of *Cryptomeria*, and his account is very incomplete. He finds that the archegonia are arranged just as in the Cupresseae, with a common jacket surrounding the group, but he reports that no ventral canal-nucleus is formed. The pollen-tube is very like that of the Cupresseae. As soon as fertilization is accomplished, the fusion-nucleus passes to the bottom of the archegonium, where a number of free nuclei are organized. Cell-walls are formed about the lower nuclei. These cells and nuclei are now arranged in three tiers, the lowest one of which forms the embryo and the middle one the suspensors. The upper tier consists of free nuclei.

Methods.

Growing on the campus of Stanford University, there are about a dozen young trees of *Cryptomeria Japonica.* Collections of cones from these trees furnished most of the material upon which the following investigation is based. Two trees growing near the Hopkins Seaside Laboratory at Pacific Grove furnished the rest of the material. The collections were made during the years 1901, 1902 and 1903. During the period of the development of the seed, from October until the following July, collections were made almost daily. Although the trees are young they produce an abundance of male and female cones every year.

The following fixing fluids were employed :

1. Flemming's strong solution.
2. Flemming's weak solution.
3. Flemming's strong solution diluted with one part water.
4. Chrom-acetic mixture.
5. Chromic acid 1% sol.

Of these, Flemming's weak solution, and strong solution diluted with one volume of water, gave the most satisfactory results, but very good preparations were also obtained by the chrom-acetic mixture. The larger quantity of osmic acid in the strong solution of Flemming caused a considerable shrinkage of the protoplasm and altogether proved unsatisfactory. When this solution was diluted with water the shrinkage was very much diminished, and very good preparations were obtained.

The fixing fluids were invariably taken into the field and the material was killed as soon as the dissections were made. For the early stages the young ovules were removed and killed without further dissection, but after pollination the integument was removed and the nucellus alone was fixed.

In all the later stages the entire prothallium was removed. Nearly all of the dissections were made while the ovules were immersed in the killing fluid.

After remaining in the fixing fluids from ten to twenty-four hours, the material was washed in running water from four to six hours. In transferring the material through the various grades of alcohol, Schleicher and Schüll's diffusion shells were used in the manner described in my work on *Sequoia* ('03). Bergamot oil preceded the infiltration of paraffin. Microtome sections from 2 μ to 8 μ in thickness were made and the albumen fixative was used. For differentiating the various cell-structures, the triple stain, safranin gentian violet and orange G, gave the most satisfactory results.

The Male Gametophyte.

The staminate cones make their appearance as early as the first week in October, although pollination does not take place until March of the following spring. During the latter part of October and the early part of November, male cones in all stages of development may be found. From collections made during this period it was found that the reduction division of the microspore mother-cell, which leads to the formation of the tetrads, occurs about the first week of November. This, however, is not always the case, for we frequently find well-developed pollen-grains and very young sporangia not yet showing the mother-cells in the same cluster of cones. Generally the tetrads have separated and the pollen-grains are formed before the first of December.

At first the microspores are more or less spherical in form, are surrounded by a thin membrane, and contain a deeply staining centrally situated nucleus (Pl. XXVII, Fig. 1). Very soon after they are formed they enlarge slightly and the thin membrane develops into a hard thick wall. During the thickening of the wall a small hook-like projection is developed from one side, as shown in Fig. 2. About four or five weeks before pollination, the nucleus of the pollen-grain enlarges and prepares for division. The spindle showing this division was not found, but material collected at this time showed two distinct nuclei in each pollen grain. One of these nuclei is much larger than the other. The larger one was generally found near the centre of the spore, while the smaller one was very frequently found lying against the spore-wall. The cytoplasm surrounding the smaller nucleus was much more dense than the rest of the cytoplasm in the spore. I am indebted to Professor Campbell[1] for calling my attention to the plasmic membrane which separates the two nuclei, and which I was at first unable to detect. Fig. 3 represents a section of the pollen-grain soon after it reaches the micropyle; by this time the plasmic

[1] Campbell's University Textbook of Botany, 1902, p. 324.

membrane between the nuclei has disappeared, and the cytoplasm surrounding the latter is very granular but uniform throughout.

In the Cycads, *Ginkgo*, *Pinus* and *Podocarpus*, one or more vestigial vegetative cells of the gametophyte have been observed in the pollen-grain. As far as I have been able to make out, no such cells are organized in *Cryptomeria*. From daily collections made during the entire period of the development of the male gametophyte, close observation failed to reveal any trace of a vegetative cell or of a nucleus representing such a cell. In this respect the pollen-grain of *Cryptomeria* resembles that of *Thuja* (Land, '01), *Taxodium* (Coker, '03), and *Sequoia* (Lawson, '04). Not only at the time of pollination, but for about three weeks after the microspores have been received at the apex of the nucellus, there are but two nuclei present in each spore. The later history of the larger of these proved it to be the tube-nucleus, and the smaller one, no doubt, represents the generative cell.

During the latter half of February, when the female flowers first become visible, the microsporangia open and the pollen is liberated. For about two weeks the branches of the trees are quite yellow from the great quantities of pollen that have fallen on them. As the female flowers are open at this time, it is practically impossible for them to escape the reception of the pollen. Longitudinal sections of the female flower taken at this stage showed the integument of the ovule extending slightly above the level of the apex of the nucellus, leaving the micropyle open. From three to eight pollen-grains were always found deposited on the apex of the nucellus. As in *Sequoia*, the pollen-grains retain this position for about three or four weeks without further changes. The upper portion of the integument grows over the pollen-grains and closes the micropyle in very much the same manner as it does in *Sequoia*. The epidermal and sub-epidermal cells lining the micropyle lengthen towards the centre of the latter until they finally meet and the micropyle is closed. Fig. 9 shows the micropyle nearly closed.

Very soon after the casting off of the thick outer wall of the pollen-grain, the tube pushes out and immediately penetrates the tissue at the apex of the nucellus. It will be remembered that in *Sequoia* (Shaw, '96; Arnoldi, '01; Lawson, '04), many of the tubes do not penetrate the nucellar tissue immediately, but grow down between the integument and the nucellus. This is clearly not the case in *Cryptomeria*. Fig. 7 shows at least four tubes penetrating the nucellar tissue at the apex. An examination of hundreds of tubes failed to reveal any indication of branching. The tubes grow directly downward towards the developing female prothallium. Fig. 8 shows a typical tube; the tube- and stalk-nuclei and the body-cell are at the tip, and are about to be discharged into the depression above the archegonium-complex. In the course of

their development, they deviate very little out of the straight line towards the female prothallium. As shown in Fig. 8, the cells of the nucellar tissue through which the tube passes become disorganized, and are probably absorbed by the developing tube. There are usually five or six pollen-tubes present, and by the time they reach the female prothallium, the upper portion of the nucellus has a very disorganized appearance (Fig. 7). As the tube advances the tube-nucleus is always found near the tip.

Soon after the penetration of the tube both the nuclei within it enlarge considerably and advance with the growing tube. The generative nucleus is always a little in the rear of the tube-nucleus. Before the tube has penetrated very far the generative nucleus divides, and the young pollen-tube has now three free nuclei. Of the two nuclei derived from the generative nucleus, it is impossible to say which is the stalk- and which is the body-nucleus, as they are of equal size. One of them, however, very soon enlarges and becomes surrounded by a dense zone of granular cytoplasm, in which innumerable starch-granules are imbedded. The future activity of this nucleus proved it to be the body-nucleus, and the sister-nucleus which failed to enlarge is no doubt the so-called stalk-nucleus.

The body-nucleus eventually becomes several times the size of the stalk-nucleus, and, as it grows, the cytoplasm surrounding it becomes much more dense. A membrane is now formed at the periphery of the dense zone, and a distinct body-cell is organized, which becomes perfectly spherical. The thickness of the dense zone of cytoplasm within the body-cell-membrane is about half the diameter of the large, centrally situated nucleus as shown in Fig. 4. The tip of the tube now contains one large spherical cell and two free nuclei. These three structures always lie near each other in a central strand of cytoplasm. The relative positions and size of these structures is shown in Fig. 4. The lower free nucleus (marked *t.n.*) is the tube-nucleus. Immediately above this is the stalk-nucleus (marked *s.n.*), and slightly in the rear is the large body-cell (marked *b.c.*). These three structures remain in close proximity to each other till about the middle of June, when the two male cells are organized. When fully mature, the nucleus of the body-cell is at least four times the size of the tube- or stalk-nuclei. It contains a very large nucleolus, and the chromatin at this time appears in the form of coarse, deeply staining granules suspended on threads of linin. The cytoplasm surrounding the body-cell and the tube- and stalk-nuclei is very coarsely granular, but free from starch. Within the body-cell large quantities of starch-grains are always present.

It takes the tube about three or four weeks to reach the female prothallium, and during this period the body-cell undergoes no more

perceptible changes. During the first week in June, however, when the tip of the tube with its contents has reached the depression just above the archegonium-complex, the nucleus of the body-cell prepares for division. It first enlarges slightly, the nucleolus disappears, and the chromatin loses its granular appearance and assumes the form of a coarse continuous thread, which winds irregularly through the karyolymph. A very careful study of the cytoplasm was made at this stage. Apart from the irregular distribution of the starch-grains, the cytoplasm surrounding the nucleus appeared to be perfectly uniform. No differentiation that would suggest the presence of the blepharoplasts reported for *Cycas* (Ikeno, '97), *Zamia* (Webber, '97), and *Ginkgo* (Hirasé, '95) could be found. Coker ('03) has recently reported the presence of light areas at the poles of the cells in *Taxodium*, but he further states that the resemblance of these areas to blepharoplasts is 'confined entirely to their position.' It must be remembered that these centrosome-like structures have only been found where the male cell develops cilia, and that their function is confined to the organization of these motile organs. Where the pollen-tube acts as the carrier of the male cells to the archegonia, motile organs are no longer necessary. Cilia in connexion with the male cells of Coniferales have never been observed, and it is therefore not surprising that the structures responsible for their formation should also be missing.

The stages showing the organization of the spindle which divides the body-cell were not found, but several sections showed the daughter-nuclei at the poles with the kinoplasmic fibrils between them. Fig. 5 shows a stage where the daughter-nuclei are completely organized, and the plate which divides the cell into two is forming at the equator. Several sections showing the development of the plate were examined, and it is apparently organized from the kinoplasmic fibrils which stretch between the daughter-nuclei. The daughter-nuclei now enlarge considerably, and each contains two or three nucleoli with the chromatin in the form of coarse granules suspended on threads of linin (Fig. 5). During the division of the nucleus, the body-cell loses its spherical form, and becomes more or less elliptical, as shown in Fig. 5.

Soon after its complete organization the cell-plate splits, and the two male cells are free and separate from each other. As shown in Fig. 6, they are flat on one side and rounded on the other. They remain close together for some time, but when they separate they become spherical in form. The nucleus of each becomes very large, its diameter being about half the diameter of the cell. The chromatin is in the form of small, irregularly shaped granules suspended in a network of linin. The two male cells are of the same shape and of equal size, and, as we shall point out under the head of fertilization, they are both functional.

With the complete organization of the male cells, the history of the male gametophyte ends, and this is true for all other Conifers that have been investigated except *Sequoia* (Lawson, '04). In this latter there is the additional step of the discharge of the male cell-nucleus into the archegonium. In *Cryptomeria* the entire male cell enters the archegonium as shown in Fig. 43. As we shall point out later, the two male cells enter separate archegonia, and therefore one pollen-tube brings about the fertilization of two egg-cells.

The general characters of the male gametophyte of *Cryptomeria* are more distinctly of the Cupresseae type than of the Taxodieae. The behaviour of the pollen-tube and its spermatogenesis are very unlike *Sequoia.* Compared with *Thuja* (Land, '02) and *Taxodium* (Coker, '03) there is a very striking similarity.

The Female Gametophyte.

The stages in the development of the macrosporangium and the integument follow very closely those in *Taxodium* (Coker, '03) and *Sequoia* (Shaw, '96, Lawson, '04). There is one striking difference, however, and that is the advanced stage in the development of the integument before the differentiation of the sporogenous cells in the nucellus. Fig. 9 shows the appearance of the nucellus and integument in section from material collected March 7. As a rule, however, the micropyle is much more open than that shown in the figure. Material collected before March 6 showed no trace whatever of sporogenous cells in the nucellus, although the integument extended considerably beyond the apex of the nucellus, and in several cases the micropyle was found to be closed, shutting the pollen-grains within. The closing of the micropyle is brought about in very much the same manner as in *Sequoia.* The sub-epidermal cells and the epidermal cells in the upper region of the integument which form the inner wall become very much elongated in a direction at right angles to the micropyle. The result is that as these cells elongate, the micropyle becomes smaller until the channel is finally closed completely.

One series of sections taken during the first week in March showed an interesting abnormality. There were two distinct sporangia developed within a single integument. This is shown in Fig. 10. These twin sporangia were not sufficiently developed to show the sporogenous cells, and as they were the only ones found out of the hundreds of preparations studied, they are no doubt abnormal and very exceptional.

The sporogenous cells become differentiated early in March. It is very difficult at first to distinguish them from the ordinary sterile cells of the sporangium, but they soon become very densely packed with starch-granules, and become five or six times as large as the sterile cells

immediately surrounding them. Their nuclei increase correspondingly, and become very conspicuous when compared with the small nuclei of the sterile tissue. There are always one or two large, deeply staining nucleoli present, and the chromatin is in the form of small granules suspended on a network of linin as shown in Fig. 12.

There are usually three or four sporogenous cells differentiated, and these are always situated near the base of the sporangium just a little above the point of insertion of the integument. The position and relative size of the sporogenous cells is well shown in Fig. 11. According to Coker ('03) but a single megaspore mother-cell is organized in *Taxodium*. In this respect *Cryptomeria* resembles *Sequoia*, where a group of five or six mother-cells are organized (Shaw, '96, Lawson, '04). Another striking difference between *Cryptomeria* and *Taxodium* is that in the latter (Coker, '03) there is a distinct zone of large-celled tissue or tapetum surrounding the large spore mother-cell, which persists up to the time of endosperm-formation. According to Coker, the function of this large-celled tissue is to nourish the young gametophyte. It is therefore regarded as a tapetum, which, instead of becoming disorganized upon the germination of the megaspore, continues to grow with the developing prothallium, and nourishes the latter until the endosperm is fully formed. I was unable to find anything that resembled this in *Cryptomeria*. Surrounding the three or four mother-cells, there is present a layer of small elongated cells which have the appearance of being crowded by the growing sporogenous cells. Soon after the spores are formed these elongated cells become disorganized and are probably absorbed by the germinating spore. The general appearance of the cells surrounding the megaspore mother-cells is shown in Fig. 12.

Upon their complete organization the three or four mother-cells immediately prepare for division. This is the reduction-division which marks the beginning of the female gametophyte. In *Pinus* (Coulter and Chamberlain, '01), the single mother-cell gives rise to a row of four potential megaspores. In *Larix europaea* (Strasburger, '79), there are at least three potential spores formed from the mother-cell. In *Larix Sibirica* Juel ('00) finds that the first division of the single mother-cell is heterotypic, and the number of chromosomes is just half the number present in the nuclei of the nucellus. Each of the daughter-nuclei now divides by a homotypic division with the same reduced number of chromosomes. There is thus formed from the single mother-cell a row of four megaspores, the lowest of which germinates and gives rise to the prothallium. In *Sequoia* (Shaw, '96, Lawson, '04) there are five or six mother-cells organized, each of which divides twice and gives rise to four potential spores. In *Taxodium* (Coker, '03) there are two cells formed as a result of the first division of the single mother-cell, but only the lower one of these divides again.

The result is that there are only three potential spores formed from the mother-cell.

As stated above, there may be three or four megaspore mother-cells organized in *Cryptomeria*. As the early stages of the spindles of the first division were not found, I was unable to study the behaviour of the chromatin. Fig. 13 shows a spindle of the first division of one of the mother-cells with the daughter-nuclei already organized at the poles. The next stage observed showed a large group of spores as illustrated in Fig. 14. The contents of these megaspores were devoid of starch, and quite different in appearance from those of the mother-cells. They varied from twelve to sixteen in number, showing without much doubt that each mother-cell gives rise to four megaspores. As shown in Fig. 14, the one that is more centrally situated becomes larger than the others, and it probably is the only one that germinates. As this central megaspore increases in size, free nuclear division proceeds at a rapid rate. Meantime the other spores show all stages of disorganization, and are very probably absorbed by the young prothallium. These early stages in the development of the gametophyte progress slowly, for about a month after the organization of the megaspores we find the condition represented in Fig. 15. It will be observed from this figure that the greatest growth of the young prothallium has taken place in the direction of the chalaza. There are a number of vacuoles present, and the numerous free nuclei are distributed irregularly throughout the strands of cytoplasm. Up to this time the conditions are very much the same as those observed in *Sequoia* (Lawson, '04).

As the young prothallium increases in length, the vacuoles increase in size, and eventually flow together, forming one very large central vacuole as in *Sequoia* (Arnoldi, '01 ; Lawson, '04), *Taxodium* (Coker, '03), *Taxus* and other Conifers. The result of this is that the cytoplasm is crowded to the wall, and appears in the form of a very thin parietal layer, in which the free nuclei lie imbedded at more or less regular intervals, as indicated in Fig. 16. A higher magnification of the parietal layer is shown in Fig. 19. It will be seen that the thickness of the cytoplasmic layer is just about equal to the diameter of the nuclei.

The most striking difference between the young female gametophytes of *Sequoia* and *Cryptomeria* up to this stage is in the entire absence in the latter of any secondary prothallia. It will be remembered that in *Sequoia* one or two secondary prothallia, while failing to produce true prothallial tissue, nevertheless reach an advanced stage of development. In *Cryptomeria* only one of the megaspores germinates, and there is consequently but one primary prothallium formed.

The next stage in the development of the prothallium showed a considerable increase in the thickness of the parietal layer of cytoplasm, and

this is accompanied by an increase in the number of free nuclei. As the free nuclei continue to divide, delicate membranes are formed between them, and they now take up a position at the inner layer of cytoplasm exposed to the cell-sap of the large central vacuole. This condition is shown in Fig. 17. The membranes formed between the nuclei are very delicate, and all lie more or less parallel to each other. A higher magnification of this stage is shown in Fig. 20. It will be seen that the primary cells thus formed are open on the inner side, exposing the cytoplasm to the vacuole. These structures are the so-called 'Alveoli,' first described by Mlle. Sokolowa ('91) in *Pinus*, *Cephalotaxus*, and *Juniperus*, and since found in other Gymnosperms by Ikeno ('98), Arnoldi ('00), and Coker ('03). Coker's objection to the term 'Alveoli' is well taken, but the term he substitutes, 'prothallial tubes,' is almost as misleading, for these structures are not always tubular. They constitute the first cells of the prothallium, and although they are open on one side and are later multinucleate, they are nevertheless cells. To avoid confusion I will therefore use the term 'primary prothallial cells' to designate these structures.

Fig. 20 indicates the manner in which the primary prothallial cells are formed. Comparing Figs. 19 and 20, it is obvious that the nuclei have divided and that a delicate membrane has been formed in the region of the equator of the spindle, midway between the daughter-nuclei. A section taken parallel to the inner surface of the parietal layer of cytoplasm is shown in Fig. 22. This is looking down into the cells, and their outline and the method in which the membrane is formed are easily made out from this view.

The primary prothallial cells thus organized elongate towards the centre of the vacuole in very much the same way as Mlle. Sokolowa ('91) has described for other Conifers ; the nuclei retaining their position at the inner exposed surface. By the time the prothallium has reached the stage shown in Fig. 18, the nuclei in the primary prothallial cells have divided repeatedly and very delicate membranes have formed between them. The method of the formation of these membranes is shown in Figs. 20 and 21. They evidently develop from the continuous fibrils of the spindle which extend between the daughter-nuclei. As numerous cross-walls are formed, all of the primary cells do not extend inward as far as the vacuole. But they all now proceed to elongate and grow towards the centre of the diminishing vacuole. As this growth continues the vacuole becomes smaller and smaller until the space it occupied is closed by the union of primary prothallial cells at the centre. During this period the nuclei divide freely so that the cells are all multinucleate. Fig. 23 represents a longitudinal section of the upper position of the prothallium at this time. As shown in the figure, the walls of the cells are extremely delicate and may not

extend all the way to the centre of the prothallium. Now these delicate membranes take no part whatever in the formation of the cell-walls of the permanent prothallial tissue. As we shall proceed to demonstrate, these permanent cell-walls originate in quite another way.

By the time the central vacuole has been completely closed by the ingrowing primary cells, the nuclei continue to divide without the formation of walls between them. As the membranes of the primary cells do not extend all the way across, the median region of the prothallium consists merely of numerous nuclei lying freely in the cytoplasm. These free nuclei, as well as those in the primary cells, now undergo a peculiar division, which results in the formation of permanent cell-walls. The manner in which these walls are formed is extremely interesting, and unlike anything that has so far been reported for the Gymnosperms. Sections of the prothallium at this time showed hundreds of the free nuclei in all stages of mitosis. The early stages in the development of the spindle clearly showed the multipolar character, indicating that they follow the same general method that prevails in the Angiosperms. As shown in Fig. 24, the mature spindle is quite narrow and sharply pointed at the ends. When the chromosomes reach the poles, numerous kinoplasmic fibrils stretch between them (Fig. 25), and by the time the daughter-nuclei are organized, these fibrils increase in number and curve outward on all sides. This condition is shown in Figs. 26, 27, 28. Ordinarily we should expect to find the cell-plate formed from these fibrils midway between the two daughter-nuclei, but this does not occur. The fibrils continue to increase in number and curve outward still further as shown in Figs. 29, 30. This process continues until both daughter-nuclei are completely surrounded by a sheath of fibrils. Of the somewhat spherical structure thus formed, the fibrils are all at the periphery and the nuclei are within, one at each end and surrounded by ordinary cytoplasm. Fig. 31 shows the appearance of one of these structures in cross-section—that is at right angles to the long axis of the spindle. The fibrils in section appear as small dots closely packed together, and arranged in a dense zone which completely encircles the two nuclei (Fig. 32). From this view the two nuclei cannot be seen at the same focus. The fibrils which compose these peculiar kinoplasmic structures fuse laterally with one another and are gradually converted into a permanent cell-membrane, which completely encloses the two nuclei. As these divisions of the free nuclei occur almost simultaneously, the prothallium at this time presents a most extraordinary appearance. The process goes on throughout the whole of the prothallium except in the region of the archegonial initials. All stages in the process could be observed in a single section. Fig. 24 represents a portion of the prothallium at this time. Although this division brings about the formation of the first permanent cell-membranes in the endosperm, no cell-plate is developed between the daughter-nuclei as

ordinarily occurs after mitosis. The result of this is that the prothallium passes through a stage when the majority of its cells are binucleate.

When first formed, the membranes surrounding the pairs of nuclei appear round or oval in section. As hundreds of them are formed they become more or less crowded and the walls become flat where they press upon each other. As they crowd against one another, the neighbouring walls become fused together, and this gives the appearance of ordinary cellular tissue. Fig. 33 shows a portion of the prothallium soon after this division. There are two distinct nuclei in each cell.

As far as I am aware such a peculiar method of endosperm-formation has not been recorded. Indeed I do not know where such a type of free cell-formation occurs in the plant kingdom. The nearest parallel to it is that which Harper ('97 and '00) has described in the development of the ascospores in the ascus of *Erysiphe* and *Pyronema*. In these Fungi, after the last division of the spore-mother cell, the kinoplasmic fibrils which radiate out from the centrosome bound off the ascospores by a process of free cell-formation. These fibrils increase in number, and as they grow in length they curve around the nucleus. By fusing together laterally the fibrils are converted into a complete membrane which surrounds the young ascospore. Between this process and that which I have described in the endosperm of *Cryptomeria*, there is a striking similarity; the main difference being that it is the astral rays in *Erysiphe* and *Pyronema* which form the membrane, while in *Cryptomeria* it is the continuous fibrils of the spindle which are converted. They also differ in the absence of the centrosome in *Cryptomeria*, and also in the enclosure of two nuclei instead of one.

The earlier stages in the development of the female prothallium in *Cryptomeria* agree very closely with those reported for many other Conifers, but this later stage, where such a peculiar form of free cell-formation precedes the formation of the permanent endosperm-tissue, is unlike anything that has heretofore been described. That it is normal in *Cryptomeria* I feel tolerably certain. It was found in a large number of preparations of material collected from several different trees and during both years, 1902 and 1903. Whether or not it occurs in other Conifers can only be learned from future investigations.

After the binucleate cellular tissue has been thoroughly established in the endosperm, nuclear division proceeds in the usual way and the cell-plates are formed between the daughter-nuclei. Some preparations, however, showed binucleate cells in the endosperm as late as the early stages of the embryo.

The Archegonia.

The archegonial initials were first observed in material collected about May 25, just before the prothallial tissue is thoroughly organized. They are always situated at the apex of the prothallium ; and are generally peripheral cells, but some were frequently found one or two layers of cells below the periphery. They are very easily distinguished from the other cells of the prothallium by their large size and deeply staining granular cytoplasm. The nucleus is also very conspicuous, being fully twice the size of the nuclei of the surrounding sterile cells. A group of the initials is shown in Fig. 34. At first the nucleus is centrally situated and the lower part of the cell is vacuolated.

The further development of the archegonium is very rapid and only a few preparations showed the most important stages. When they first become differentiated, the nucleus is nearly always centrally situated. About the time the vacuole develops, the nucleus moves towards the periphery of the cell and immediately prepares for division. Several preparations showed the spindle, and in each case it was found very near the periphery and always parallel with the long axis of the cell. This division results in the organization of the central cell and the primary neck-cell of the archegonium. A complete wall cuts off the primary neck-cell and the central nucleus moves back to a position just above the vacuole. From the beginning the primary neck-cell is many times smaller than the central cell.

Very soon after the primary neck-cell has been cut off, the central cell enlarges considerably, especially at the base, and the region surrounding the vacuole becomes very much wider than the narrow tapering region towards the neck. Meantime the cytoplasm becomes very densely granular, and the nucleus, which is centrally situated, increases very much in size, and the condition of the chromatin shows that the nucleus is preparing for further division.

During the development of the central cell, the primary neck-cell undergoes some interesting changes. It does not increase in size, but its cytoplasm is densely granular and stains very readily and can therefore be easily distinguished from the neighbouring sterile cells. The nucleus now very soon divides, and a wall is formed between the daughter-nuclei at right angles to the wall which cuts off the primary neck-cell from the central cell. Fig. 35 shows the primary neck-cell just after its nucleus has divided ; the wall between the daughter-nuclei has not yet been organized. The archegonium has now two neck-cells, and these were very easily distinguished in longitudinal sections. Indeed longitudinal sections of all later stages showed but two cells in the neck. A study of cross-sections, however, showed very conclusively that each of the two neck-cells divide

again at right angles to the first division. The mature archegonium has therefore at least four cells in the neck. These are shown very well in Fig. 38, as they appear in cross-sections. In but a single case was I able to find a variation from this number, and that was in a longitudinal section where four were observed, suggesting that there may have been eight altogether. It appears that in the Conifers the number of neck-cells is not always constant. According to Coker ('01), they may vary from two to twenty-five in *Podocarpus*. In *Sequoia* (Lawson, '04) they may be two to four. In *Taxodium* (Coker, '03) they vary from two to sixteen or more, and they also vary in *Tsuga* (Murrill, '00). In *Cryptomeria* the neck consists typically of a single tier of four cells (Fig. 38).

By the time the neck-cells have been organized the central nucleus enlarges considerably, and shows all the characteristics of a nucleus preparing for division. Fig. 41 shows a section of the nucleus in this condition. The large nucleolus has disappeared and the chromatin has assumed the form of definite chromosomes. The spindle showing the actual division of the central nucleus, and which gives rise to the egg and ventral canal-nucleus, was not found; but from the observations made it seems tolerably certain that such a division takes place. Many archegonia at this stage showed two distinct nuclei, and one of these is shown in Fig. 36. The results of investigations during the last few years make it appear that the cutting off of the ventral canal-cell or nucleus is very general, if not universal, among the Conifers. Such a cell or nucleus has been found in *Juniperus* (Strasburger, '79, Belajeff, '93); *Pinus* (Blackman, '98, Chamberlain, '99, Ferguson, '01); *Taxodium* (Coker, '03); *Podocarpus* (Coker, '02); *Tsuga* (Murrill, '00); *Thuja* (Land, '02); *Sequoia* (Lawson, '04); and *Picea* (Miyake, '03). In many of these forms all the stages of mitosis have been carefully followed, so that there can be little doubt that such a division of the central nucleus occurs in these Conifers. Owing to the fact that Arnoldi denies the existence of a ventral canal-cell or nucleus in *Cryptomeria*, I have been very careful in my observations. Although I was unable to find the spindle, there was nevertheless enough evidence to convince me that such a division takes place. In a large number of archegonia, just before fertilization, two distinct nuclei were observed in the cytoplasm. That this second nucleus was not the male nucleus in the act of fertilizing the egg was very obvious, from the fact that the neck-cells were in no way disturbed, and in nearly every case the male cells were to be seen in the pollen-tube above the archegonia. When we consider that the stage immediately preceding this showed the central nucleus preparing for division, I have little hesitation in considering the second nucleus in the archegonium to be the ventral canal-nucleus. In Fig. 36, the ventral canal-nucleus is already showing signs of disorganization. It presumably lasted but a short time, for it was not found in the stages during or after fertiliza-

tion. Fig. 37 represents a typical mature archegonium ready for fertilization. The egg-nucleus is at this time always centrally situated, and below this is a large vacuole. With the exception of the character of the neck-cells the mature archegonium of *Cryptomeria* is strikingly like that of *Thuja* (Land, '01) and *Taxodium* ('03), and, as we shall see below, the grouping of the archegonia is distinctly of the Cupresseae type.

In regard to the distribution of the archegonia, my observations agree very closely with those of Arnoldi ('01). They are grouped in exactly the same way as in the Cupresseae. The number varies from eight to fifteen, and they nearly always form a single compact group at the apex of the prothallium as shown in Fig. 39. The necks of the archegonia are not on a level with the tip of the prothallium, but open into a very distinct depression, as indicated in Fig. 39. In longitudinal sections only four or five of the archegonia may be seen, but cross-sections show the entire number and also the way they are arranged. Fig. 40 represents a cross-section of the prothallium in the region of the nuclei of the archegonia.

As reported by Arnoldi ('01), there is a common jacket surrounding the group of archegonia. The jacket-cells extend nearly as far as the neck, and for a considerable distance constitute but a single layer. Towards the base there may be two or three layers of jacket-cells, but at the base and below the archegonia there are several layers of them. Not infrequently the jacket-cells were found extending a little way up between the archegonia, and occasionally, as shown in Fig. 39, they extended quite as far as the neck-cells, thus completely separating one or two of the archegonia from the main group.

The jacket-cells themselves showed some interesting features. The cytoplasm is always densely granular and stains exactly like the cytoplasm of the egg. The nuclei are very conspicuous and stain much more intensely than those of the other sterile cells of the prothallium. The jacket-cells at the base of the archegonia are small and are crowded closely together, while those extending up between the archegonia vary considerably as to their size ; some growing to fully half the size of the archegonium. By the time that the archegonia are mature, nearly all of the jacket-cells are multinucleate. All stages of nuclear division were found in the cells, and they showed the characteristics of ordinary normal mitosis. There may be as many as five or six nuclei in each jacket-cell. Another conspicuous characteristic of these cells was the presence of a large vacuole towards one end (Fig. 42). If the larger of the jacket-cells were not multinucleate, they would be strikingly like small archegonia.

It will be remembered that in *Sequoia* the jacket-cells are very irregularly distributed, and that in certain stages it is quite impossible to distinguish them from young archegonia. In my work on *Sequoia* ('04) I pointed out the common origin and general similarity of the

jacket-cells and archegonial initials, and suggested that the jacket-cells were sterile archegonia. The fact that the jacket-cells are multinucleate in *Cryptomeria* does not argue against this view, but rather strengthens it. If the jacket-cells are archegonial initials morphologically, and their generative functions changed to one of nourishing, we ought not to expect nuclear activity to be entirely suppressed. From this point of view, I regard the various nuclei in the jacket-cell as representing the abortive nuclei of the egg, ventral canal-cell and neck-cells.

In this connexion, it is interesting to note that in *Sequoia*, where the archegonia are very numerous, the jacket-cells are less highly differentiated; and in forms like *Pinus*, where the archegonia are very few, the jacket-cells become very highly specialized as nourishing cells, and even have (according to Goroschankin, '83) a direct communication with the cytoplasm of the central cell.

Fertilization.

We have already described how the pollen-tube grows directly downward through the tissue at the apex of the nucellus until it reaches the tip of the prothallium. There may be as many as four or five pollen-tubes, and their ends reach the depression above the archegonium-complex some little time before the archegonia are completely organized. The tubes all grow towards this common point, and when they reach the apex of the prothallium there may be as many as ten male cells situated immediately outside the neck-cells. Fertilization is therefore very easily accomplished.

In many Conifers the process of fertilization becomes very much complicated by the entrance of other pollen-tube structures than the male cell into the archegonium. In *Pinus* nearly the whole of the contents of the pollen-tube passes into the archegonium (Goroschankin, '83; Dixon, '94; Blackman, '98; Ferguson, '01; Coulter and Chamberlain, '01); a similar condition has been found in *Taxodium* (Coker, '03; *Cephalotaxus* (Arnoldi, '00), and in *Picea excelsa* (Miyake, '03). In *Picea vulgaris*, Strasburger ('84) reports that two male cells enter one archegonium. In *Thuja*, Land ('02) finds that the tube- and stalk-nuclei may occasionally enter the archegonium, but they more generally remain outside and become disorganized in the cavity above the archegonium-complex. From these recorded observations it appears that the fertilization of many Conifers at the time of fusion of the male and female nuclei becomes very much complicated by the presence of other pollen-tube structures in the archegonium which take no essential part in actual fertilization. In *Sequoia* (Lawson, '04), however, quite a different and much more simple process prevails. Here only the nucleus and a very small amount of cytoplasm of the male cell enters the archegonium, so that at the time of fusion only the

two sex-nuclei are present in the archegonium. In this respect *Sequoia* differs from all other Conifers that have been investigated.

In *Cryptomeria* only one male cell enters the archegonium. Although a great many preparations showing all stages of fertilization were studie d no exception to this rule was found. Just before fertilization, the archegonia are very similar to those described by Coker ('03) for *Taxodium*; they differ only in the character of the neck-cells. There is a large central nucleus containing a chromatin network and one or two nucleoli. Just below the nucleus there is a large vacuole. Fig. 37 shows a mature archegonium ready for fertilization. Cross-sections of the neck at this time showed a distinct space between the neck-cells, indicating that they were preparing for the entrance of the male cell by separating from each other, as shown in Fig. 38. The actual entrance of the male cell was not observed, but several preparations showed it just inside the archegonium. In these cases the neck-cells were forced from their position and were more or less disorganized. Upon its entrance, the male cell loses its spherical form. As shown in Fig. 43, it is nearly twice as long as broad, and completely fills the upper part of the archegonium. The cytoplasm is very densely granular and contains an abundance of starch-grains. The membrane surrounding the male cell remains intact for some little time after it enters the archegonium, and its nucleus is just about half the size of the egg-nucleus.

As the male cell advances towards the egg-nucleus, the membrane surrounding it disappears and its cytoplasm unites freely with that of the egg. The male nucleus enlarges and immediately moves towards the female nucleus. At this time the male nucleus is not quite as large as the female, but their structure is similar. The chromatin is in the form of small granules suspended on an irregular network of linin. They also stain equally dense with safranin or gentian violet. The only distinction between the male and female is their size and position.

The male nucleus now flattens itself against the female in the manner shown in Fig. 44. As it does so, it evidently increases in size, for the next stage showed the condition illustrated in Fig. 45. As indicated in the figure, the male nucleus has so far forced in the wall of the female that it is almost completely enveloped by the latter.

During this period, the cytoplasm of the two sex-cells becomes very intimately united, and the starch-granules brought in by the male cell collect and form a very dense zone around the fusing nuclei. This condition is strikingly similar to that described by Coker ('03) for *Taxodium*.

In *Pinus*, according to Blackman ('98), the first segmentation-spindle begins to develop before the sex-nuclei lose their identity. This has been at least partially confirmed by Chamberlain (1899) and Ferguson ('01). A similar condition has been found by Woycicki ('99) in *Larix*. In these

forms the chromatin of the male and female nuclei may be distinguished inside the wall of the female nucleus; the fusion of the two taking place very slowly. In *Cryptomeria* the process of fusion is more rapid. As shown in Fig. 45, the male and female nuclei only retain their identity by the presence of the nuclear membrane between the two. The chromatin contents of the nuclei are structurally alike, and as soon as the membrane separating them breaks down the male can no longer be distinguished from the female. In this particular process, *Cryptomeria* resembles *Sequoia*. When the membrane between the nuclei disappears, the chromatic contents of the two mingle together and form the common network of the fusion-nucleus. The fusion-nucleus was frequently met with, and this has convinced me that there is at least a short resting period before the organization of the first segmentation-spindle. Fig. 49 shows the fusion-nucleus with the chromatin evidently in the resting condition. It has been reported that in *Taxodium* (Coker, '03) and in *Taxus* (Jäger, '99) the fusing nuclei travel to the base of the egg before the first division occurs. In *Cryptomeria* this is clearly not the case, for the first segmentation-spindle was always found at the centre of the egg, just about the point where the fusion of the nuclei took place.

It has been stated above that each fertilized archegonium receives but a single male cell, although there are two of the latter formed in each pollen-tube. We have now to complete the history of the second male cell. In many Conifers, especially in those forms where the entire contents of the pollen-tube pass into the archegonium, only one of the male cells is functional. This has been observed in *Pinus* (Blackman, '98), *Cephalotaxus* (Arnoldi, '00), *Picea* (Miyake, '03), *Podocarpus* (Coker, '02), and it may sometimes occur in *Taxodium* (Coker, '03). In these cases a pollen-tube can bring about the fertilization of but one archegonium. In other forms where both male cells are functional, as in *Juniperus* (Strasburger, '79), *Sequoia* (Lawson, '04), and generally in *Taxodium* (Coker, '03), two archegonia may be fertilized from the contents of one pollen-tube. In *Cryptomeria*, where so many male cells from the various pollen-tubes are gathered together in the depression immediately above the archegonium-complex, it was difficult to distinguish the second male cell from those of the other tubes, as they are all of the same size and shape. As nearly all of the archegonia were fertilized, however, it seems extremely probable that both male cells are functional, and that two archegonia are fertilized by one pollen-tube.

The Embryo.

Although the fusion of the male and female nuclei results in a distinct resting fertilized nucleus, the resting period is a short one. The uniform chromatin network of the fusion-nucleus very soon undergoes a change

which is characteristic of nuclei preparing for division. It assumes the form of definite chromosomes. The stages in the formation of the first segmentation-spindle were not observed, but the mature spindle was frequently met with. Arnoldi ('01) reports that the first division takes place at the base of the archegonium, Coker ('03) reports the same condition for *Taxodium*. I was unable to confirm the observations of these writers, for every case examined showed the first segmentation-spindle in the middle of the archegonium just about the place where the fusion of the sex-nuclei occurred.

The dense zone of starch-granules which surround the fusion-nucleus persists until the completion of the first division. Fig. 46 shows the first segmentation-spindle completely surrounded by the zone of starch-granules. This gives the impression that the spindle is organized within the nuclear membrane, but a very close examination failed to reveal a trace of the latter. The first spindle is a little larger than those formed in the pro-embryo and embryo. It is very sharply pointed at the poles and broad at the equator, as shown in Fig. 46. Only a few of the chromosomes are shown in this figure; the majority of them were found in the sections immediately following the one from which the figure was drawn.

The chromosomes of the pro-embryo are very long, V-shaped structures. They are always attached to the spindle-fibrils at the point where the arms of the V meet. They are just about one-fourth the length of the spindle, for when they are situated at the equator, they extend half-way to the pole. Fig. 47 shows a spindle of the second division of the pro-embryo with the chromosomes approaching the poles. On account of the presence of the two long arms which overlapped each other more or less, it was practically impossible to accurately estimate the number of the chromosomes. There are approximately eighteen or twenty in the embryo. Comparing the typical embryo-spindle as shown in Fig. 47 with a typical spindle from the female prothallium as shown in Fig. 48, the difference in the number of the chromosomes in the two is very obvious. Hundreds of spindles in all stages in both the gametophyte and sporophyte were examined, and this difference was always very marked. The gametophyte-spindle had not only fewer chromosomes, but it was only about half as wide at the equator as the sporophyte-spindle. This is well illustrated in Figs. 47 and 48. Both these figures were drawn by the aid of the camera lucida and at the same magnification. As near as could be estimated there are nine or ten chromosomes in the gametophyte, and eighteen or twenty in the sporophyte of *Cryptomeria*.

The result of the first segmentation is shown in Fig. 50; two free nuclei are formed and they move towards the base of the archegonium. As they move downward both nuclei divide and we have four free nuclei, as shown in Fig. 51. So many preparations showed this condition that

I am convinced that the first two divisions at least take place before the base of the archegonium is reached, although Arnoldi reports that the fusion-nucleus passes to the bottom of the archegonium before dividing. One preparation, as shown in Fig. 52, showed six free nuclei lying one behind the other. It seems to me very improbable that the uppermost of this row of nuclei originated at the base of the archegonium.

In his work on *Taxodium*, Coker ('03) makes special emphasis of the starch brought into the egg by the male cell. He reports that the protoplasm and starch of the male cell form a distinct layer around the egg-nucleus. This later becomes separated from the egg and forms the greater part of the young embryo. In *Cryptomeria* a distinct zone of starch was observed surrounding each of the free nuclei of the pro-embryo. The quantity present, however, is very much greater than that brought in by the male cell, and I am therefore convinced that only a very small proportion of the starch present in the developing embryo comes from the male cell. Viewed in the light of what occurs in *Sequoia* (Lawson, '04), where all the starch of the male cell remains outside of the archegonium, and becomes disorganized in the pollen-tube, the point which Coker has raised is not of very great importance.

By the time the second division is completed, two or more of the free nuclei have settled in the base of the archegonium and they all prepare for the third division. Fig. 52 shows the manner in which the two lower nuclei are situated and the upper two have not yet reached the base. Each nucleus is surrounded by a dense zone of starch-granules. The starch is carried down with the nuclei and forms a very sharply differentiated region as shown in Figs. 53, 54, and 55.

The third division results in the formation of eight nuclei, which are arranged in tiers as shown in Fig. 56. In these latter stages it was impossible to see all the nuclei in a single section, but by studying the series, it was comparatively easy to distinguish eight nuclei. Immediately after the third division the continuous fibrils of the spindles persist, and present the appearance of radiating systems, which connect the daughter-nuclei with one another. In the first two divisions the entire spindles disappear immediately after the daughter-nuclei are organized, but after the third division the fibrils persist (Fig. 54 and 55), and the first cell-membranes of the embryo are formed between the nuclei. As shown in Fig. 55, these cell-walls of the upper tier are formed parallel with the long axis of the archegonium, and the cells thus formed are open on the inner or upper side.

The next division only concerns the upper tier of nuclei. The spindles are arranged at right angles to those of the preceding division, as shown in Fig. 56. A large number of preparations showed this stage, with the nuclei in all stages of mitosis. Cell-walls are now formed between the daughter-

nuclei of the middle and upper tier, but not between the free nuclei of the upper tier. The result of this division is shown in Fig. 57. We have now in the pro-embryo two tiers of cells and one tier of free nuclei. The cytoplasm of the cells is very densely granular, containing an abundance of starch. It stains very deeply, and presents a very sharp contrast to the clear cytoplasm of the archegonium above, in which the tier of free nuclei lie embedded.

Arnoldi's ('01) account of the development of the pro-embryo in *Cryptomeria* is rather meagre, but the four stages which he figures agree with my own observations. With the exception of the first two divisions, Coker's ('03) account of the pro-embryo of *Taxodium* agrees very closely with that here given for *Cryptomeria*.

The next stage in the development of the embryo is the elongation of the middle tier of cells. The development of the suspensors from these cells was followed very closely; many preparations showing all the essential stages in this formation. Fig. 58 shows that cells of the middle tier have become much longer than the original archegonium, and as they grow, they carry the lower tier of cells downward through the tissue of the prothallium. At first the suspensors elongate in a straight line, but, as shown in Fig. 58, they soon become more or less curved. As they increase in length, this curvature becomes more marked, until they assume a distinct winding form. This curvature and winding in and out of the suspensors may be explained on the assumption that the rate of growth of the cells is very much greater than the rate with which they can be forced through the solid cellular tissue of the prothallium. As they wind in and out in all directions, it is impossible to observe their exact length or direction of growth in a single section. Fig. 59 represents a longitudinal section of the older suspensors with an embryo at the tip. It will be seen that a portion of the suspensors has been cut away in sectioning on account of the direction of their growth. It will also be observed that the suspensors are enormously long, and if they were stretched out their full length they would reach over half the length of the prothallium.

In the stages older than that shown in Fig. 59, it was impossible to trace back the suspensors to their point of origin. With so many suspensors from the various archegonia, all growing together and winding in and out in all directions, the upper region of the prothallium showed nothing but a confused tangle of long suspensor cells.

It was very difficult to estimate accurately the number of embryos developed from one archegonium; my preparations showed a marked variation in this respect. The suspensors from several archegonia were sometimes found growing downward close together, and in such cases it was impossible to say definitely whether a series of suspensors came from one or more archegonia. By the time the suspensors reached the stage

shown in Fig. 59, the tips of the suspensors usually separated from each other. There were generally one or two embryo-cells at the end of each suspensor. The two cells at the tip were merely the two-celled stage of the embryo proper. In no case was I able to find more than one embryo developed from a single suspensor as Coker ('03) has recently reported for *Taxodium*. One embryo was generally developed from one suspensor, but very frequently a single embryo was found at the united tips of two or sometimes three suspensors.

In the later stages, distinct embryonal tubes are developed from the cells nearest the suspensors, in much the same fashion as they do in *Taxodium* (Coker, '03). They are, however, not as numerous or as long as in *Taxodium*.

While differing in certain minor details, the development of the embryo of *Cryptomeria* and *Taxodium* is very similar. To *Sequoia*, where there are no free nuclei formed in the pro-embryo, *Cryptomeria* bears no resemblance whatever.

Summary.

The reduction-division which leads to the formation of the tetrads takes place during the latter part of October, although pollination does not take place until March of the following spring. At the time of pollination the microspore contains a tube-cell and a generative cell. Cells or nuclei representing the vegetative tissue of the male gametophyte are not formed.

There are usually four or five microspores deposited on the nucellus at the base of the micropyle. These all germinate and produce pollen-tubes, which penetrate the nucellar tissue at the apex. At the time of penetration the generative nucleus divides, so that the young pollen-tube contains the tube-, stalk-, and body-nuclei.

The body-nucleus very soon enlarges and becomes surrounded by a dense zone of cytoplasm and starch-grains. A membrane is formed at the periphery of this zone. The pollen-tube now contains one large cell and two free nuclei.

After the tip of the pollen-tube has reached the depression above the archegonium-complex, the body-cell divides and gives rise to two male cells. The two male cells when mature are spherical in form, of equal size, and both are functional. They enter separate archegonia.

There are three or four macrospore-mother-cells differentiated in the nucellus at a point just a little above the insertion of the integument. Each mother-cell divides twice, and there are consequently from twelve to sixteen macrospores formed.

Only one of the macrospores germinates and develops into the female

gametophyte. No distinct tapetum is present. Upon germination the nucleus of the macrospore divides and the spore enlarges. Free nuclear division now proceeds at a rapid rate, and the young prothallium elongates in the direction of the chalaza. There are now several vacuoles present, and the nuclei are distributed along the intervening strands of cytoplasm. The vacuoles enlarge and eventually flow together. The very large central vacuole increases enormously, and forces the cytoplasm to the wall. The prothallium now consists of a large central vacuole and a parietal layer of cytoplasm, along which the free nuclei are distributed at more or less regular intervals.

As the prothallium increases in size, the parietal layer of cytoplasm becomes thicker and the free nuclei divide. Between the daughter-nuclei thus organized delicate membranes are formed. The structures thus formed are the first cells of the prothallium, and they are open on the inner side. The nuclei at this time always occupy a position at the periphery of the cytoplasm on the side exposed to the sap of the vacuole. These primary prothallial cells now elongate and grow inward towards the centre of the vacuole. During this growth free nuclear division proceeds, and numerous cross-walls are formed, but the cells nearest the vacuole are always open on the inner side.

The primary prothallial cells soon become multinucleate, and by this inward growth eventually close up the space occupied by the sap of the vacuole. The membranes of these primary cells are very delicate and incomplete, they do not extend across the prothallium, and eventually take no part whatever in the formation of permanent cell-walls of the cellular endosperm.

The cell-walls are formed as a result of a peculiar method of free cell-formation. The manner in which these walls are formed is unlike anything that has so far been reported for endosperm-formation. Hundreds of the free nuclei divide about the same time. When the daughter-nuclei are formed at the poles of the spindle, the kinoplasmic fibrils stretching between them increase in number and curve outward on all sides. No cell-plate is formed between the daughter-nuclei. The fibrils continue to increase in number, and curve out still further. This process continues until both daughter-nuclei are completely surrounded by a sheath of fibrils. The fibrils are all at the periphery of the nearly spherical structures thus formed. By fusion together laterally, the fibrils are gradually converted into a membrane which completely encloses the two nuclei. This process of free cell-formation goes on throughout the whole of the prothallium except in the region of the archegonial initials. As hundreds of these structures are formed they become crowded, and the walls become flat where they press upon one another. Through this pressure the neighbouring membranes fuse together, and this gives the appearance of ordinary

cellular tissue. The prothallium thus passes through a stage when the majority of its cells are binucleate. After this binucleate cellular tissue has been organized, nuclear division proceeds in the usual way, and cell-plates are formed between the daughter-nuclei.

The archegonial initials make their appearance just before the prothallial tissue is thoroughly organized. They are nearly always peripherical cells. There are four neck-cells, and a ventral canal-nucleus is cut off before fertilization. The archegonia are arranged as in the Cupresseae, that is, in a single group at the apex of the prothallium. They are surrounded by a common layer of jacket-cells, but these latter are sometimes found between the archegonia. The jacket-cells are multinucleate, and their characters suggest that they may be sterile archegonia.

A single male cell enters the archegonium, and after the wall surrounding it breaks down the sex-nuclei fuse. The first segmentation-spindle is organized in the centre of the archegonium just about the place where the fusion of the sex-nuclei occurred. After the second division the four free nuclei pass to the base of the archegonium and undergo another division, but meantime the nuclei have been arranged in two tiers. Walls are now formed between the nuclei, and then the nuclei of the upper tier divide. The walls formed between these latter are at right angles to the long axis of the archegonium, but the upper tier forms no membranes between its nuclei. The embryo now consists of two tiers of cells and one tier of free nuclei. The middle tier develops into long, tortuous suspensors, which carry down the embryo cells at their tips. There may be one or several embryos developed from a single archegonium.

As near as could be estimated, there are nine or ten chromosomes in the nucleus of the gametophyte, and eighteen or twenty in the sporophyte.

The gametophytes and embryo of *Cryptomeria* are distinctly of the Cupresseae type.

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EXPLANATION OF FIGURES IN PLATES XXVII–XXX.

Illustrating Dr. Lawson's paper on *Cryptomeria*.

All the figures were drawn with the aid of the camera lucida. The following oculars and objectives were used :—

Figs. 1, 2, 3, 4, 5, 6, 12, 13, 14, 35, 41, 42, 45, Zeiss oc. 1, obj. oil imm. $\frac{1}{12}$.
Fig. 8, Zeiss oc. 3, obj. 7.
Figs. 9, 10, 11, 15, 39, 40, 59, Zeiss oc. 2, obj. 3.
Figs. 7, 16, 17, 18, Zeiss oc. 3, obj. 3.
Figs. 19, 20, 21, 22, 25, 26, 27, 28, 29, 30, 31, 32, Zeiss oc. 3, obj. oil imm. $\frac{1}{12}$.
Figs. 23, 34, 36, 37, 38, 43, 44, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, Zeiss oc. 4, obj. 7.
Figs. 24, 33, Zeiss oc. 5. obj. 7.
Figs. 46, 47, 48, Zeiss oc. 5, obj. oil imm. $\frac{1}{12}$.

Fig. 1. A cross-section of a microspore soon after the separation of the tetrads. Nov. 1, 1903. × 1000.

Fig. 2. A microspore showing the hook-like projection. Drawn from living spore. Dec. 1, 1903. × 1000.

Fig. 3. A section of a microspore soon after it reaches the apex of the nucellus. March 7, 1903. × 1000.

Fig. 4. The body-cell *t.*, stalk-nucleus *s.n.*, and tube-nucleus *t.n.* as they are situated at the tip of the pollen-tube. June 2, 1903. × 1000.

Fig. 5. The body-cell undergoing division with the stalk-nucleus situated just below it. June 9, 1903. × 1000.

Fig. 6. The two male cells just separating from each other. June 5, 1903. × 1000.

Fig. 7. A longitudinal section of the apex of the nucellus, showing at least four pollen-tubes penetrating the nucellar tissue. May 26, 1902. × 150.

Fig. 8. A longitudinal section of a pollen-tube with the tip just about to discharge its contents in the depression above the archegonium-complex. June 2, 1902. × 300.

Fig. 9. A longitudinal section of a young megasporangium and integument, showing the method of closing of the micropyle. March 7, 1903. × 170.

Fig. 10. A longitudinal section of two young megasporangia within a single integument. March 7, 1903. × 170.

Fig. 11. A longitudinal section of a megasporangium showing the position of the sporogenous cells. March 7, 1903. × 170.

Fig. 12. From a longitudinal section of a megasporangium showing four megaspore-mother-cells. March 7, 1903. × 1000.

Fig. 13. The spindle of the reduction-division of the megaspore-mother-cell. The daughter-nuclei are already organized at the poles. March 7, 1903. × 1000.

Fig. 14. A longitudinal section showing at least fourteen megaspores. The large centrally situated spore is probably the only one that germinates. April 6, 1903. × 1000.

Fig. 15. A longitudinal section of the nucellus, showing a large single embryo-sac with numerous free nuclei. May 12, 1902. × 170.

Fig. 16. A longitudinal section of a young female prothallium, showing the large central vacuole and the parietal layer of cytoplasm, in which numerous free nuclei are imbedded at more or less regular intervals. May 26, 1902. × 150.

Fig. 17. The same at a later stage. The free nuclei in the parietal layer of cytoplasm have divided and delicate membranes formed between them. The primary cells thus organized are open on the side towards the vacuole. The nuclei are arranged at the periphery of the cytoplasm and the latter is exposed to the sap of the vacuole. May 29, 1903. × 150.

Fig. 18. The same at a little older stage, showing the inward growth of the primary prothallial

cells. Numerous cross-walls have already formed, but the cells that extend as far as the vacuole are still open on the inner side. The nuclei in these cells still retain their position at the periphery of the cytoplasm exposed to the fluid of the vacuole. May 26, 1902. × 150.

Fig. 19. A longitudinal section of the parietal layer of cytoplasm from the stage shown in Fig. 16, but at a much higher magnification. May 26, 1903. × 1500.

Fig. 20. The same at a little later stage, where the nuclei have just divided. Delicate membranes are formed between the daughter-nuclei, and the primary cells or 'alveoli' are thus organized. They are open on the side exposed to the vacuole. May 29, 1903. × 1500.

Fig. 21. The same at a later stage, showing that cross-walls are formed at this early stage. May 26, 1902. × 1500.

Fig. 22. A section of the parietal layer of cytoplasm taken parallel with the inner exposed surface, showing how the walls are formed separating the nuclei. May 29, 1903. × 1500.

Fig. 23. A longitudinal section of the upper end of the prothallium, showing the multinucleate nature of the primary prothallial cells, and also that the walls of these cells are incomplete. May 26, 1902. × 350.

Fig. 24. A portion of a longitudinal section of the prothallium, showing the process of free cell-formation. May 29, 1903. × 480.

Fig. 25. One of the free nuclei undergoing division and preparing for free cell-formation. May 29, 1903. × 1500.

Fig. 26. The same at a later stage. May 29, 1903. × 1500.

Fig. 27. A still later stage of the same, showing the formation of the kinoplasmic fibrils between the daughter-nuclei. May 29, 1903. × 1500.

Fig. 28. The same, showing how the kinoplasmic fibrils curve outward. May 29, 1903. × 1500.

Fig. 29. The same at a little later stage. May 29, 1903. × 1500.

Fig. 30. The two daughter-nuclei completely enclosed by the kinoplasmic fibrils. May 29, 1903. × 1500.

Fig. 31. A section taken at right angles to the long axis of the spindle of the stage shown in Fig. 30. The cross-sections of the kinoplasmic fibrils appear as small dots. By fusing together laterally, these fibrils form a membrane. May 29, 1903. × 1500.

Fig. 32. A section taken same as in Fig. 31. The two nuclei cannot be seen at the same focus. May 29, 1903. × 1500.

Fig. 33. A portion of the prothallium soon after free cell-formation. The membrane formed by the lateral fusion of the kinoplasmic fibrils encloses both daughter-nuclei, so at this time the cells of the prothallium are binucleate. May 29, 1903. × 480.

Fig. 34. A longitudinal section of the apex of the young prothallium, showing a group of archegonial initials. May 29, 1903. × 350.

Fig. 35. A longitudinal section of the upper portion of an archegonium, showing the division of the neck-cell. The membrane between the two nuclei is not yet formed. June 5, 1903. × 1000.

Fig. 36. A longitudinal section of an archegonium showing the large egg-nucleus *e.*, and the small ventral canal-nucleus *v.c.* June 1, 1903. × 350.

Fig. 37. A longitudinal section of a typical mature archegonium ready for fertilization. June 1, 1903. × 350.

Fig. 38. A cross-section through the necks of the archegonia, showing four distinct neck-cells in each neck, and also the clefts between these cells. June 2, 1902. × 350.

Fig. 39. A longitudinal section of the upper portion of the prothallium, showing the typical way in which the archegonia are grouped. The jacket-cells surround the entire group, but occasionally run up between the archegonia. June 1, 1903. × 170.

Fig. 40. A cross-section of the prothallium through the region of the nuclei of the archegonia, showing the way in which the latter are grouped. There are twelve archegonia shown in the section. June 7, 1903. × 170.

Fig. 41. A section of the central nucleus preparing for the division which gives rise to the ventral canal-nucleus. June 2, 1902. × 1000.

Fig. 42. A group of jacket-cells, showing their multinucleate character. June 9, 1903. × 1000.

Fig. 43. An archegonium showing the male cell just after it has penetrated. The latter fills the entire upper portion of the archegonium and the membrane surrounding it is still intact. June 1, 1903. × 350.

Fig. 44. An archegonium showing the fusion of the male and female nuclei. The nuclear membrane between the two persists for some time. June 5, 1903. × 350.

Fig. 45. A later stage of the same, showing that the female nucleus almost completely envelops the male before the membrane between them breaks down. The structure of the chromatin of the two nuclei are very similar at this time. June 1, 1903. × 1000.

Fig. 46. The first segmentation-spindle surrounded by a dense zone of starch-granules. June 9, 1903. × 2000.

Fig. 47. A spindle of the second division of the pro-embryo, showing the large number of long V-shaped chromosomes on their way to the poles. June 9, 1903. × 2000.

Fig. 48. A spindle of the division of one of the free nuclei of the endosperm, showing that there is clearly half the number of chromosomes in the gametophyte that there is in the sporophyte as shown in Fig. 47. May 29, 1903. × 2000.

Fig. 49. An archegonium showing the fusion-nucleus surrounded by a zone of starch-granules. June 9, 1903. × 350.

Fig. 50. An archegonium showing two free nuclei; the result of the first division of the fusion-nucleus. June 9, 1903. × 350.

Fig. 51. An archegonium showing the result of the second division after fertilization. The pro-embryo now consists of four free nuclei. June 9, 1903. × 350.

Fig. 52. An archegonium showing six free nuclei in the pro-embryo. June 9, 1903. × 350.

Fig. 53. An archegonium showing four nuclei of the pro-embryo. Two of the nuclei have settled at the base of the archegonium. The zone of starch is carried to the base with the nuclei. June 9, 1903. × 350.

Fig. 54. The base of an archegonium showing four free nuclei lying in a sharply differentiated starch area. Kinoplasmic fibrils radiate out from the nuclei preparatory to forming the membranes between the latter. June 9, 1903. × 350.

Fig. 55. A later stage than Fig. 54, showing that the first membranes between the free nuclei of the pro-embryo are formed parallel to the long axis of the archegonium. June 9, 1903. × 350.

Fig. 56. A later stage of the same, showing that the second series of membranes is formed at right angles to the long axis of the archegonium. June 9, 1903. × 350.

Fig. 57. A later stage of the same, showing that the embryo now consists of two tiers of well-defined cells and one tier of free nuclei. June 29, 1903. × 350.

Fig. 58. A later stage, showing that the middle tier of cells has developed into a series of long suspensors, which carry the lower tier, or embryo proper, down through the endosperm. June 7, 1903. × 350.

Fig. 59. A later stage, showing the long tortuous suspensors with the embryo at the tip. June 25, 1902. × 170.

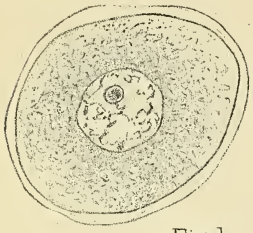


Fig. 1.

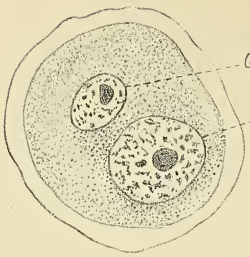


Fig. 3.

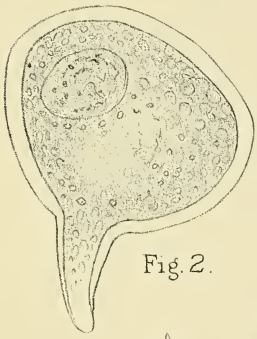


Fig. 2.

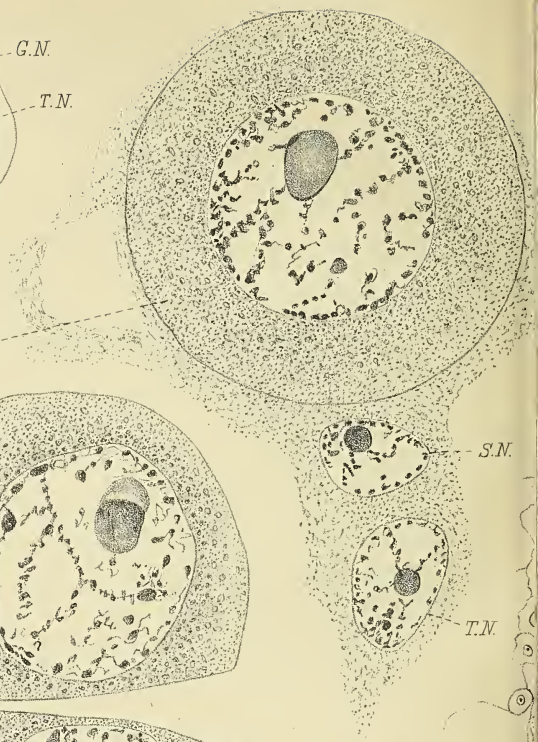


Fig. 4.

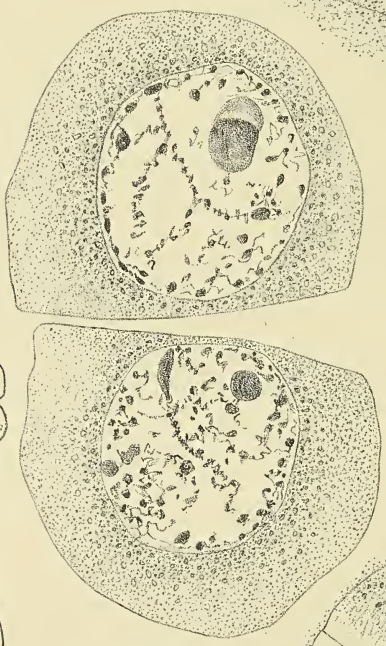


Fig. 6.

Fig. 7.

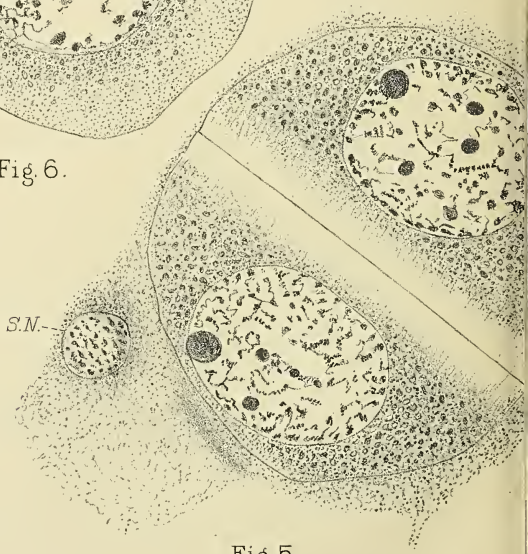


Fig. 5.

A. A. Lawson del.

LAWSON.—CRYPTOMERIA JAPONICA.

Fig. 8.

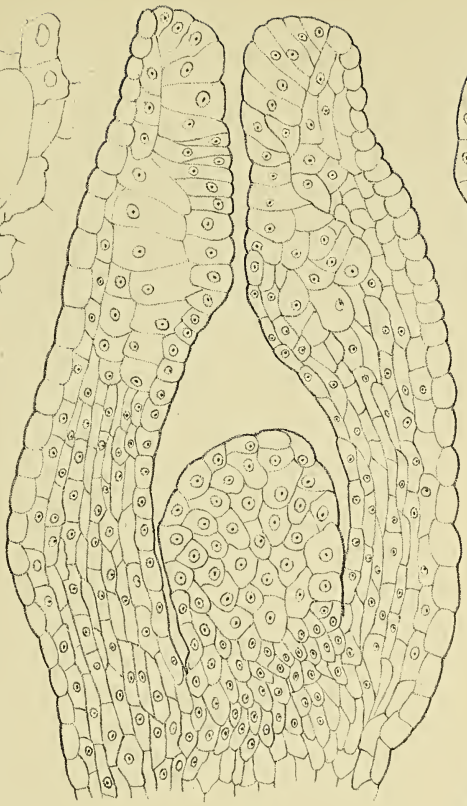
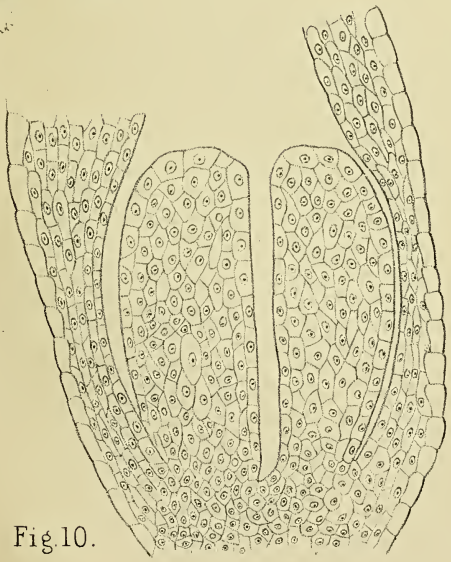


Fig. 9.

Fig. 11.

Fig. 13.



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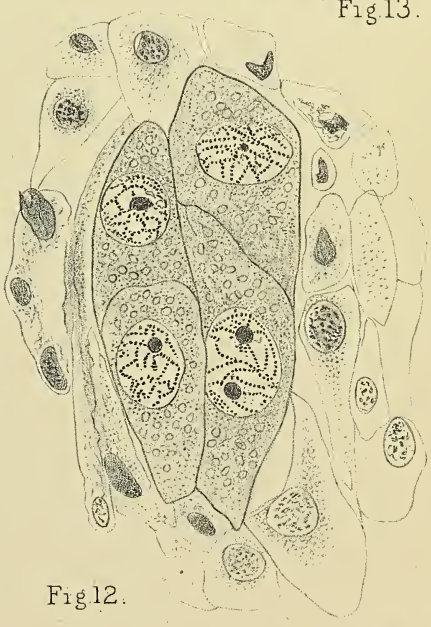

Fig. 12.

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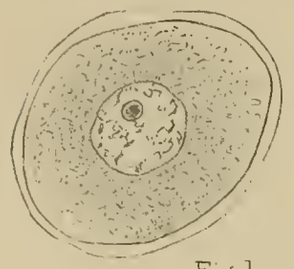


Fig. 1.

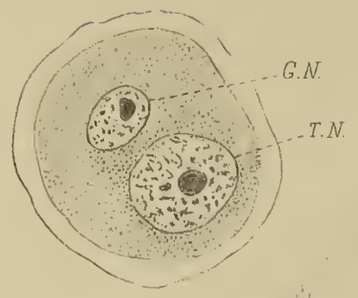


Fig. 3.

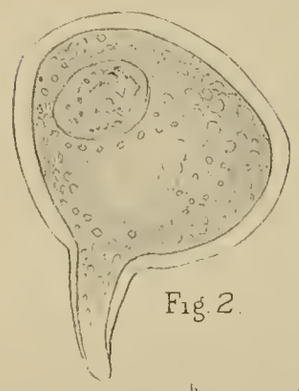


Fig. 2.

Fig. 4.

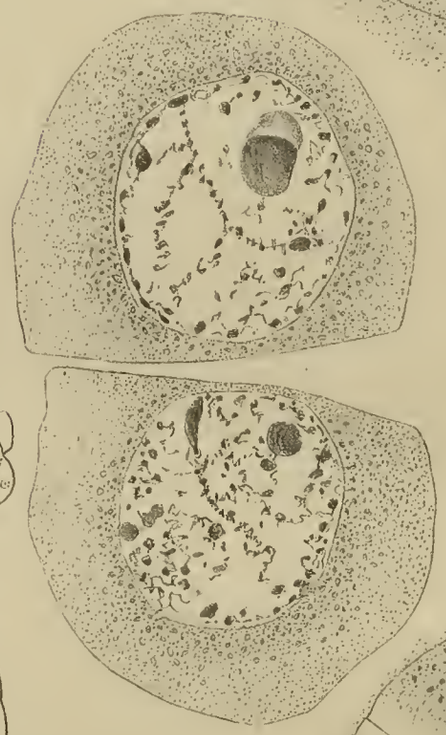
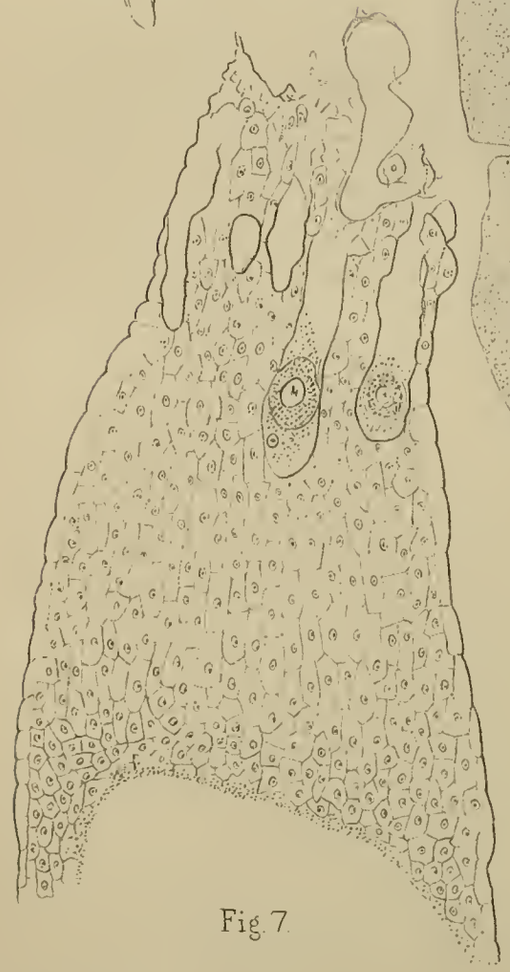


Fig. 6.



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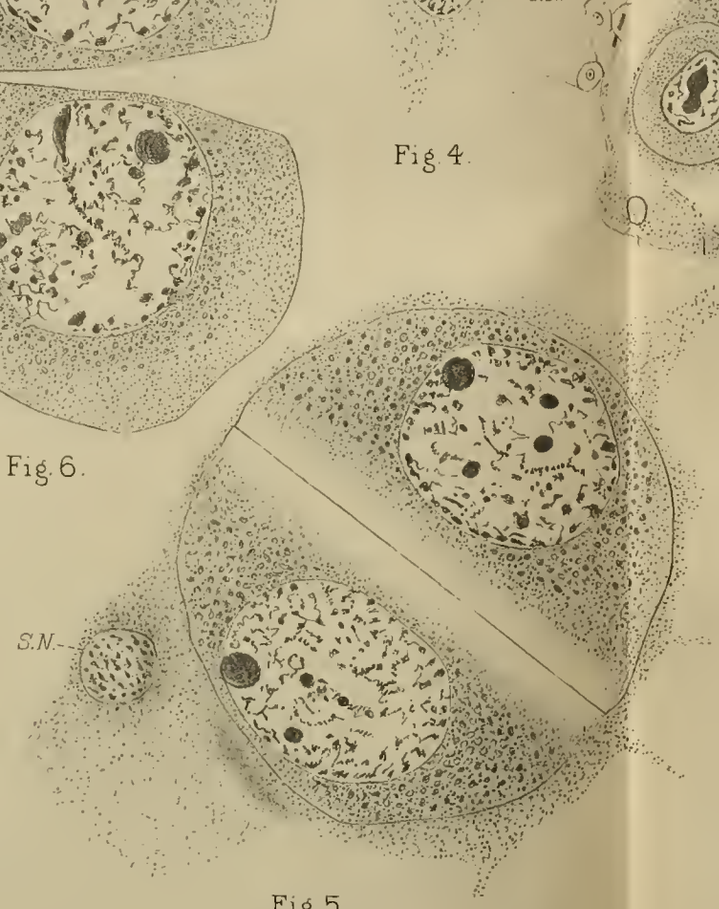


Fig. 5.

Fig. 8.

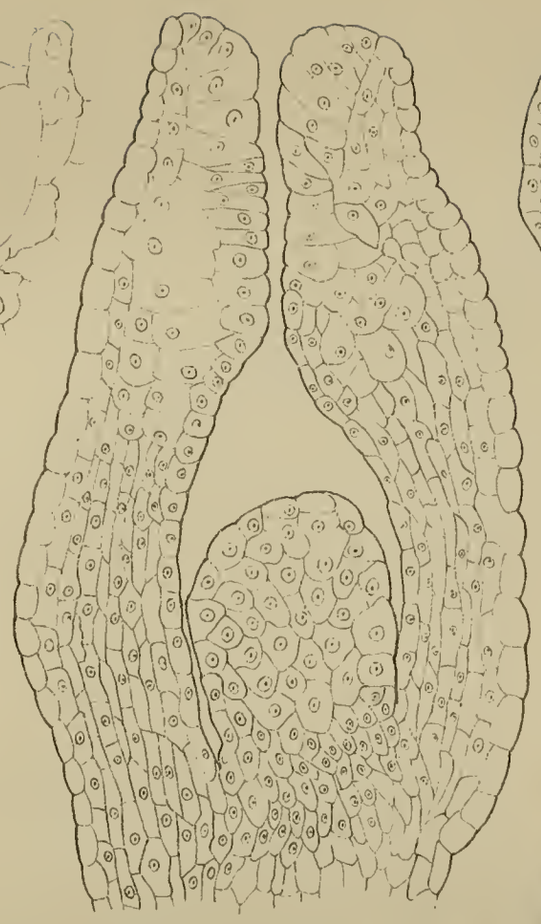
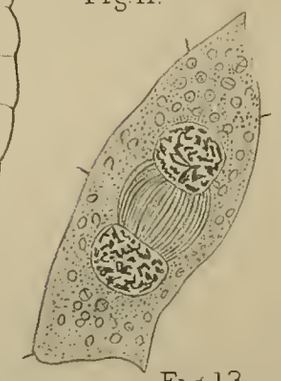


Fig. 9.

Fig. 11.



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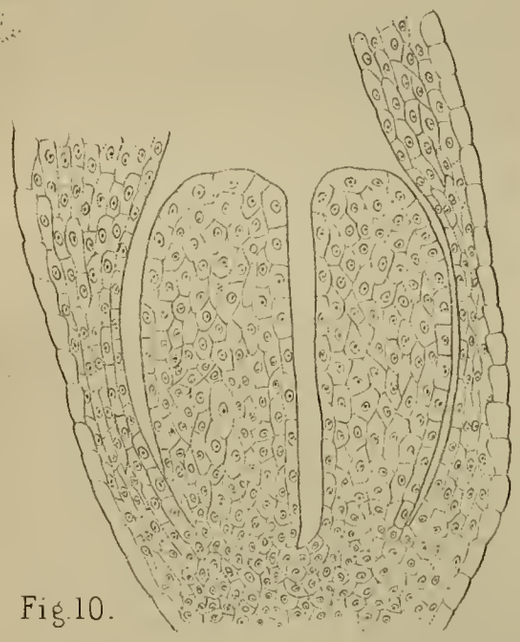

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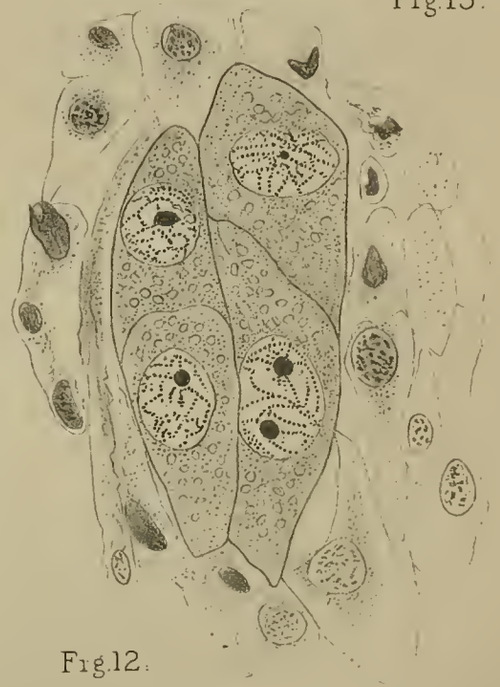

Fig. 12.

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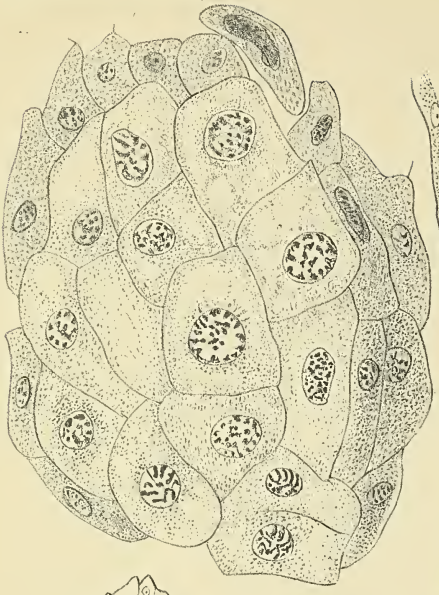

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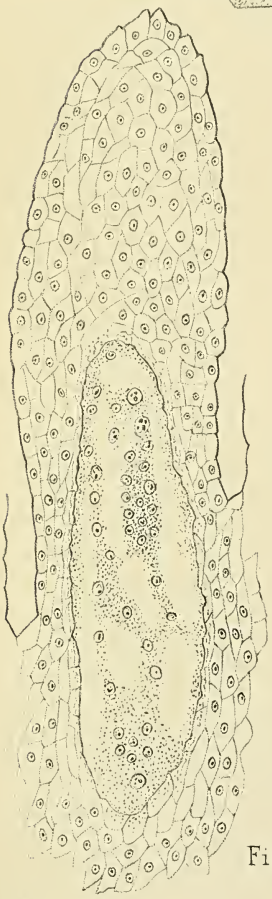

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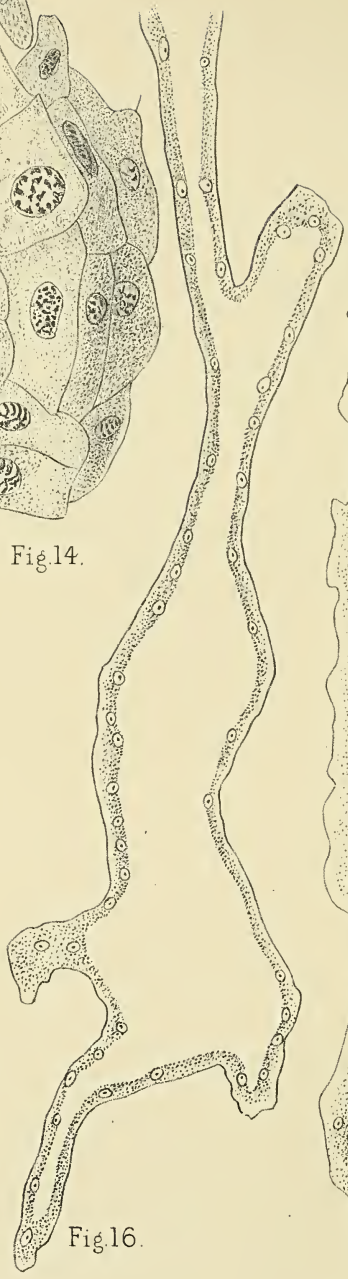

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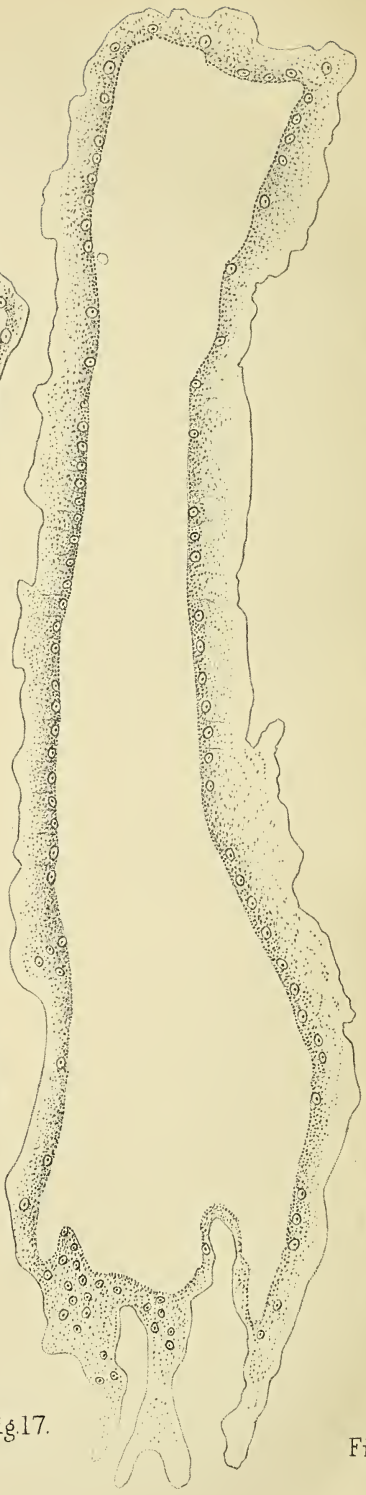

Fig.17.

Fig.18.

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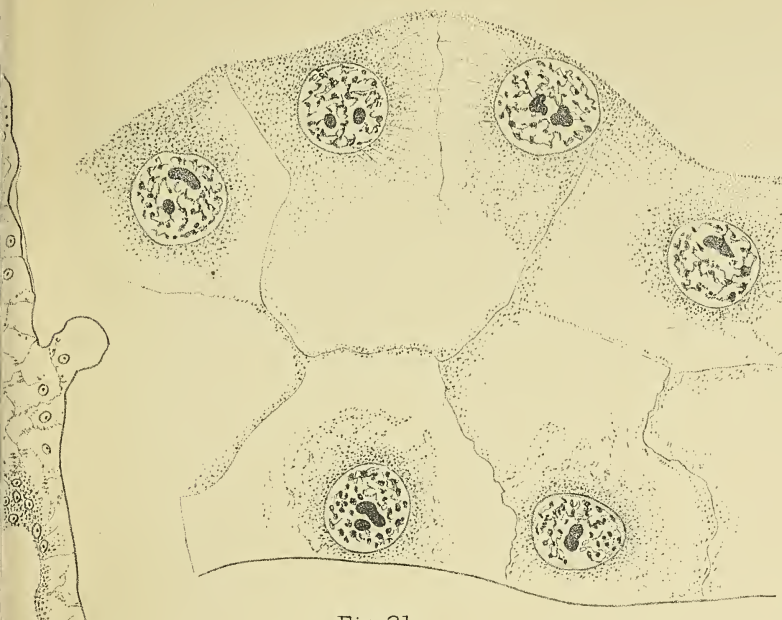

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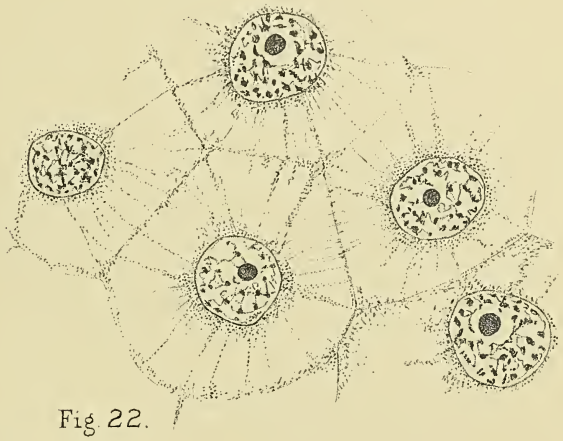

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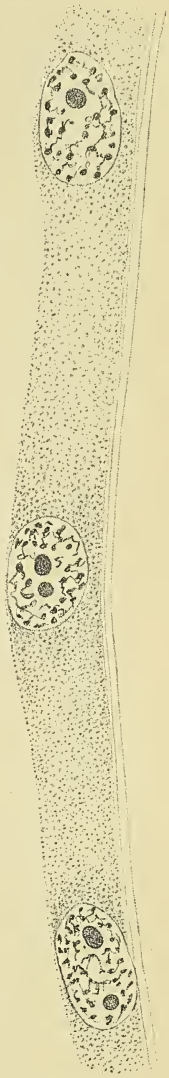

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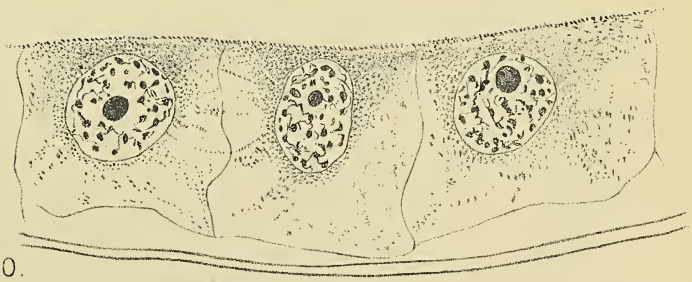

Fig. 20.

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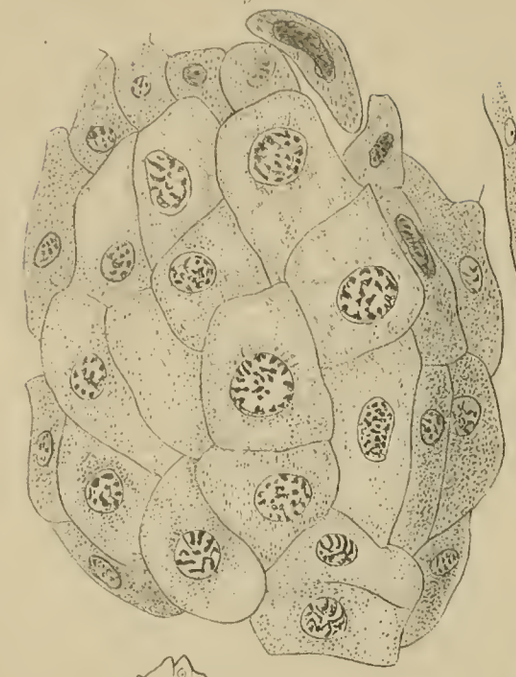
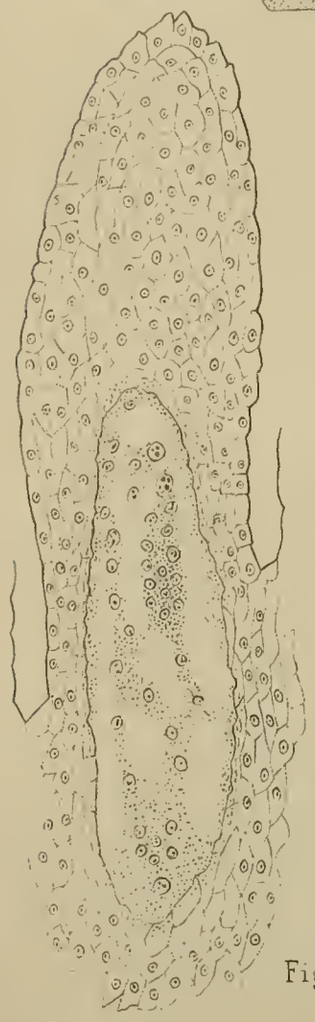


Fig. 14.



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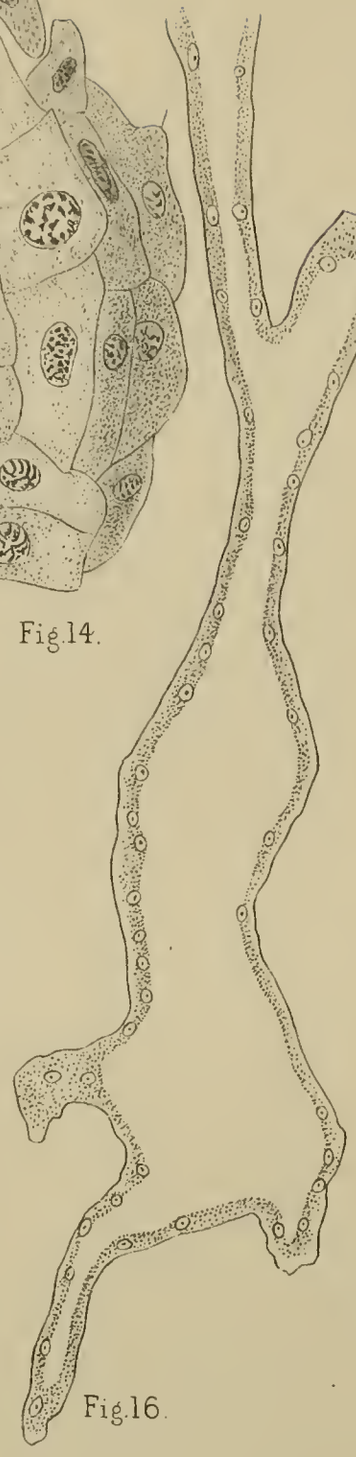

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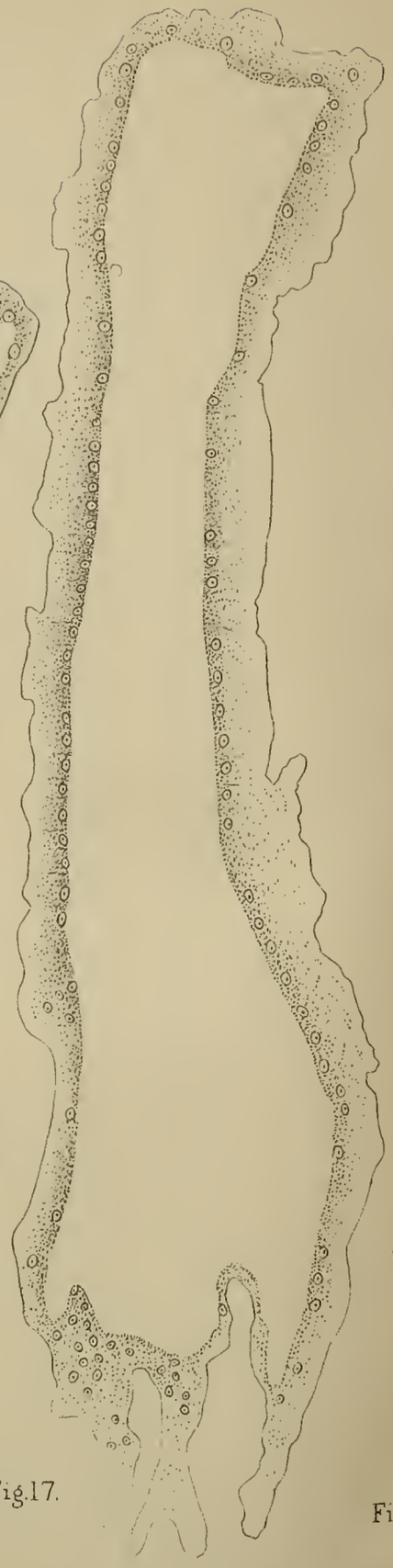

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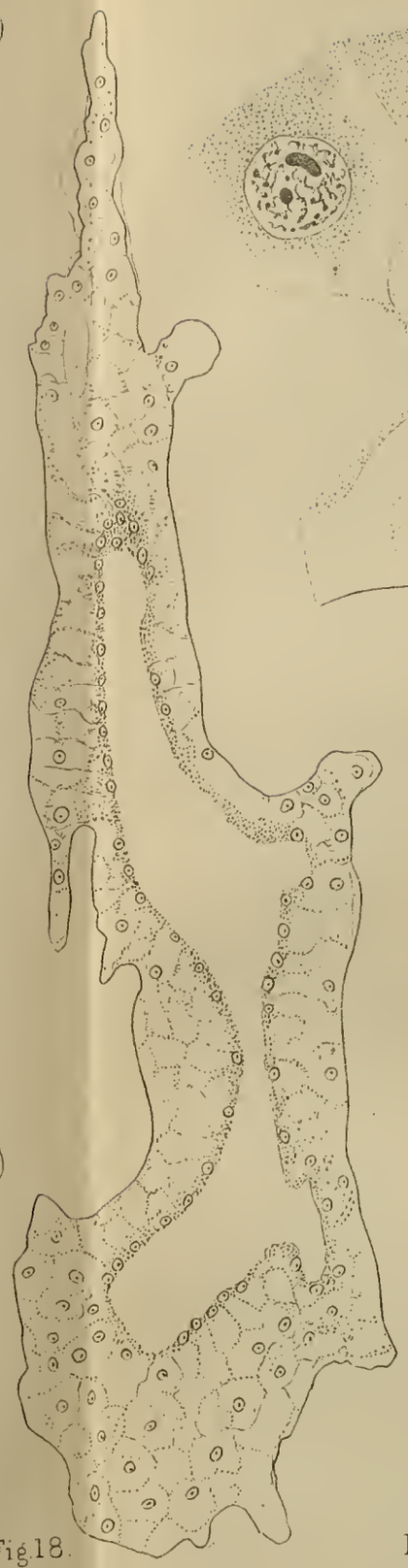

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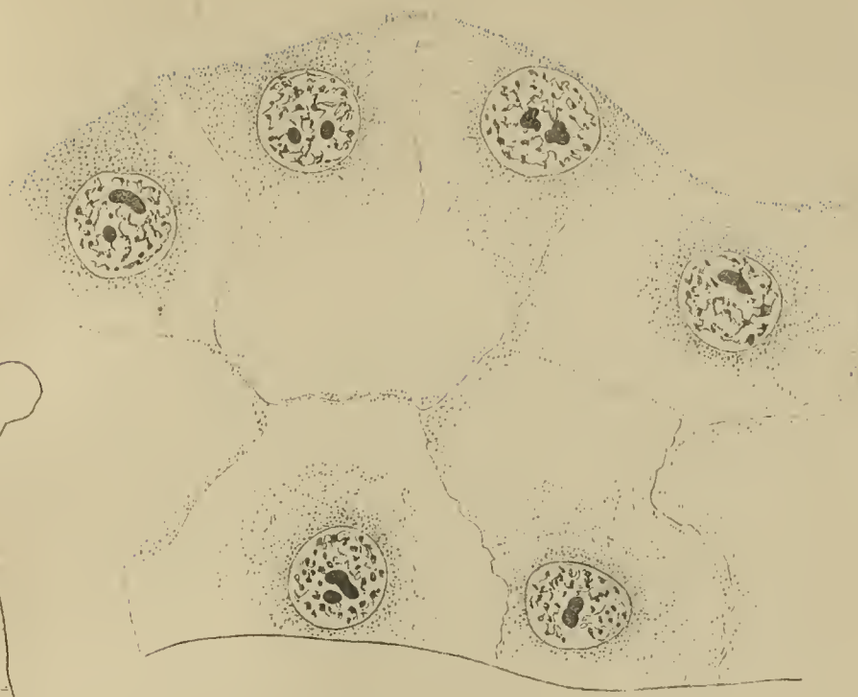

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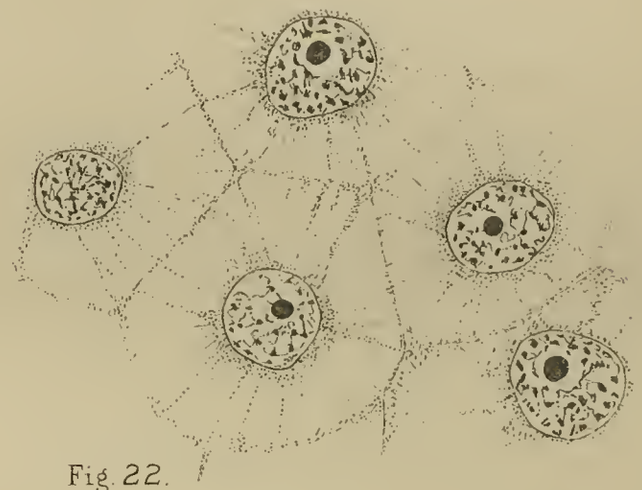

Fig. 22.

Fig. 19.

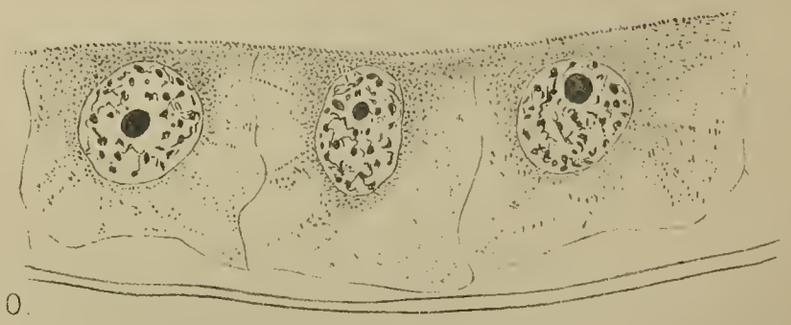

Fig. 20.

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Fig. 23.

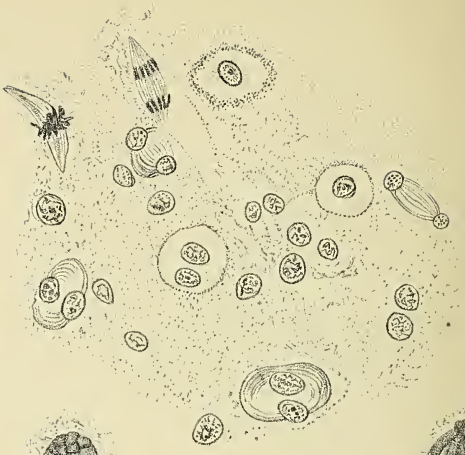

Fig. 24.

Fig. 26.

Fig. 25.

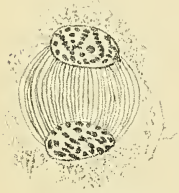

Fig. 28.

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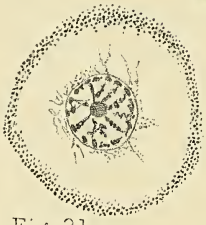
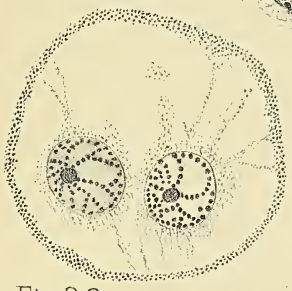


Fig. 31.

Fig. 34.



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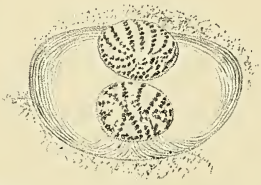
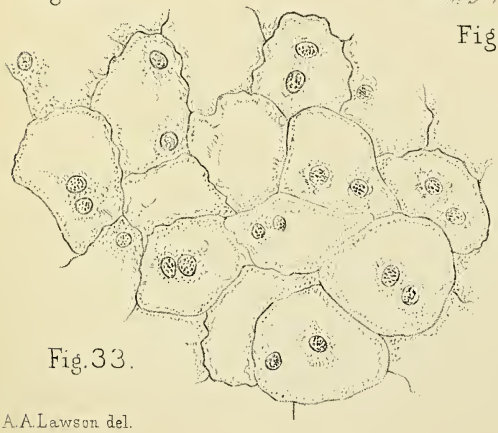


Fig. 30.

Fig. 27.



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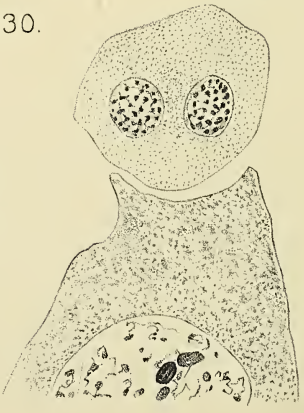

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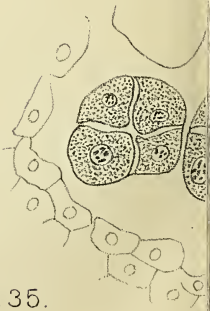

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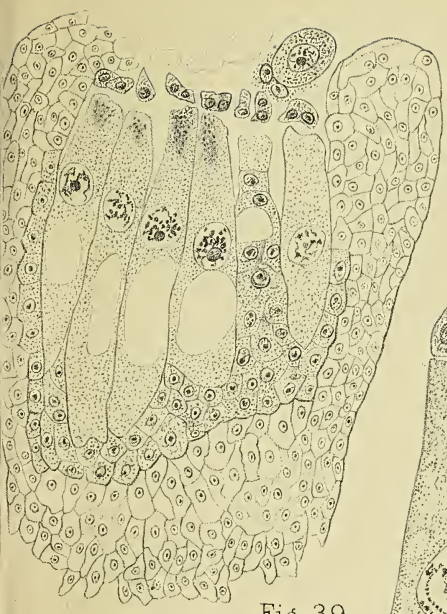

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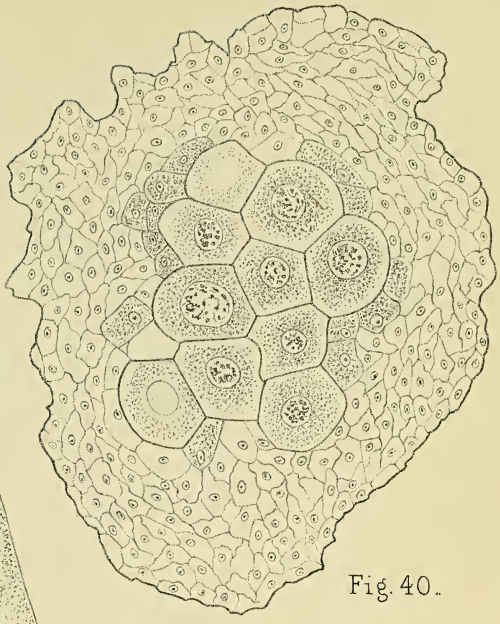

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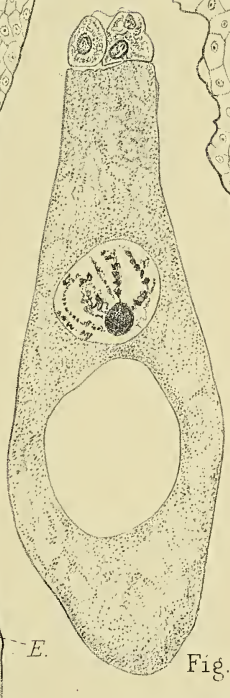
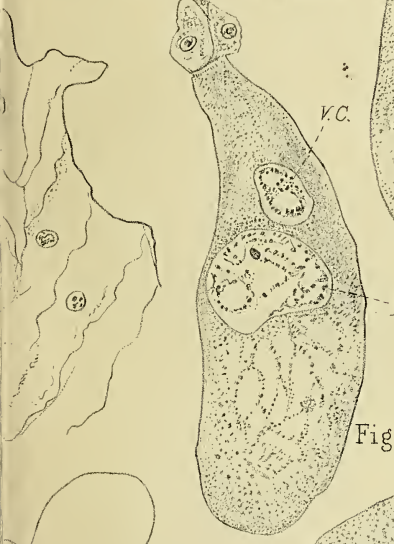


Fig. 37.

Fig. 41.



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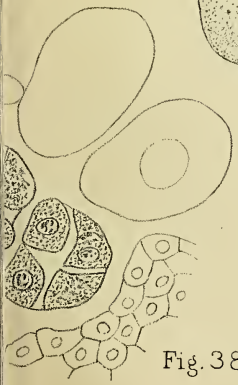

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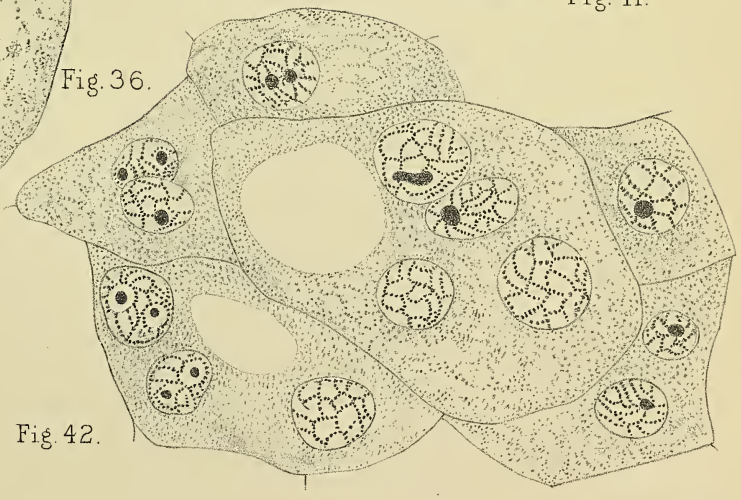

Fig. 42.

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Fig. 23.

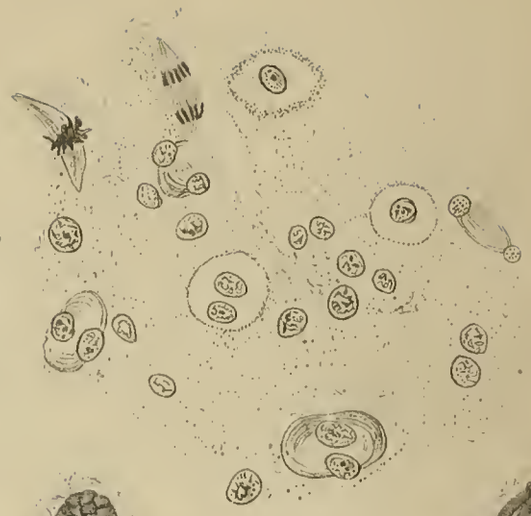


Fig. 24.

Fig. 26.

Fig. 25.

Fig. 39.

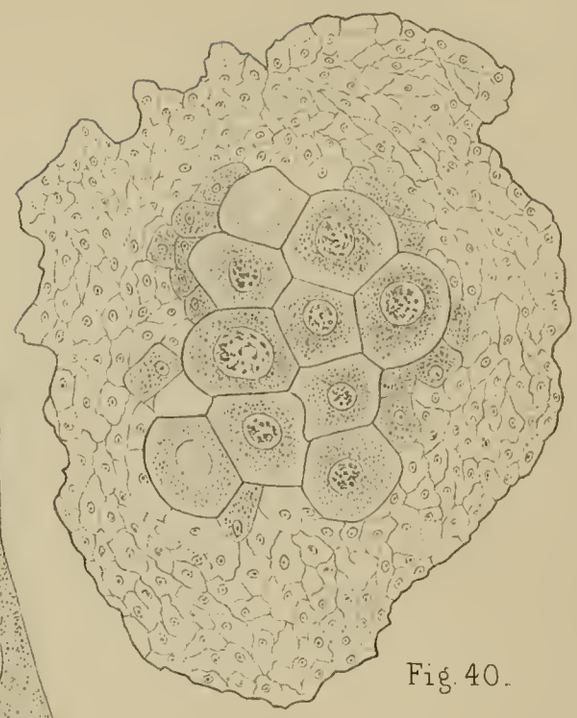


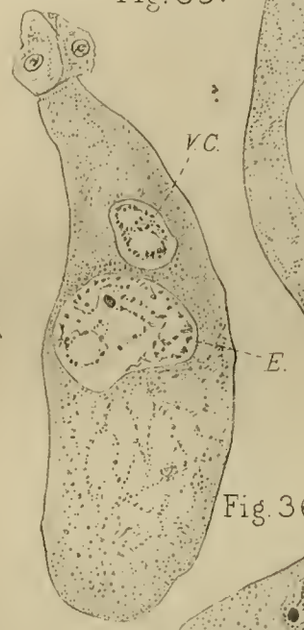
Fig. 40.

Fig. 28.

Fig. 29.

Fig. 31.

Fig. 34.



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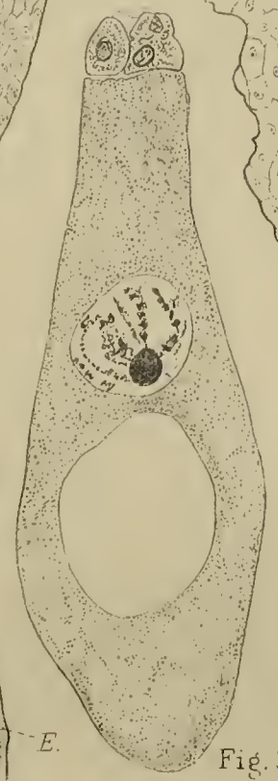


Fig. 37.

Fig. 41.

Fig. 32.

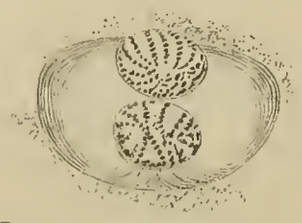
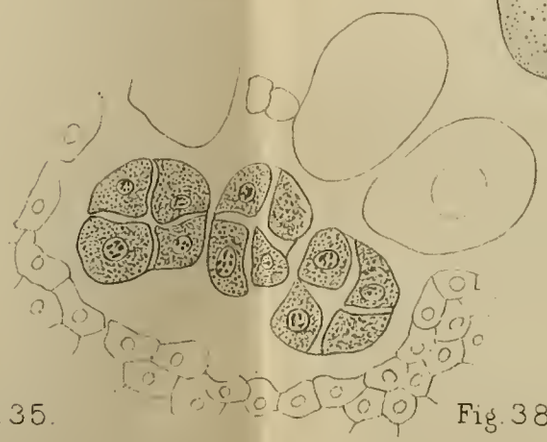


Fig. 30.

Fig. 27.

Fig. 33.

Fig. 35.



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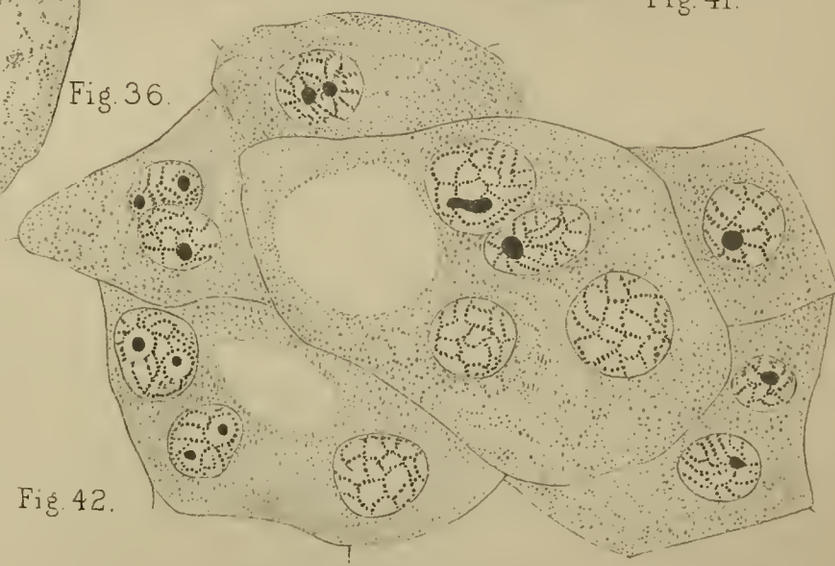

Fig. 42.

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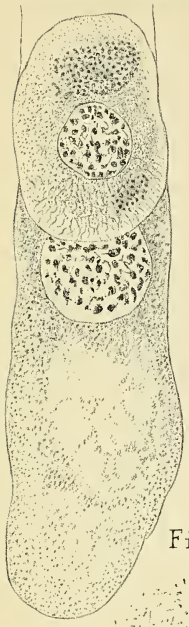

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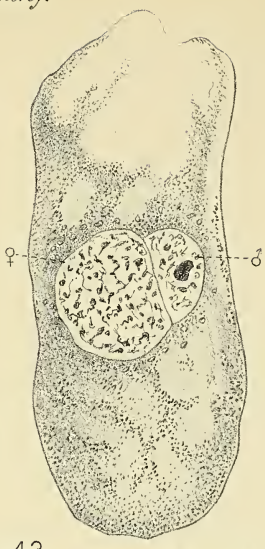
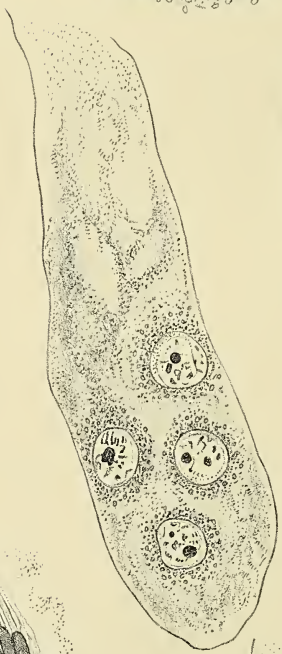


Fig. 44.

Fig. 46.

Fig. 45.



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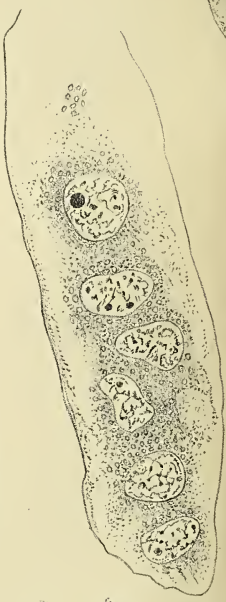

Fig. 5

Fig. 47.

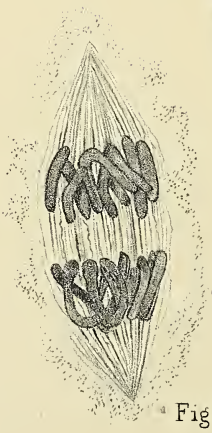

Fig. 48.

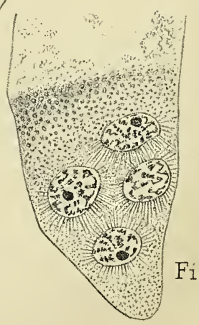

Fig. 54.

A.A.Lawson del.

LAWSON.– CRYPTOMERIA JAPONICA.

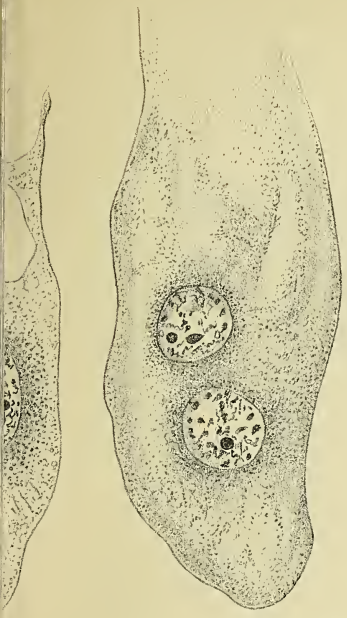

Fig. 50.

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Fig. 53.

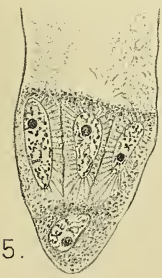

Fig. 55.

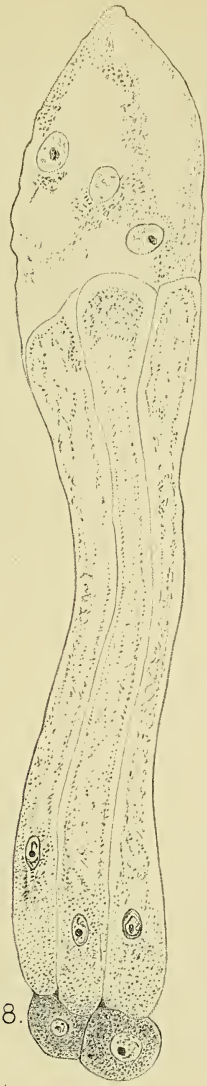

Fig. 58.

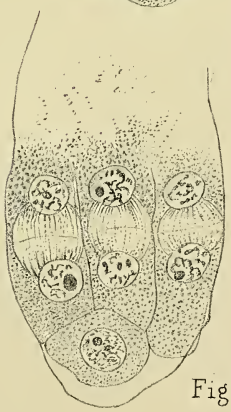

Fig. 56.

Fig. 59.

Fig. 57.

Huth lith. et imp.

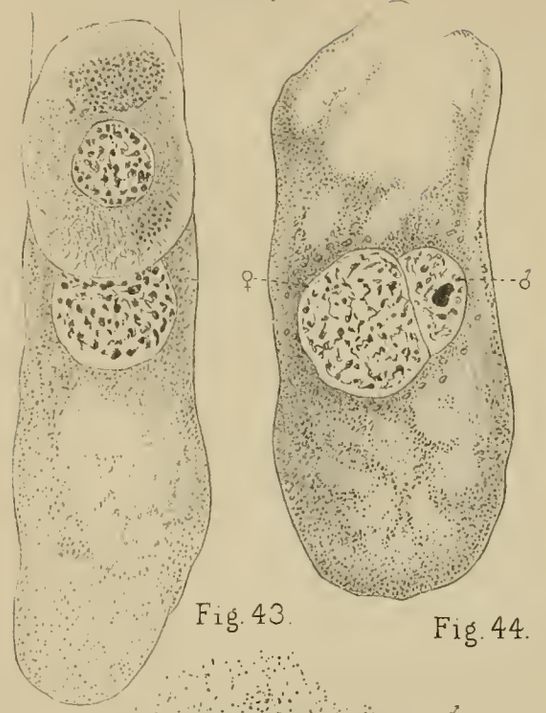

Fig. 43.

Fig. 44.

Fig. 46.

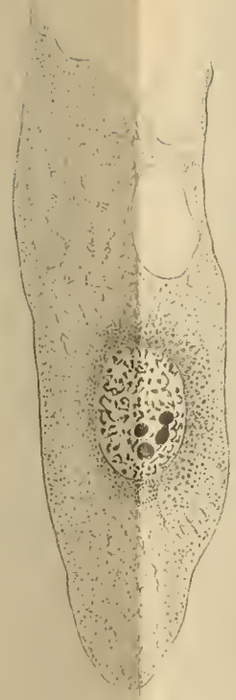

Fig. 49.

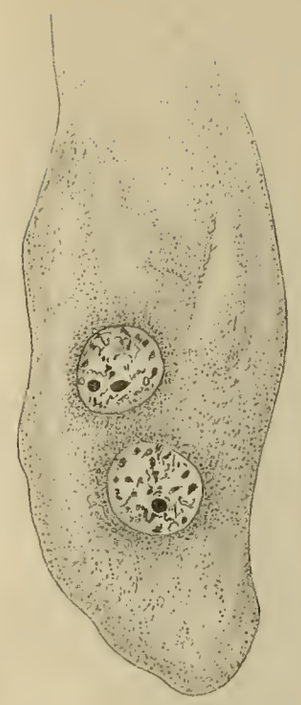

Fig. 50.

Fig. 58.

Fig. 59.

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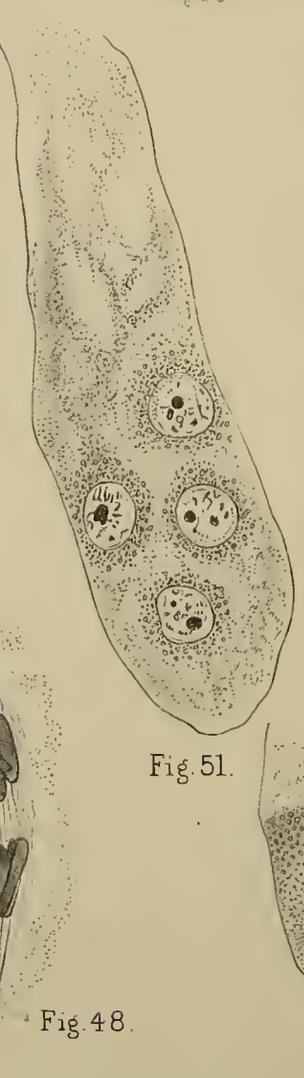

Fig. 51.

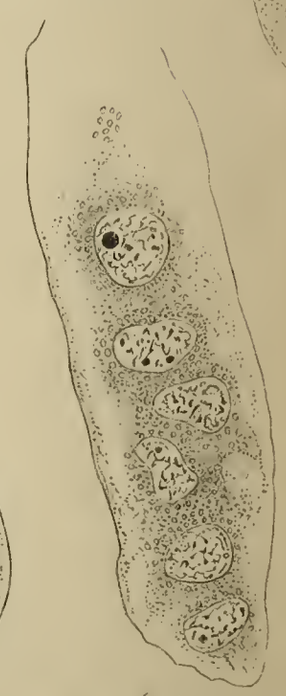

Fig. 52.

Fig. 48.

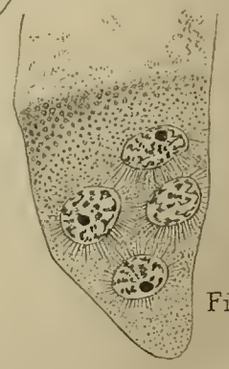

Fig. 54.

Fig. 53.

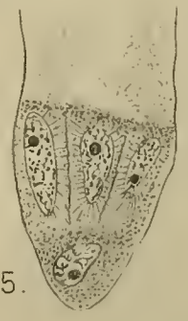

Fig. 55.

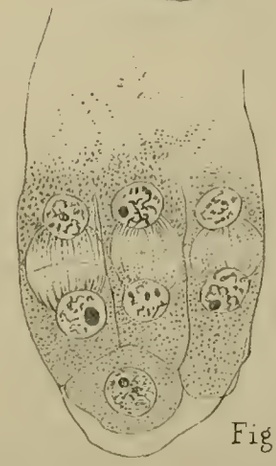

Fig. 56.

Fig. 57.

A.A. Lawson del.

Huth lith. et imp.

LAWSON.– CRYPTOMERIA JAPONICA.

Spore-Formation in Leptosporangiate Ferns [1].

BY

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With Plate XXXI and a Figure in the Text.

PREVIOUS accounts of the formation of the spores in Ferns differ from one another as regards essential features; so that an independent examination was undertaken as a preliminary to work upon the nuclei of the gametophyte generation.

The investigation recently made by Farmer and Moore [2] into the reduction-phenomena of certain plants and animals led to a new interpretation of the processes undergone in the formation of the chromosomes at the heterotype division, indicating the occurrence of a transverse fission of the chromosomes at that division. A more extended examination of the Leptosporangiate Ferns was therefore made, with results which I think leave no doubt that the essential features of the phenomena described by Farmer and Moore occur throughout this group of plants.

The species which I have examined are *Pteris tremula*, *Scolopendrium vulgare*, *Asplenium marinum*, the so-called hybrid between *Scolopendrium vulgare* and *Asplenium Ceterach*, *Onoclea sensibilis*, *Davallia capense*, *Fadyenia prolifera* (all included among the Polypodiaceae); *Alsophila excelsa* and *Dicksonia davallioides* (Cyatheaceae). The processes of spore-formation are exactly similar in all these types, except as regards the number of chromosomes present [3].

1. Earlier Work upon Spore-Formation in Ferns.

Calkins [4], working upon *Pteris tremula* and *Adiantum cuneatum*, described a process of tetrad ('Vierergruppen') formation at the heterotype division, very similar to that observed in various animals by several authors [5]. From this he drew the conclusion that a transverse division of the chromosomes, involving a qualitative reduction, took place in the Ferns.

[1] An early account of the results given here appeared in the *Roy. Soc. Proc.* vol. lxxiii. p. 86 (Feb. 4, 1904).

[2] Farmer and Moore, '03.

[3] See p. 447.

[4] Calkins, '97.

[5] See Wilson, '02, p. 246 et seq.

Stevens[1], using *Scolopendrium vulgare*, *Cystopteris fragilis* and *Pteris aquilina*, described the division of the chromosomes by two longitudinal fissions—the first provided for by the longitudinal fission of the spireme thread in the spore-mother-cell, the second provided for by an exactly similar fission of the chromatin thread of the daughter-nuclei. Strasburger[2] suggested that the process in these Ferns is the same as that in *Osmunda*, where tetrad-like bodies occur; and recognized Stevens's mistake in passing over the early appearance of the longitudinal fission which provides for the second (homotype) mitosis. In the essential point his view agrees with that of Stevens in indicating the absence of a qualitative reduction-division of the chromosomes.

2. Methods.

Fresh material was teased and examined after treatment with either acetic iodine green, acetic methyl green or acetic carmine.

The fixing reagents used in the earlier part of the work were: (1) absolute alcohol; (2) Hermann's platino-acetic-osmic; (3) Flemming's weak solution (chrom-osmic-acetic), used both cold and hot; (4) Merkel's chromo-platinic solution; (5) 1 to 2 % of chromic acid in water. Of these, the last proved the most satisfactory. During the later part of the work a mixture was used, consisting of two parts of absolute alcohol to one part glacial acetic acid. Small pieces of material placed in this for from 15 to 20 minutes gave excellent results.

From absolute alcohol the material was transferred to a mixture of alcohol and xylol, and thence, after a few minutes, to pure xylol, in which it was allowed to remain for periods varying from 15 minutes to 24 hours. Two changes of paraffin were used, the time allowed for penetration ranging from 2½ hours up to 2 days.

The sections varied in thickness from 5 to 20 μ.

The stains found most useful were: (1) Heidenhain's iron-alum-haematoxylin; (2) carbolic fuchsin and Licht Grün; and (3) Flemming's triple stain (aniline-safranin, gentian violet, orange G).

The best results were obtained from material preserved in acetic-absolute, washed for about ½ hour in each of four changes of redistilled absolute alcohol, transferred to xylol for 15 minutes and allowed to remain for from 2½ to 4 hours in melted paraffin[3].

The outlines of the nuclear structures were much sharper in material prepared in this way and stained with Heidenhain's haematoxylin, than in material which had been subjected to the prolonged process of embedding.

[1] Stevens, '98.

[2] Strasburger, '00, p. 79.

[3] I should like to take this opportunity of acknowledging my indebtedness to Professor Farmer for the advice as to methods which he has so kindly given me.

3. Observations.

The chromosomes of the somatic mitoses are so crowded that it is not easy to determine their exact number. In the species of the Polypodiaceae which were examined it is possible to count about 60 chromosomes; no evidence was obtained of the occurrence of a number approaching that given by Calkins for *Pteris tremula* and *Adiantum cuneatum* (120–130). The reduced number of chromosomes is 32; there is therefore a strong presumption that 64 chromosomes are present in the somatic mitoses, as stated by Stevens. In *Alsophila* the number of chromosomes is larger, the reduced number apparently being about 60.

After the vegetative divisions of the archesporium are complete the spore-mother-cells undergo a period of rest and growth. The first indication of their approaching division is the transformation of the reticulum of the nucleus into a much-coiled spireme thread, which at this stage is evenly distributed throughout the nucleus (Pl. XXXI, Fig. 1). The spireme now undergoes a longitudinal fission (Fig. 2); the two halves of the divided thread generally lie close to one another, but may diverge to a certain extent in some places (Fig. 7). The fission of the thread is quickly followed by its contraction towards one side of the nucleus, which is accompanied by a pulling-out of the thread into a series of loops[1] (Figs. 3, 4 and 5).

As the polarity of the spireme becomes more pronounced the limbs of each loop approach one another (Figs. 6, 7, 11–14) and in many cases become closely applied to, or even twisted upon one another. In the earlier stages of this process, the double nature of the chromatin thread is quite clear (Figs. 6–14), and is revealed even in the least satisfactory preparations if by chance the thread has been cut across (Fig. 10). In the later stages this structure becomes more obscure, but in some, at least, of the loops of any nucleus indications of the double nature of each limb can be found (Figs. 12, 13, 14).

The segmentation of the longitudinally-divided thread into chromosomes takes place in such a way that each chromosome has its origin in one of these loops[2], and thus forms a U-shaped body, the limbs of the U being twisted upon one another to varying degrees in the different chromosomes of the same nucleus (Fig. 15). The approximation towards one another of the distal ends of the limbs of each U, often resulting in the appearance of the 'ring' type of chromosome, is a common feature of the heterotype division in Ferns (Figs. 16–21).

[1] See also Calkins, l. c., Pl. 295, Fig. 3.

[2] In the contracted condition of the spireme the loops are so closely crowded together that the segmentation into chromosomes can only be clearly followed in those loops which project from the central mass.

As Farmer and Moore have pointed out [1], the increasing difficulty of recognizing the original longitudinal fission in the limbs of the chromosomes during these successive stages has led to an incorrect interpretation of their structure. The two limbs of which each chromosome consists were interpreted as being the result of the original longitudinal fission in the now very much shortened and thickened chromosomes. A similar conception led to the interpretation of the 'ring' type of chromosome as being due to the divergence of the halves into which each chromosome was separated by that fission. The examination of numerous preparations of the stages intermediate between that of the looped spireme and that of early metaphase reveals the incorrectness of this interpretation, inasmuch as the original longitudinal fission can be recognized *in each limb* of the chromosome (Figs. 17, 18, 20, 21). In the same way favourable preparations reveal the double nature of the 'ring' chromosomes, which, as has been pointed out, differ from the other forms only in the degree to which the approximation of the limbs towards one another has been carried [2].

The chromosomes may acquire a striking resemblance to the tetrads which are characteristic of the heterotype division of certain animals [3]. The resemblance becomes particularly marked at about the time when the nuclear wall breaks down (Figs. 22 and 23), and is due only to a more or less temporary splaying of the ends of the chromosomes (compare Figs. 16–23 with those of Calkins, l.c., Pl. 295, Fig. 6, I, J, K). Calkins's interpretation of the processes resulting in the tetrad-like appearance as indicating the transverse fission of the chromosomes is therefore incorrect.

The spindle-fibres are attached to the limbs of the chromosomes near the distal ends of the latter (Figs. 22, 23, 24); as the daughter-chromosomes are drawn apart the familiar ⫫-shaped figures are obtained, and the final separation takes place at a point corresponding with the apex of the original loop.

The exact time when this *transverse* fission, which separates the two limbs of each loop, takes place is not easily determined and appears to be variable. In many cases it appears to have been completed before metaphase is reached, so that the chromosomes as they move toward the equatorial plate each consist of two separate parallel rods which represent the limbs of the original U (Fig. 23) [4]. In others the separation only becomes visible later and appears to be synchronous with the commencement of the contraction of the spindle-fibres, and consequent divergence of the limbs of the chromosomes (Figs. 24, 24 *a*) [5].

During the latter part of the period just described—as the chromo-

[1] Farmer and Moore, l. c.

[2] Compare Figs. 17–21 with those of Calkins, l. c., Pl. 295, Figs. 4 and 5.

[3] Wilson, l. c., p. 246 et seq.

[4] Cf. Calkins, l. c., Pl. 295, Fig. 6, A, B.

[5] Cf. Calkins, l. c., Pl. 295, Fig. 6, I, J, K.

somes move towards the equatorial plate—a longitudinal fission becomes once more clearly apparent in each limb (Figs. 24, 24 *a*), so that, seen in face, the diverging daughter-chromosomes form a ◊-shaped body (Figs. 25, 26). This fission has been interpreted by many authors as a *second* longitudinal fission, appearing very early as a provision for the second maturation division.

In the small chromosomes of the Ferns it is impossible in all cases to trace the presence of the original longitudinal fission through the late prophase condition up to the beginning of metaphase. Nevertheless, a study of the successive forms assumed by the chromosomes indicates that the gradual obliteration is apparent rather than real; for it can still be recognized by means of the slightly bifid ends of the limbs of the chromosomes. These appearances are sufficiently convincing as to the correctness of the interpretation of the so-called second longitudinal fission, as nothing more than a reappearance of the original fission undergone by the spireme in the early stages of prophase.

During anaphase the daughter-chromosomes preserve their V-shaped appearance (Fig. 27), and finally aggregate closely together at the poles of the spindle (Fig. 28), where they finally occupy a space resembling a vacuole in the cytoplasm (Fig. 29).

This space appears to be bounded by a nuclear membrane, which, however, only persists for a very short time. The chromosomes fuse end to end in the manner described in several instances by Strasburger [1], forming a thread which does not pass into a reticular structure (Fig. 29).

The second (homotype) division follows very rapidly upon the completion of the heterotype division, and is provided for by the longitudinal fission already noticed in the diverging chromosomes of the heterotype division (Figs. 30, 31).

If, as seems likely, this fission is in reality that which appeared in the prophase of the heterotype division, the latter division may, as Farmer and Moore have pointed out, be considered a process intercalated between the earliest and the later stages of the homotype division.

The result is then a transverse true reduction-division of the bivalent chromosomes which characterize the heterotype division. This work therefore provides an extension to another group of plants of the results obtained by Farmer and Moore in certain plants and animals.

4. General Considerations.

In connexion with the segregation of characters, which takes place at the formation of the gametes in Mendelian hybrids, the occurrence of

[1] Strasburger, '00.

a qualitative reduction in plants as well as in animals is extremely important, as affording a possible provision for that purity of the gametes in respect of allelomorphic characters, which is demanded by Mendel's hypothesis.

There is strong evidence in support of the theory which infers a correspondence between the development of certain characters in the soma of the zygote and the presence of certain chromosomes or groups of chromosomes in its nuclei.

Boveri's experiments upon multiple fertilization of the eggs of Sea Urchins [1] have shown that the presence of certain combinations of chromosomes is essential to normal development. This direct evidence of a qualitative differentiation among the chromosomes is supported indirectly by the evidence of the individuality of the chromosomes derived from cytology. Of the latter it is only necessary to mention here the work of Sutton upon *Brachystola magna*, in which there is morphological differentiation (in size) between the chromosomes.

The chromosome group of the presynaptic germ-cells was shown to consist of two equivalent series of chromosomes. In synapsis the homologous members of the two series fuse with one another in pairs; at the reduction-division the chromosomes which have fused together are relegated to different germ-cells. 'We are virtually able to recognize each chromosome in eleven consecutive cell-generations. . . . No continuous spireme is formed; and although after each division there is a brief interval, during which chromosomic boundaries can no longer be traced, the regular correspondence, unit for unit, of the mother-series with the daughter-series established a high probability that we are dealing with morphologically distinct individuals [2].'

'If, as the facts in *Brachystola* so strongly suggest, the chromosomes are persistent individuals in the sense that each bears a genetic relation to one only of the previous generation, the probability must be accepted that each represents the same qualities as its parent element [3].'

Wager [4] has recently suggested that the part played by the nucleolus during mitosis has hitherto received insufficient recognition.

He says: 'We have in *Phaseolus* a phenomenon which, if found to be a widely spread one, must modify our conception of the significance of the chromosomes and nucleolus in heredity [5]. . . . The nucleolus as well as the chromosomes will have to be taken into account in any new hypothesis which may be put forward [6].'

In the spore-mother-cells of the Ferns [7] the nucleolus is smaller relatively to the size of the nucleus than in many plants, and is therefore

[1] Boveri, '02. [2] Sutton, '02, p. 34. [3] l. c., p. 39.
[4] Wager, '04. [5] l. c., p. 50. [6] l. c., p. 53.
[7] These observations apply also to *Osmunda regalis* as well as to the Ferns mentioned on p. 445.

not very favourable for observation. In preparations stained with aniline-safranin, gentian violet, orange G[1] the nucleolus of the young spore-mother-cell is brilliant red, while the reticulum has a purple tinge. At a later stage the spireme shows increasing affinity for the safranin.

The nucleolus is always in close contact with the thread, with which in some cases it appears to be in direct continuity (see Wager's figure, loc. cit., Pl. V, Fig. 16)[2]. When this is the case the part of the thread in the immediate vicinity of the nucleolus stains a brighter red, and is without the purple tinge which characterizes the remaining portions. The contraction of the spireme invariably takes place in such a way that the nucleolus is closely surrounded by the aggregated thread. The nucleolus becomes vacuolated, and its staining capacity diminishes, while the chromosomes continue to take the stain strongly. The nucleolus is visible for some little time after the segregation into chromosomes has taken place, as a spherical body lying free in the nuclear cavity, but it disappears completely at about the time that the spindle-fibres first become visible.

No nucleolus has been found in the daughter-nuclei, so that all trace of this body is lost from shortly after the formation of the chromosomes of the heterotype division until its reappearance in the nucleus of the young spore. This absence is not surprising, since the daughter-nuclei scarcely enter the resting condition (see p. 449); the manner of its reappearance in the young spore-nucleus scarcely suggests its direct formation by a fusion of the chromosomes into nucleolus-like masses[3]. The appearance is rather that of a fusion of the chromosomes resulting in the formation of a thread, which is becoming reticular at the time when the nucleolus (or nucleoli) reappears in close connexion with it.

In the Ferns the relative proportion of the nucleolus to the reticulum as regards size and staining capacity differs considerably from that in *Phaseolus* and certain other plants[4]. The transference of stainable matter from the nuclear thread to the nucleolus in the resting condition of the nucleus would appear, therefore, to vary in the degree to which it is carried out in different plants; and the reverse process, which always precedes mitosis, seems to indicate that in the fission of the chromosomes some provision is made for the distribution of nuclear matter in a way which would not be accomplished by a direct division. The work of Sutton, already quoted (p. 450), apparently points to an individuality of the

[1] This was found to be more satisfactory than haematoxylin owing to the differential staining of the nucleolus and spireme.

[2] The figures illustrating this paper were all completed before the appearance of Wager's paper directed special attention to the relations of the nucleolus, so that the figures specially illustrating these points must unfortunately be omitted.

[3] Wager, l. c.

[4] The structure of the nucleus in e.g. *Lathyrus odoratus* is very like that of *Phaseolus* as figured by Wager.

chromosomes, which, I think, must be looked upon as independent of the transference of stainable matter from or to the nucleolus.

Cannon has suggested a 'cytological basis for the Mendelian laws[1]' founded upon the occurrence of a qualitative reduction-division, and predicted the discovery of a qualitative reduction in plants. A similar suggestion was independently made by Sutton [2].

Cannon's hypothesis consisted in the assumption that in fertile hybrids, as well as in pure races, 'the chromosomes derived from the father and the mother unite in synapsis, and separate in the metaphase of one of the maturation divisions . . . so that the end is attained that the chromatin is distributed in such a way that two of the cells receive pure paternal, and two cells pure maternal chromosomes, and no cells receive chromosomes from both the father and the mother.'

Thus enunciated the hypothesis is applicable only to monohybrids (de Vries); it is insufficient to explain the phenomena observed in the offspring of Mendelian hybrids whose parent-races differ from one another in respect of more than one pair of allelomorphic characters.

This was recognized by Sutton, who therefore paid particular attention to the positions assumed by the chromosomes in *Brachystola*. He says: 'The results gave no evidence in favour of the parental purity of the gametic chromatin as a whole. On the contrary, many points were discovered which strongly indicate that the position of the bivalent chromosomes in the equatorial plate of the reducing division is purely a matter of chance, that is, that any chromosome pair may be with maternal or paternal chromatid indifferently towards either pole, irrespective of the positions of the other pairs, and hence that a large number of different combinations of maternal and paternal chromosomes are possible in the mature germ-products of an individual.'

Häcker [3] and Rückert [4] have shown that in *Cyclops*, as in *Ascaris* and other forms [3], the germ-nuclei do not fuse completely in fertilization, but give rise to two groups of chromosomes, which lie side by side in the succeeding mitoses. Häcker [5] has since traced the autonomy of the paternal and maternal chromatin in *Cyclops* from fertilization up to the formation of the mother-cells of the gametes.

In the primary oöcyte the twelve tetrads are arranged in two groups, each consisting of six tetrads, which occupy parallel planes across the 'provisorische Teilungsfigur.'

At a later stage the two groups are separated by a partition-wall, which extends across the nucleus, and Häcker suggests that those of the tetrads which lie upon one side of this wall are of paternal, those upon the other side of maternal origin. In the 'secundären Keimbläschen' the

[1] Cannon, '02. [2] Sutton, '03. [3] Häcker, '92.
[4] Rückert, '95. [5] Häcker, '03.

chromosomes of the two groups pass between one another ' in einer ganz gesetzmässigen Quadrillen-ähnlichen Ordnung [1],' with the result that ' die Eizelle in gleichmässiger Mischung grossväterliche und grossmütterliche Elemente erhält [2].'

These later results apparently indicate that in *Cyclops* the gametes *always* contain chromosomes derived from both parents.

The view adopted here of the significance of the reduction-division appears then to be open to the objection that no provision is made for the production, among the different kinds of gametes, of a certain number bearing Mendelian characters derived exclusively from one parent.

This objection involves the assumption, which is not at present justified by evidence, that *each* pair of chromosomes corresponds with a pair of allelomorphic characters, and that the correspondence is independent of any effect due to the presence or absence of other chromosomes [3].

There is, however, another possible interpretation of the phenomena observed in *Cyclops*. Häcker supposes that of the two groups of tetrads

$$\frac{ab}{ab}\quad\frac{cd}{cd}\quad\frac{ef}{ef}\quad\frac{gh}{gh}\quad\frac{ik}{ik}\quad\frac{lm}{lm}$$

$$\frac{no}{no}\quad\frac{pq}{pq}\quad\frac{rs}{rs}\quad\frac{tu}{tu}\quad\frac{vw}{vw}\quad\frac{xy}{xy}$$

the series $\frac{ab}{ab}, \frac{cd}{cd}$ . . . originates from one parent,

the series $\frac{no}{no}, \frac{pq}{pq}$ . . . from the other.

If this is so, the plane of separation between the paternal and maternal groups is now at right angles to the position it occupied in the earlier stages [4].

In *Cyclops strenuus*, *Heterocope robusta* and *Diaptomus gracilis*, the tetrads are at first *evenly distributed* throughout the nuclear cavity of the oocyte [5]. In the first-mentioned form there appears *at a later stage* a distinct tendency towards the separation of the tetrads into two groups [6]. In the other forms this is less marked, but may perhaps be indicated

[1] Häcker, l. c., p. 342. [2] Ibid., p. 374.

[3] The degree to which the analysis of the nucleus has been carried (Boveri, '02) only admits of the application of the conclusions to *combinations* of chromosomes, of which a large number are possible even under the conditions which obtain in *Cyclops*, if Häcker's suggestion is correct. Further the characters exhibited by certain heterozygotes appear to be only explicable as corresponding with a combination of chromosomes. To use a physical analogy, a combination of chromosomes may be compared to a chemical compound as distinct from a mechanical mixture. But in citing heterozygotes as an instance of the effect of a combination of chromosomes it must not be forgotten that we are dealing with a zygote-nucleus and not with a reduced one.

[4] See Häcker, l. c., Figs. 28, 29 (*Diaptomus denticornis*); Rückert ('95), Figs. 1–3, 6, 7 (*Cyclops strenuus*). [5] Rückert ('94). [6] l. c., p. 303, and Figs. 15 and 20.

by the distribution of the tetrads shown by Rückert in his Figs. 25 (*Heterocope*) and 34 (*Diaptomus*).

The question therefore suggests itself whether each tetrad may not consist of two univalent chromosomes, one of paternal, the other of maternal origin. This view agrees with that which is generally accepted of the significance of the bivalent chromosomes, but leaves unexplained for the present the later separation of the tetrads into groups. If it is correct, the movements of the chromosomes described by Häcker will

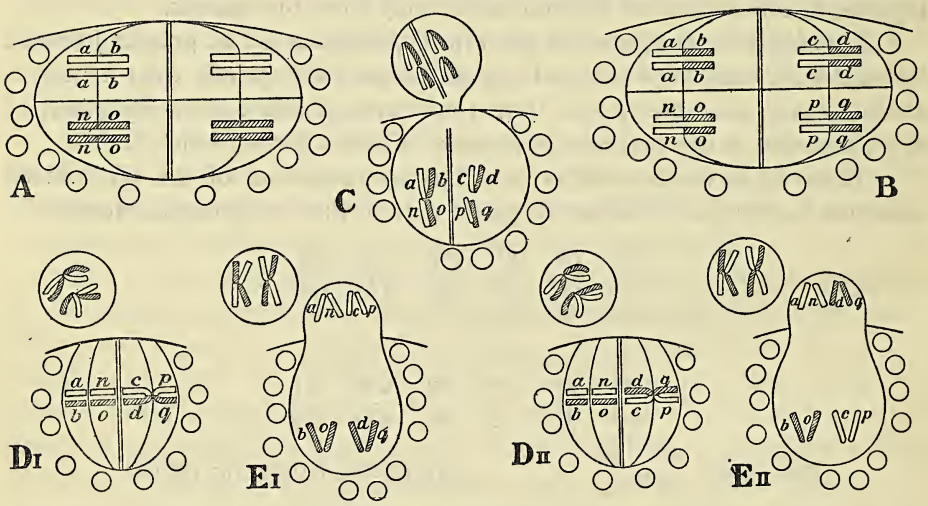

FIG. 43. Diagrams of the reduction-division in *Cyclops*.

The chromosomes are white or shaded to indicate their suggested origin from the two parents. A, from Häcker. B–E, the interpretation suggested in the text. A and B, the oöcyte. C, the formation of the first polar body indicating the distribution of the chromatin which would follow from B. The orientation assumed by the chromosomes in the next stage may be as in DI, as in the converse of DI, or as in DII, with the result that the egg may contain chromatin from one parent only (EI and its converse), or any of the combinations of paternal and maternal chromatin (EII), which become possible with a larger number of chromosomes than the four represented in the diagrams.

provide for any arrangement of the paternal and maternal chromatin in the gametes, since the different relative positions assumed by the chromosomes in the 'secundären Keimbläschen[1]' permits either the paternal or the maternal element to be turned indifferently towards either pole. (See Fig. 43 in text.)

The regularity observed by Häcker in the movements of the chromosomes at the reduction-division may perhaps be an indication, in a limited degree, of a more comprehensive symmetrical design extending throughout the nuclear divisions which provide for the production of the different types of gametes in Mendelian hybrids. 'It is impossible to be presented with the fact that in Mendelian cases the cross-bred produces on an

[1] See Häcker ('03), Pl. 19, Figs. 36–38.

average *equal* numbers of gametes of each kind, that is to say, a symmetrical result, without suspecting that this fact must correspond with some symmetrical figure of distribution of the gametes in the cell-divisions by which they are produced [1].'

Rosenberg's earlier observations upon the hybrid *Drosera longifolia* × *D. rotundifolia*, whose parents differ from one another in the number of chromosomes, gave somewhat indefinite and variable results [2]. The chromosomes derived from the two parents do not apparently present any morphological differentiation by which their lineage can be recognized; but, even in the absence of this, Rosenberg's later results [3] agree entirely with the expectation based upon the theory of the individuality of the chromosomes and the union in pairs of paternal and maternal chromosomes in synapsis.

The reduced number of chromosomes in *D. longifolia* is ten, in *D. rotundifolia* twenty. In the somatic mitoses of the hybrid thirty (i. e. 10+20) chromosomes occur. In the pollen-mother-cells and in the embryo-sac mother-cell there always occur twenty chromosomes, of which ten are large, ellipsoidal, with a central constriction, and ten are smaller, without a constriction [4]. At metaphase the ten large chromosomes occupy the equator of the spindle, the small ones are distributed somewhat irregularly upon both sides of the equatorial plate. The large chromosomes divide and the daughter-chromosomes move towards opposite poles of the spindle, where they are enclosed by a nuclear membrane; with them may be included any of the small chromosomes which lie near the poles, but some are usually left behind in the cytoplasm.

Rosenberg suggests the following explanation. *D. rotundifolia* is represented in the hybrid by only ten chromosomes, *D. longifolia* by twenty. Each of the chromosomes derived from the parent *D. rotundifolia* fuses with one derived from the other parent, *D. longifolia,* giving rise to the ten double chromosomes. The remaining ten chromosomes of *D. longifolia* find no corresponding chromosomes of the other parent, and remain as the ten small chromosomes, which do not divide at the first division. Accepting the views of Strasburger and Guignard upon the division of the chromosomes by two longitudinal fissions, Rosenberg supposes that the fusion in synapsis has been lateral instead of end to end, as is usually the case. It perhaps seems more probable that further work in the light of Farmer and Moore's interpretation of the changes undergone in the prophase of the heterotype division will reveal similar phenomena in the *Drosera* hybrid.

On the hypothesis that the segregation of characters occurs at the reduction-division, we shall expect that the mitoses in a Mendelian

[1] Bateson ('02), p. 30.
[2] Rosenberg ('03).
[3] Rosenberg ('04).
[4] Ibid.

hybrid will be perfectly regular, and in our present condition of inability to recognize qualitative differences between chromosomes alike in form, we should further expect that the mitoses will differ in no *visible* way from those of the pure paternal and maternal races. Cannon has shown this to be the case in race-hybrids of *Pisum sativum*, a result which is confirmed by my own observations upon race-hybrids of *Lathyrus odoratus*, for the material of which I am indebted to Mr. Bateson.

The sterility which characterizes many hybrids follows upon the abortive development of the sex-cells, and the suggestion has been made that this may be due to the inability of the hybrid to separate, in the formation of the gametes, the characters which were united in the hybrid zygote. It is well known that sterile plant-hybrids are particularly characterized by abortive development of the pollen, or (in the case of the hybrid Fern described by Farmer) of the spores.

Among the offspring of a race-hybrid of *Lathyrus odoratus* fertilized with its own pollen, Mr. Bateson obtained a number of individuals which failed to form good pollen. In the plants with coloured flowers the sterility was, with a few exceptions, correlated with the development of a somatic character—the sterile plants generally possessing a green leaf axil, while the fertile coloured plants with rare exceptions had red axils. In these plants the divisions of the vegetative cells are quite normal, as are also those of the archesporium up to the formation of the pollen-mother-cells. The irregularity makes its appearance only in the heterotype division.

The longitudinal fission of the spireme takes place quite normally, but the segmentation into chromosomes is, if carried out at all, irregular, and the pollen-mother-cells degenerate. Since the equation divisions are quite normal, this would seem to indicate that the union of the chromosomes in synapsis is such as to prevent any subsequent separation, the result being that no sex-cells can be organized, since the essential condition of a qualitative separation of the chromatin is not fulfilled.

Postscript.

Since the above was written Strasburger's paper 'Ueber Reduktionsteilung' (Sitzungsberichte d. k. preussischen Akad. der Wissenschaften, Mar. 24, 1904) has been received. Strasburger finds that a true reduction-division takes place in *Lilium spp.*, *Tradescantia virginica* and *Galtonia candicans*, the last-named affording very clear evidence. In the pollen-mother-cells of *Galtonia* six bivalent chromosomes are formed by the segmentation of the spireme thread. Each of these undergoes a transverse fission into two equal limbs. Not until after this fission does the bending of the limbs upon one another take place, so that the bivalent chromosomes form II-shaped, instead of U-shaped, bodies. In this plant then the transverse fission takes place at a very early stage. This condition may be compared with the variation in the time of fission which has been observed in the Ferns (p. 448 and Figs. 15, 16, 23).

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EXPLANATION OF PLATE XXXI.

Illustrating Mr. Gregory's paper on Spore-formation in Ferns.

The figures were drawn with a camera lucida. A Zeiss aprochromatic objective 2 mm. 1.4 N.A. was used for all except Figs. 32–34, which were drawn with Zeiss homog. immers. K. Compensating oculars 8, 12, and 18.

Fig. 1. *Onoclea sensibilis.* Spore-mother-cell, showing spireme before the longitudinal fission has taken place. × 1000.

Fig. 2. *Asplenium Ceterach.* Longitudinal fission of the spireme. × 1500.

Fig. 3. *Onoclea sensibilis.* Contraction of the double spireme thread towards one side of the nucleus has commenced. In some places the two halves of the thread diverge from one another. × 1500.

Fig. 4. *Onoclea sensibilis.* The contraction of the spireme with the formation of a series of loops. × 1000.

Fig. 5. *Davallia Capensis.* Longitudinal fission and contraction of the spireme. × 1000.

Figs. 6, 7, 8, and 9, show later stages in the formation of the loops. The twisting of the limbs upon one another is shown in Figs. 8 to 10.

Fig. 6. *Onoclea sensibilis.* × 1500.

Fig. 7. *Asplenium marinum.* × 1500.

Fig. 8. *Scolopendrium vulgare* × *Asplenium Ceterach.* × 1500.

Fig. 9. *Asplenium Ceterach.* × 1500.

Fig. 10. *Onoclea sensibilis.* The longitudinal fission is rather less evident than in the earlier stages. × 1500.

Fig. 11. *Onoclea sensibilis.* Showing the looped spireme. (Some of the loops are omitted for the sake of clearness. The chromatin granules are represented somewhat diagrammatically.) × 1000.

Fig. 12. *Asplenium marinum.* The limbs of the loops are closely approximated to one another. × 1500.

Fig. 12 *a.* Two of the loops (*a* and *b* of Fig. 12) indicating the original longitudinal fission. × 2250.

Fig. 13. *Fadyenia prolifera.* A late stage in which the longitudinal fission is clearly visible in the cut ends of the thread. × 1000.

Fig. 13 *a.* From another nucleus of the same sporangium, showing the longitudinal fission of the spireme. × 2250.

Fig. 14. *Fadyenia prolifera.* The spireme shortly before segmentation into the chromosomes takes place. × 1500.

Fig. 15. *Onoclea sensibilis.* The segmentation of the spireme into chromosomes. In some instances there are clear indications of the double nature of each limb. × 1000.

Fig. 16. *Scolopendrium vulgare* × *Asplenium Ceterach.* Later stage, showing the various forms assumed by the chromosomes in the heterotype division. × 1000.

Fig. 17. Chromosomes from the same nucleus. × 2250.

Fig. 18. *Asplenium marinum.* Chromosomes of the heterotype division. × 2250.

Fig. 19. *Onoclea sensibilis.* As Fig. 16.

Figs. 20, 21. *Onoclea sensibilis.* Chromosomes of the heterotype division. The longitudinal fission may be indicated in surface view (Fig. 20) or through the bifid ends of the limbs of the chromosomes (Fig. 21). × 2250.

Fig. 22, 23. *Davallia capense.* Two stages in the breaking down of the nuclear wall and the development of the spindle. There are indications of the original longitudinal fission in the limbs of some of the chromosomes. Fig. 22, × 1000; Fig. 23, × 2250.

Fig. 24. *Davallia capense.* Formation of the spindle and movement of the chromosomes towards the equatorial plate. The limbs of the chromosomes are already slightly drawn apart, and their longitudinal fission is clear. × 1000.

Fig. 24 *a.* Part of the same. × 2250.

Fig. 25. *Asplenium marinum.* Metaphase of the heterotype division. × 1500.

Fig. 26. The same. The section is tangential to the spindle and shows the chromosomes in face view. × 2250.

Fig. 26 *a.* The same stage in *Onoclea sensibilis.* The chromosomes seen in profile. × 2250.

Fig. 27. *Asplenium marinum.* Tangential section of the nucleus in anaphase of the heterotype division. × 1500.

Fig. 28. *Scolopendrium vulgare* × *Asplenium Ceterach.* Late anaphase. × 1000.

Fig. 29. *Onoclea sensibilis.* Telophase. Formation of a nuclear wall. × 1000.

Fig. 30. From the same sporangium, showing a slightly later stage in which the nuclei have entered upon the homotype division. × 1000.

Fig. 31. *Onoclea sensibilis.* Telophase of the homotype division. × 1500.

Figs. 32, 33, and 34. *Scolopendrium vulgare* × *Asplenium Ceterach.* Three stages in the development of the spore. All × 1500.

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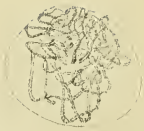

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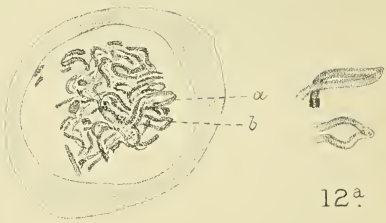

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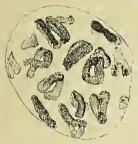

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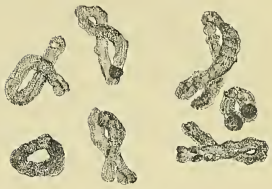

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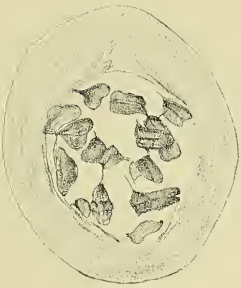

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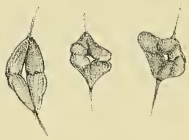

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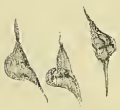

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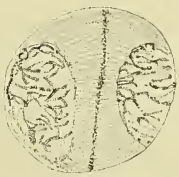

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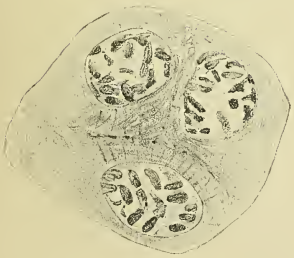

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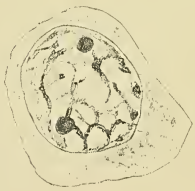

34.

Huth lith. et imp.

A Monograph of the genus Inocybe, Karsten.

BY

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With Plate XXXII.

THE word *Inocybe* was first used by Fries[1] to designate a section of the genus *Agaricus*, which at the time included the majority of gill-bearing Fungi.

Karsten was the first to use *Inocybe* in a strictly generic sense[2]; in fact this author raised all the sectional names employed by Fries in dividing up his huge genus *Agaricus* to generic value; a step which has received the assent of most modern mycologists.

The species of *Inocybe* are by common consent looked upon as difficult to recognize in the field; in fact all past attempts to do so have resulted in failure and almost hopeless confusion, and I think it will be generally conceded that the Friesian method of study, depending on naked-eye, or at most, pocket-lens characters, is wholly inadequate. One principal reason for this state of things is the great variability presented by the pileus of the same species under different weather conditions. A species may have the surface of the pileus normally smooth and silky; or in other words, the specimen on which a species was originally founded happened to have a pileus of this nature; consequently this feature constitutes one of the salient characters in its diagnosis, where macroscopic features alone are taken into consideration. Unfortunately all who have had a considerable amount of experience in the field, know perfectly well that a Fungus usually having a pileus of the nature defined above may, under other conditions of growth, have the surface of the pileus broken up into scales. Now this change technically removes the Fungus from the section 'Velutini,' and places it in the section 'Laceri.' As already stated, an experienced

[1] Syst. Myc. Fung. I, p. 254 (1821).

[2] Rysslands, Finlands, och den Skandinaviska Halföns *Hattsvampar*; forming vol. xxxii, Finlands Natur och Folk, p. 453 (1879).

[Annals of Botany, Vol. XVIII. No. LXXI. July, 1904.]

mycologist is not *always* deceived by such a superficial change, but less experienced persons often are. The beginner fails to locate in the 'Laceri' a Fungus which in reality belongs to the 'Velutini,' and a 'new species' is the result.

Other causes leading to confusion, and quite unnecessary multiplication of names, are as follows.

Personally I consider that colour should not constitute part of a specific diagnosis; however, as a matter of fact, by almost common consent it does so. Where the colours are clear and well defined, as in many genera, there is no serious objection to the practice, but in the genus *Inocybe*, where the colour of dozens of closely allied species is some shade of brown, the attempts of different authors to convey to others the particular shade of brown is, to say the least, perplexing. When a definition of odours is attempted matters are still worse. As an example, Weinmann (Fung. Ross, p. 194) says of *Inocybe Trinii*, his own species, 'odore valde suavis et fere caryophyllaceus!' Bresadola (Fung. Trid., ii, p. 14), speaking of the same Fungus, says, 'odore forti terreo.' Numerous such differences of opinion are extant, and the obvious conclusion is that mycologists cannot define the characteristics of a smell in words.

Schaeffer, Persoon, Bulliard, Bolton, Krombholz, and other pioneers in mycology, have left evidence of their untiring enthusiasm in the pursuit of their favourite study, in the form of coloured figures of many hundred different kinds of Fungi. These they named and described to the best of their ability; but it must be admitted that in numerous instances the figures are somewhat grotesque, and in the best cases rarely give more than a general idea of the species intended, from an artistic rather than a scientific standpoint. Again, the principal characteristic of the specific descriptions furnished by the above-named authors is their extreme brevity, which in many instances renders identification of the exact species they had in view somewhat uncertain, or often practically impossible for ordinary mortals to accomplish.

Unfortunately for mycology there have of late years always been two or three extraordinary people amongst us, who appear to be firmly convinced that their special function on earth is to indicate, without possibility of error, the exact species the old authors had in view. By such clairvoyants, species of Fungi that have been known by a particular name for a considerable period of time, and universally accepted, are suddenly discovered to be nothing more than the identical kind figured and described by some ancient author. An exchange of names follows, and the discoverer rewards himself by adding his own name after the new combination.

Even this method could be accepted if there was any hope for finality, but in the absence of types, and the figures and descriptions being as stated above, there is room for no such hope.

Not unfrequently the confusion alluded to is intensified by changing the specific name, on the ground that the one originally used was not classically correct.

A law of their own formulating is the justification for this act.

By such tactics the champions of scientific accuracy, and of justice to old authors, carefully eliminate not only the original specific name, but also that of the original author; and why? Solely for the purpose of posing as the founder of a species; and in their anxiety to accomplish this object, scientific accuracy and justice to the old authors are alike forgotten.

This method of procedure goes behind the rule, that the name following a species should be that of the person who first placed it in the right genus.

I am daily expecting the advent of the person who will boldly contest the validity of all specific names conferred up to the present, on the ground that their characteristics are not defined in classical Latin. At all events his argument would possess the merit of being as logical as many of those used at present for a similar purpose.

The genus *Inocybe* contains 112 distinct species, and as a preliminary to the preparation of this monograph, 417 specimens were examined microscopically for the purpose of obtaining camera drawings and measurements of the spores, cystidia, and basidia. These specimens included those contained in the Kew Herbarium, supplemented by types and authentic specimens kindly communicated by other mycologists.

As a result of this detailed examination of the structure of the hymenium in the various species, the fact has become apparent that perhaps in no other genus included in the Agaricaceae are characters of specific value more distinctly marked, than those presented by the spores, cystidia and basidia in the genus *Inocybe*.

For systematic purposes the spores may be divided into two primary groups: (1) epispore smooth; (2) epispore rough, that is, furnished with projections of some kind. In the first group, the most general form I have called *pip-shaped*, on account of the resemblance to the pip or seed of an apple. A second type of smooth spore is that of a long, narrow ellipse with obtuse ends; this is termed *elliptico-cylindrical*. This form of spore is in some species very slightly curved. In a third form, only known in one species—*I. rhombospora*, Massee, from India—the spores are distinctly rhomboidal in outline, and much compressed laterally (Fig. 4). In the second group the spores are either *globose* or *irregularly oblong*. In all there is a more or less pronounced *apiculus*, or narrowed end, corresponding to the point of attachment of the spore to the sterigma. The ornamentation of the epispore is included under two heads: (1) *Spinulose*, when the epispore bears slender pointed spines. Up to the present this type of epispore ornamentation is confined to one species—*I. Gaillardi*, Gillet,

a Fungus common to Europe and America (Fig. 11). (2) *Nodulose*; this type of epispore marking varies considerably in different species. The most frequent form is where the epispore is sparsely covered with large, blunt warts or papillae; in a second type the nodules are only very slightly raised, giving the spore a wavy outline when seen in optical section (Fig. 6); a third type has the nodules elongated into blunt finger-shaped papillae (Fig. 12). The cystidia are present under two forms: *ventricose*, having a pronounced swelling some distance below the apex (Fig. 8); *fusoid*, when more or less spindle-shaped (Fig. 10). The tips of cystidia are sometimes crowned with a brownish mass resembling a conglomeration of small crystals. This feature is by some mycologists considered as of importance, and finds a place in specific descriptions. This character is however absolutely valueless; the accumulation is simply nothing more than mucilage which escapes from the interior of the cystidium after the deliquescence of the thin portion of wall at its apex. If the air continues to be moist the escaping mucilage remains liquid and numerous falling spores are caught, forming a dense mass at the apex of the cystidium (Fig. 9). On the other hand, if the air is dry when the mucilage escapes, it dries up and contracts into a rugged mass. When the mucilage once dries it is afterwards insoluble in water (Fig. 8).

True cystidia are only met with on the surface of the gills. Their walls are very thick and highly refringent, and when free from colour are very apt to be overlooked, even when specially sought after. This difficulty can be overcome by running in under the cover-glass a weak solution of the stain called 'azurblau,' dissolved in water, and adding potassic hydrate until the solution assumes a clear red colour. The cystidia are the first to take up the stain, which gradually extends to all the tissues. This stain will be found of general use in the examination of Fungi.

The margins of the gills are frequently whitish, and under a pocket-lens appear minutely *fimbriate*. This appearance is due to the presence of large clavate or fusoid cells, which are in some species arranged in little groups, when the margin of the gill is described as *serrulate*.

These marginal cells are often as large as cystidia, from which they differ in having thin walls, and not deliquescing at the apex and exuding mucilaginous matter. They also differ in origin, being modified elements of the hymenium, basidia and paraphyses; whereas cystidia originate from tramal cells, and push up between the elements of the hymenium, until they eventually project some distance above the level of the hymenium. Cystidia are sometimes spoken of as excretory organs, but in reality nothing definite is known as to their functions, which presumably differ in different genera, judging from the variety in structure presented.

It is premature to speak of the geographical distribution of *Inocybe*, considering that out of 112 known species, 80 occur in Europe, and 36 in

America, all from the United States. The following table gives the distribution so far as at present known:—

Europe.	*Asia.*	*Africa.*	*America.*	*Australasia.*
Total 80.	Total 3.	Total 1.	Total 36.	Total 3.
Endemic 69.	All endemic.	Endemic.	Endemic 25.	Endemic 2.

Eleven species are common to Europe and America, and one Australasian species is also European.

In the following arrangement of the species the primary sections depend on the presence or absence of cystidia, and on the rough or smooth epispore. There will undoubtedly be a difference of opinion as to the relative value of the above characters in the discrimination of species. On the other hand, perhaps all will agree as to the advantage of having the characters of cystidia and spores, obtained from type specimens, added to the original diagnosis of species from which they were originally omitted. This I have been enabled to do through the generosity of the following mycologists in giving or lending type specimens, and to each of whom I express my thanks:—Dr. E. Boudier, Professor Dr. F. R. Kjellman, Dr. P. Hariot, Professor C. H. Peck, Dr. L. Romell.

Inocybe, Karsten.

Pileus symmetrical, flesh thin, covered with a fibrillose veil which becomes either longitudinally cracked, or broken up into squamules or squarrose scales, dry or rarely viscid; gills adnate, adnexed or nearly free, brownish or dingy; spores pale brown, epispore smooth, warted or spinulose; cystidia often present; stem central, slender, fibrillose, often peronate with squamules or squarrose scales up to the imperfectly defined annular zone.

The genus *Inocybe* is most closely allied to *Hebeloma*, under which at one time it was included. The last named differs in the constantly viscid, pelliculose veil which is never fibrous and silky, whereas in *Inocybe* the veil covering the pileus is always distinctly silky or fibrous, even when viscid.

The majority of species grow on the ground in woods or damp, shady places.

Key to the Species.

A. **Spores rough.**

I. *Cystidia present.*
  * Stem whitish, or pallid.
  ** Stem coloured.

II. *Cystidia absent.*

[*Spores rough, no knowledge of cystidia.*
  * Stem whitish, or pallid.
  ** Stem coloured.]

B. **Spores smooth.**

III. *Cystidia present.*

* Stem whitish, or pallid.

† Gills brownish, ochraceous or cinnamon.

†† Gills tinged olive.

††† Gills tinged violet.

** Stem coloured.

† Gills ochraceous, brown or cinnamon.

†† Gills tinged olive.

††† Gills tinged violet.

IV. *Cystidia absent.*

* Stem whitish, or pallid.

† Gills brownish, ochraceous or cinnamon.

†† Gills tinged olive.

** Stem coloured.

† Gills brownish, ochraceous or cinnamon.

†† Gills tinged olive.

[*Spores smooth, no knowledge of cystidia.*

* Pileus dark-coloured.

** Pileus light-coloured.]

The two sections in square brackets include those species respecting which our knowledge is at present incomplete, no information being included in the original diagnosis respecting cystidia.

Abbreviations used in the specific descriptions :—

P. = pileus; *g.* = gills; *s.* = stem; *sp.* = spores; *c.* = cystidia.

A. Spores Rough.

I. *Cystidia present.*

* *Stem whitish, or pallid.*

fibrosa, Karst., Hattsv., p. 460; Sacc., Syll. v, p. 779; Bres., Fung. Trid., i, tab. 56; *Ag. fibrosus*, Sowerb., Fung., tab. 414; *Ag. fastigiatus*, Britz., Derm. Süd. Bay. p. 4, f. 27; *Ag.* (*Ino.*) *inditus*, Britz., Hym. Südb., p. 150, f. 143; *I. inedita*, Sacc., Syll. v, p. 780.

P. campanulate, then expanded and gibbous, silky, whitish to pale yellow-brown, edge cracking, 6–10 cm., flesh thick, white; g. nearly free, narrowed behind, dingy ochre; s. solid, stout, narrowed and floccose upwards, whitish, 7–11 cm.; sp. irregularly oblong, apiculate, slightly nodulose, 10–12 × 7–7·5 μ; c. ventricose, 45–60 × 12–15 μ.

In pine woods, &c. Britain, France, Germany, Austria, Russia, Finland, Sweden, Holland.

One of the largest species. Differs from *I. perlata* in having warted spores. (Sowerby's type examined.)

Bresadolae, Massee; *I. repanda* (Bull.) Bres., Fung. Trid., pl. 119, f. 1. P. campanulate, then expanded and umbonate, edge sinuate and wavy, lubricous, whitish and covered with rosy-tawny fibrils, disc even and rosy-tawny, 3–6 cm.; g. crowded, white, then dull cinnamon becoming rufous, edge white-fimbriate, rounded behind and free; s. solid, whitish, pruinose, at length tinged with rosy-tawny, apex striate, base ventricose or slightly turbinately bulbous, 3–5 cm. long, 5–6 mm. thick; sp. elongated, tuberculose, 8–10 × 6 μ; c. ventricose, 60–70 × 17–20 μ. Flesh white, tinged red when broken. Smell pleasant.

On the ground. Austria.

Notwithstanding Bresadola's rider 'Speciem hanc genuinam *Ag. repandum*, Bull. sistere vix dubitaret,' I cannot admit the identity. This question I consider to have been settled by Berkeley more than half a century ago, and moreover Bresadola has obviously disregarded Bulliard's text relating to his Fungus, and relied entirely on figures in his determination. Berkeley's original account of the Fungus he considered to be Bulliard's plant, and which he placed in the section *Entoloma*, is as follows:—

Pileus 1–2 inches broad, conic, obtuse, at length expanded, very fleshy, the margin incurved and lobed, pale whitish ochraceous, with a few streaky shades, clothed with a very close, adpressed indistinct silkiness. *Gills* pale dull-rose, broad in front. *Sporules* round, rose-coloured. Stem 1½ inch high, 3 lines thick, white, beautifully adpresso-sericeus, composed of fibrous cells, distinct from those of the pileus. *Odour* like that of fresh meal. My specimens agree precisely with Bulliard's plant quoted above, except that the colour is not so lively. He says expressly that the seminal powder is 'rougeatre,' which can hardly apply to any species of the subgenus *Inocybe* (Eng. Flor., v, Fungi, p. 78).

Rolland (Bull. Soc. Myc. Fr., xix, 333, pl. 16, figs. 1–3, 1903) has collected some specimens in France, respecting which he writes:—'Dans ces champignons, où il nous est impossible de voir autre chose que le véritable type de *l'Inocybe repanda* Bull., les spores sont ovales et lisses.'

This makes the fourth species that has been stated to be undoubtedly the *Agaricus repandus*, Bull.

asterospora, Quél., Flor. Myc., p. 100 (1888); Sacc., Syll. v, p. 780; *Ag.* (*Ino.*) *asterosporus*, Quél., Bull. Soc. Bot. Fr., xxvi, p. 50; Soc., Sci. Nat. Rouen, 1879, tab. 2, f. 6; Cke., Ill., pl. 385; *I. subrimosa*, Sacc., Syll. ix, p. 100; *Clypeus subrimosus*, Karst., Symb. ad Myc. Fenn., xxviii, in Med. Soc. Faun. et Flor. Fenn., 1888, p. 38 (non Cooke, Ill. pl. 402, as stated by Karsten).

P. campanulate, then expanded and umbonate, even and almost glabrous, becoming rimose and silky-fibrous, from brownish to dingy cinnamon, 2–5 cm.; g. emarginate, ventricose, dingy cinnamon; s. cylindrical, minutely emarginately bulbous, almost glabrous, whitish, sometimes becoming tinged red and streaked with brown fibrils, 5–8 cm.; sp. subglobose, coarsely stellately-nodulose, 10–13 μ; c. ventricose, fairly numerous, 60–75 × 12–16 μ.

On the ground in woods, &c. Britain, France, Finland, United States ('5514, C. Wright, Connecticut,' under *I. rimosa* in Herb. Kew.).

Superficially resembling *I. rimosa*, with which it was at one time confounded,

differing in the nodulose spores. Near to *I. margarispora*, which differs in the absence of cystidia.

(Specimen of *I. asterospora* from Quélet, and of *I. subrimosa* from Karsten examined.)

proximella, Karst., Symb. Myc., ix, p. 44; Sacc., Syll. v, p. 781.

P. conico-convex, then expanded and umbonate, even, then longitudinally fibrously cracked, pallid, the disc and especially the umbo passing into rusty brown or bay, 2–4 cm.; g. adnate, crowded, ventricose, pallid then tan, finally brown; s. stuffed, slightly narrowed upwards, usually ascending from the base, sometimes wavy, subfibrillose, pallid, flesh white, 6–8 cm.; sp. irregularly oblong, slightly nodulose, 8–10 × 5–7 μ; c. ventricose, 55–70 × 12–16 μ, abundant.

On the ground in woods. Finland.

Superficially resembling *I. asterospora*, but distinguished by the ventricose gills, and more especially by the irregularly oblong spores.

(Type specimen from Karsten examined.)

praetervisa, Quél., in Bresad., Fung. Trid., i, p. 35, tab. 38; Quél., Flor. Myc., p. 99; Sacc., Syll. v, p. 782.

P. conico-campanulate, then expanded and broadly umbonate, margin often lobed and split when old, lubricous, soon beautifully longitudinally rimose, fibrillose, disc glabrous, ochraceous tan, sometimes brownish towards the edge, 3–6 cm.; g. crowded, almost free, white then greyish-cinnamon, edge fimbriate; s. solid, terete, glabrous or subfibrillose, apex pruinose, white then straw-colour, base minutely marginately bulbous, 4–7 cm.; sp. irregularly-oblong, nodulose, 10–11 × 5–6 μ; c. ventricose or fusoid, 55–75 × 20–30 μ. Flesh white.

On the ground among herbage in pine woods. France.

Distinguished from allies by the lubricous pileus.

eriocephala, Sacc., Syll. v, p. 791; *Ag. eriocephalus*, Fries, Mon., i, p. 351; Fries, Icon. Sel., ii, p. 9, tab. 110, f. 4.

P. hemispherical, then convex, obtuse, silky, dry, white with a dull yellow tinge, and with white downy flecks, especially near the margin, 1–1·5 cm.; g. adnate, narrow, pallid then rusty; s. fistulose, silky-fibrillose, or sometimes squamulose, whitish, 5–8 cm.; sp. irregularly oblong, apiculate, very slightly nodulose, some almost smooth, 6–7 × 5 μ; c. ventricose, scattered, 40–50 × 10–13 μ.

On rotten wood. Sweden.

Marked by the pale, obtuse pileus more or less covered with downy flecks, and the small, very slightly nodulose spores.

(Specimen from Fries examined.)

albodisca, Peck, 51 Rep. State Mus., p. 290 (1897); Sacc., Syll. xvi, p. 90.

P. conical or campanulate, umbonate, smooth and whitish at the disc when fresh and moist, elsewhere dingy yellowish-brown or lilac-brown, paler and slightly fibrillose or silky when dry, longitudinally rimose, about 2·5 cm.; g. rounded behind, somewhat crowded, whitish then rather rusty; s. equal, solid, striate, glabrous or slightly pruinose at the apex, pallid, 3–5 cm.; sp. suboblong, slightly nodulose, 7–9 × 5–6 μ; c. abundant, some very ventricose, others almost fusiform, 40–60 × 14–20 μ.

Under spruce and balsam fir-trees. United States (North Elba, Essex Co.).

Easily distinguished from all other species of this genus known to me, by the whitish umbonate apex of the pileus (Peck).

(Peck's type examined.)

curvipes, Karst., Hedw., 1890, p. 176; Sacc., Syll. ix, p. 97.

P. convex, then expanded, unequal, obtuse, adpressedly fibrillose or fibrillosely squamulose, becoming glabrous, brown or fuscous becoming paler, 2–2·5 cm.; g. adnexed, seceding, crowded, whitish then brownish; s. solid, curved, flexuous or twisted, narrowed downwards, fibrillose, pallid, about 3 cm.; sp. irregularly elliptic-oblong (angulato-ellipsoideis), 9–15 × 5–7 μ; c. fusoid-ventricose, 60–70 × 19–22 μ.

On naked earth. Finland.

decipiens, Bres., Fung. Trid., ii, p. 13, tab. cxviii; Sacc., Syll. xi, p. 51.

P. convex, then expanded and umbonate, floccosely-silky, disc even then breaking up into squamules, ochre-cinnamon, 3–5 cm.; g. crowded, broad, ventricose, sinuate, adnexed, cinnamon; s. glabrous, pallid, slightly striate, stuffed, base minutely marginato-bulbous, 4–5 cm.; sp. irregularly oblong, slightly tuberculate, 11–14 × 6–8 μ; c. ventricose, 50–70 × 15–25 μ.

Gregarious. Austria.

Allied to *I. lucifuga*.

cicatricata, Ellis and Everh., Journ. Myc., v, p. 25 (1889); Sacc., Syll. ix, p. 100.

P. broadly and obtusely conical or conic-campanulate, expanding to convex, densely grey fibrilloso-rimose except the smooth disc, which is livid when moist, 2–2·5 cm.; g. ascending, adnexed with a slight decurrent tooth, becoming sub-sinuate and brownish-cinnamon; s. short, stout, sub-bulbous, solid, nearly white, tomentose, then darker, 1·5–3 cm.; sp. very irregularly and coarsely nodulose, usually more or less elongated, 10–12 × 7–8 μ; c. broadly fusoid, not abundant, 45–55 × 12–15 μ.

Gravelly sand near filbert-trees. United States (Newfield, N.Y.).

Rather close to *I. Renneyi*.

Ellis gives the spore measurement as 7–9 × 5–6 μ, but in the specimen quoted below I find them larger.

(Ellis and Everh., N. Amer. Fung. Ser. ii, 1901, examined.)

infida, Massee; *Ag.* (*Heb.*) *infidus*, Peck, 27 Rep. State Mus., p. 95 (1874); *Ag. umbratica*, Quél., Assoc. Fr. 1883, tab. 6, f. 7; Sacc., Syll. v, p. 787; *I. leucocephala*, Boud., Soc. Bot. Fr. 1885, tab. 9, f. 1; Sacc., Syll. v, p. 765; *I. commixta*, Bres., Fung., Trid., i, p. 53, tab. 48, f. 2; Sacc., Syll. v, p. 787.

Entirely white. P. conico-campanulate then expanded and umbonate, silky-fibrillose, or more or less squamulose, white, or slightly tinged with grey or yellow, margin often splitting, 1·5–3 cm.; g. free, crowded, greyish-cinnamon; s. solid, minutely pruinose, apex scurfy, white, 3–5 cm.; sp. irregularly globose-oblong, nodulose, 9–10 × 6–7 μ; c. fusiform or subventricose, 40–50 × 12–15 μ. Smell earthy, strong.

On the ground in woods, &c. United States, France, Austria.

Superficially indistinguishable from the white form of *I. geophylla*, from which

the present species differs in the nodulose spores. Probably widely distributed, but passed over as *I. geophylla*. The pileus varies from silky-fibrillose to squamulose.

(Peck's type examined.)

trechispora, Karst., Hattsv., p. 465; Sacc., Syll. v, p. 789; *Ag.* (*Ino.*) *trechisporus*, Berk., Outl., p. 156, tab. 8, f. 6: Cke., Ill., pl. 403 A; *Ino. paludinella*, Sacc., Syll. v, p. 788; *Ag.* (*Ino.*) *paludinellus*, Peck, 31 Rep. State Mus., p. 34 (1878).

P. convex, then almost plane and umbonate, viscid at first then dry and silky, pallid or whitish, umbo often tinged ochre, 1·5–2·5 cm.; g. emarginate, whitish then greyish cinnamon; s. equal, pallid, often slightly flexuous, with a mass of white mycelium at the base, 3–5 cm.; sp. irregularly oblong, nodulose, 7–8 × 5–6 μ; c. fusoid or subventricose, stout, fairly abundant, 40–50 × 12–18 μ.

In woods in damp places. Britain, United States (Sandlake), and '5513, C. Wright, Con.,' in Herb. Kew.

Somewhat resembling *I. geophylla*, differing in the nodulose spores. Peck's Fungus agrees beautifully with Berkeley's in all essential features. Cooke's fig. 9, *I. trechispora*, is copied from Berkeley's original sketch, but the umbo is too dark, and the mass of white mycelium is not sufficiently emphasized in the reproduction. Saccardo's spore measurement of the spores—14–15 × 5–7 μ—is wrong, and has been copied from some one who has mistaken the species.

(Berkeley's and Peck's types examined.)

** *Stem coloured.*

fasciata, Sacc., Syll. ix, p. 95; *Ag.* (*Ino.*) *fasciatus*, Cke. and Massee, Grev., xvii, p. 52; Cke., Ill., pl. 1173.

P. campanulato-convex, silky, disc rufous the remainder pale tan, everywhere covered with minute dark squarrose scales, 5–7 cm.; g. adnexed, rounded or sinuate, narrowed in front, crowded, pallid; s. equal or slightly narrowed downwards, fibrillose, reddish within and without at the base, pallid above, solid, 5–7 cm.; sp. irregularly elliptical, minutely nodulose, 10 × 6 μ; c. ventricose, scanty, 40–50 × 12–15 μ.

On the ground among grass. Britain.

Densely caespitose, a feature which distinguishes the present from any other known species of *Inocybe*.

(Type examined.)

lanuginosa, Karst., Hattsv., p. 454; *Ag. lanuginosus*, Bull., Champ. Fr., tab. 370; *Ag.* (*Ino.*) *lanuginosus*, Fries, Syst. Myc., i, p. 257; *Ag. sabuletorum*, Berk. and Curtis, Grevillea, xix, p. 103; *Ino. sabuletorum*, Sacc., Syll. v, p. 765.

P. convex, then expanded, obtuse, velvety, the pile becoming matted together into little squamules which stand erect at the disc, umber or brown then yellowish, 1–2 cm.; g. sinuate or free, thin, ventricose, becoming clear brown, edge white, minutely fimbriate; s. solid, slender, fibrillosely squamulose or downy, brown, apex white and mealy, 2–3 cm.; sp. irregularly oblong, apiculate, with somewhat acute warts, 9–12 × 8 μ; c. fusoid, not very prominent, scattered, 40–50 × 13–15 μ.

On the ground in woods, &c. Britain, France, Austria, Russia, Sweden, Holland, United States ('Rav. No. 1239, Car. Inf.,' in Herb. Kew. under *I. lanuginosa*, and *Ag. sabuletorum*, B. and C.). Sandy pine woods, Car. (M.A.C.).

This species, as defined above, is the form accepted by European mycologists generally, and is represented in Roumeg., Fung. Gall., exs. 3814. In Brit. Fungus-Flora, ii, p. 183, the spores are incorrectly said to be smooth.

(Berkeley's type of *I. sabuletorum* examined.)

maritimoides, Peck, 38 Rep. State Mus., p. 87; Sacc., Syll. v, p. 771.

P. subconic or convex, dry, obtuse, densely squamulose, scales erect or fibrillose, minute, edge fibrillose, dusky brown, 1–2·5 cm.; g. adnate, rounded behind, ventricose, whitish then brownish-ochre; s. equal, solid, fibrillose, paler than p., 2·5 cm.; sp. ovate-oblong, very slightly nodulose, some practically smooth, 5–6 × 4·5–5 μ; c. ventricose, 36–45 × 10–12 μ, scattered.

Under trees. United States.

(Peck's type examined.)

calospora, Quél., in Bres., Fung. Trid., i, p. 19, tab. 21; Sacc., Syll. v, p. 773; *I. rigidipes*, Peck, 51 Rep. State Mus., p. 289 (1897).

P. convex or campanulate, then expanded and umbonate, fibrillose with darker squamules at the disc, yellowish-brown or tawny grey, edge paler, 1·5–2·5 cm.; g. sinuate, almost free, tawny-ochre or brownish; s. slender, pale then reddish, or coloured like p., 4–5 cm.; sp. globose, with numerous rather long, slender, cylindrical papillae, 10–12 μ; c. not numerous, subcylindrical or slightly fusiform, 45–55 × 11–14 μ.

On the ground in woods and shady places. France, Britain (Wothorpe), United States (Menands, Albany Co.).

Peck noted the affinity of his species with *I. calospora*, but pointed out that the colour of his Fungus differed in being tawny grey. The two however appear to belong to the same species. The spores and cystidia are identical.

(Type of Peck's species examined.)

stellatospora, Mass.; *Ag.* (*Hebeloma*) *stellatosporus*, Peck, 26 Rep. State Mus., p. 57; Sacc., Syll. v, p. 798.

P. convex, rough with numerous squarrose scales, brown, 2·5 cm.; g. pallid then brown; s. equal, scaly, colour of p., 5 cm.; sp. subglobose, rather coarsely nodulose, 7–8 μ; c. broadly fusiform, tapering into a long, slender pedicel, thin-walled, 70–80 × 14–20 μ.

On the ground in woods. United States (Croghan).

Bears a close resemblance to *I. mutata*, but the persistent scales and rough spores distinguish it (Peck).

(Peck's type examined.)

putilla, Bres., Fung. Trid., p. 81, tab. 88; Sacc., Syll. xi, p. 50 (written *pusilla*).

P. conico-campanulate, then expanded and umbonate, fibrillosely-silky, then becoming torn into cracks, clay-colour or greyish-brown, or fuscous becoming pale, margin persistently lurid whitish, 1·5–3 cm.; g. somewhat crowded, sinuato-adnate, whitish then greyish-tan, edge crenulate; s. stuffed, very faintly tinged rose, white-fibrillose becoming glabrous, apex white scurfy, 3–4·5 cm.; veil white, very evident when young; sp. coarsely nodulose, 8–10 × 6–7 μ; c. fusiform, 60–70 × 15–20 μ; flesh of stem tinged rose. Smell strong, earthy.

On the ground under hazel, &c. Austria.

Allied to *I. perbrevis*.

Gaillardi, Gillet, Rev. Myc., v, p. 31 (1883); Gill., Champ. Fr., Hymen, a fig.; Sacc., Syll. v, p. 773; Pat., Tab. Anal., p. 11, f. 8; *subfulva*, Peck, 41 Rep. State Mus., p. 66 (1888); Sacc., Syll. ix, p. 96; *Ino. echinocarpa*, Ellis and Everh., Journ. Myc., v, p. 25 (1889); Sacc., Syll. ix, p. 95.

P. conico-campanulate, then expanded and umbonate, pilosely-squamulose, the disc bristling with larger and stronger scales, tawny-yellow to rusty, 1–2 cm.; g. nearly free, ventricose, broadish, rather crowded, brownish-cinnamon, edge whitish; s. slender, fibrillose, about colour of p., 1·5–3 cm.; sp. subglobose or very slightly elongated, covered with long, slender spines, 10–12 × 8–9 μ; c. scanty, not very prominent, subcylindrical, 40 × 9–12 μ.

On the ground under trees, &c. France, United States.

Readily distinguished by the nature of the spores, which are sparsely covered with long, very slender, pointed spines, and the disc of the pileus with squarrose scales. The cystidia are rare, and apt to be overlooked.

This species appears to be not uncommon in the United States, for in addition to having been collected by Peck and Ellis, it is present in the Kew Herbarium, under *I. trechispora*, 'Rav., 2845, Car. Inf.'; under *I. lacera*, 'Rav., 1588, Car. Inf.'; also under *I. lacera*, 'C. Wright, 5526, Connecticut.'

(Type from Peck of *I. subfulva*, also *I. echinocarpa*, in E. and E., N. A. Fung., ser. 2, no. 1904, examined.)

Trinii, Karst., Hattsv., p. 463; Sacc. Syll., v, p. 781; Mass., Brit. Fungus-Flora, ii, p. 197; *Ag.* (*Ino.*) *Trinii*, Fries, Hym. Eur., p. 233; Cke., Ill., pl. 428 B. *Ag. Trinii*, Weinm., Hym.- et Gastero-mycetes Ross., 1836, p. 194.

P. hemispherical, obtuse, whitish with a rufous tinge, due to longitudinal rufous fibrils, tawny when dry, 1–2 cm.; g. rounded behind and adnexed, ventricose, dusky, cinnamon, edge white-flocculose; s. equal, stuffed, covered with loose reddish or rufous fibrils, apex with white meal, 4–6 cm.; sp. subglobose or somewhat oblong, nodulose, 9–10 μ or 9–10 × 6–8 μ; c. ventricose, abundant, 50–60 × 14–17 μ. Smell pleasant, strong, resembling that of clove-pinks.

Among grass. Russia, Britain.

My conception of *I. Trinii* is as described above, and as figured by Cooke, l. c., which agrees well with Weinmann's diagnosis. The fragrant, strong, clove-pink odour was very strongly marked in the fresh plants from which Cooke's figures were drawn, and persists for some time after drying.

No species of *Inocybe* appears to be so little understood among mycologists as the present species. This I attribute to their not being conversant with Weinmann's own description of his plant, which is as follows:—

'*A. Trinii Weinm.* Pileo carnoso-membranaceo, hemispherico, albido, rufescente-fibrilloso, obtuso; lamellis rotundatis, adfixis, obscure cinnamoneis, margine albo-flocculosis; stipite aequali, farcto, rutilante-fibrilloso, apice albo-pulverulento.

'Solitarius. Odor valde suavis et fere caryophyllaceus !—

'Pileus ½′ et paulo ultra lat. longitudinaliter fibrosus. Lamellae 2″ fere latae. Stipes 2–3′ long., 1–1¼″ cras., fibrillis longitudinalibus obsitus. Sporidia sordide ferruginea.'

From this description it will be gathered that the Fungus is quite small, even for

an *Inocybe*. No reference is made to the plant becoming red when broken, as insisted upon by other people.

I. Trinii, Weinm., Bresadola, Fung. Trid., ii, p. 14, tab. 120, with a whole string of synonyms, and with 'odore forti terreo' and smooth spores, is *I. Godeyi*, Gill.

maritima, Karst., Hattsv., p. 457 (1879); Sacc., Syll. v, p. 771 (1887); *Ag.* (*Ino.*) *maritimus*, Cke., Ill., pl. 392; *Ag. maritimus*, Fries, Obs. Myc., ii, p. 41 (1818).

P. hygrophanous, convex then almost plane and umbonate, flocculosely fibrillose, subsquamulose, brownish mouse-colour or umber, paler and hoary when dry, 2–2·5 cm.; g. rounded and adnexed, then almost or quite free, broadish, grey then rusty; s. solid, equal, straight, fibrillose, slightly paler than p., apex naked, 1·5–2·5 cm.; sp. irregularly oblong, apiculate, nodulose, 10–11 × 7–8 μ; c. ventricose, 45–55 × 12–18 μ, not uncommon.

Often caespitose. Damp sand on sea-shore, also on ground in woods. Sweden, Britain, Germany, Solomon Islands (Dr. Guppy).

Distinguished by the umber hygrophanous pileus becoming pale and hoary when dry. Allied to *I. lanuginosa*.

(Specimen from Fries examined.)

umbrina, Bres., Fung. Trid., i, p. 50, pl. 55; Sacc., Syll. v, p. 772.

P. convexo-campanulate, becoming almost plane and umbonate, chestnut-brown, somewhat viscid, fibrillosely woolly, then beautifully rimose, disc sometimes verruculose, 2–3·5 cm.; g. sinuato-adnate, crowded, dingy citrine, then rufous-cinnamon, edge darker; s. stuffed, then partly hollow, equal, base slightly bulbous, fibrillose, somewhat paler than p., apex obsoletely white-scurfy, 4–6 cm.; sp. globose or irregularly oblong, coarsely nodulose, 7–8 × 5–6 μ; c. ventricose, 60–70 × 14–18. Greyish-brown veil very evident in the young plant.

Gregarious or subcaespitose in pine woods. Austria.

When young resembling *I. carpta*, and when old resembling *I. asterospora*, but distinct from both (Bres.).

umboninota, Peck, 38 Rep. State Mus.; Sacc., Syll. v, p. 780.

P. broadly campanulate or expanded, prominently umbonate, rimoso-fibrillose, dusky brown, 3–5 cm.; g. whitish, then rusty brown; s. equal or the base very slightly thickened, solid, fibrillose, paler than the pileus, apex pruinose, 3–3·5 cm.; sp. irregularly oblong, very slightly nodulose, 6 × 4·5–5 μ; c. very slightly ventricose or subcylindrical, 50–60 × 11–14 μ, abundant.

On the ground among moss in woods. United States (Caroga).

Differs from *I. rimosa* in the nodulose spores, and from *I. asterospora* in the very prominent umbo and different spores.

(Peck's type examined.)

rufoalba, Sacc., Syll. v, p. 787; *Ag.* (*Ino.*) *rufoalbus*, Pat. and Doass., Rev. Myc., 1886, p. 26; Pat. Tab. Anal., fig. 548 (1886).

P. convex, umbonate, brown, covered with a delicate white silky tomentum, which gives to the pileus a white appearance, except the umbo, which is always brown, up to 1 cm.; g. almost free, reddish-brown; s. slender, equal, reddish, covered

everywhere with a white silkiness that hides the colour, 1–3 cm.; sp. irregularly oblong, nodulose, 9–10 × 4–5 μ; c. ventricose.

On the ground. France.

Allied to *I. scabella.*

Renneyi, Sacc., Syll. v, p. 788; *Ag.* (*Ino.*) *Renneyi,* B. and Br., Ann. Nat. Hist., no. 1761; Cke., Ill., pl. 520 A.

P. hemispherical, slightly fibrillose, disc brown, remainder pale fawn-colour, 1·5–2 cm.; g. rounded behind and almost free, dingy ochraceous; s. slightly narrowed downwards, fibrillose, solid, paler than p., 3–5 cm.; sp. angularly oblong, slightly nodulose, one end pointed, 11–13 × 7–8 μ; c. rather thin-walled, fusoid, 40–50 × 12–16 μ, much scattered and apt to be overlooked.

On the ground. Britain.

Allied to *I. cicatricata,* an American species.

(Type specimen examined.)

Var. **major,** Cke., Ill., pl. 520 B.

Coloured like the typical form, but larger; p. campanulate, up to 2·5 cm.; g. broadly adnate, cinnamon colour; s. equal; sp. slightly nodulose, 13–17 × 10 μ; c. as in the typical form.

In fir woods. Britain.

(Type examined.)

subexilis, Peck, 38 Rep. State Mus., p. 87; Sacc., Syll. v, p. 785.

P. convex or subcampanulate, then expanded and umbonate, edge fibrillose, at first pale chestnut, then yellowish or subochraceous, up to 1 cm.; g. narrow, rather crowded, rounded behind, subventricose, whitish then subochraceous; s. solid, flexuous, slightly pruinose, rosy-white then yellowish, slightly striate, about 2 cm.; sp. subglobose, slightly nodulose, 6 or 6 × 5 μ; c. slightly ventricose, numerous, 45–60 × 12–15 μ.

Mossy ground in woods. United States (Caroga).

(Peck's type examined.)

fulvella, Bres., Fung. Trid., ii, p. 16, tab. cxix, f. 2; Sacc., Syll. xi, p. 51.

P. subhygrophanous, conico-campanulate then expanded and umbonate, floccosely silky, disc glabrous, tawny, remainder at first yellowish olive, then yellowish or brownish olive, 6–12 mm.; g. rather distant, ventricose, pale lilac then ochraceous cinnamon, edge fimbriate, rounded behind and nearly free; s. stuffed, narrowed downwards, glabrous, apex white-pruinose, lilac then rufescent, 2–2·5 cm.; sp. irregularly oblong, tuberculose, 8–9 × 5–6 μ; c. ventricose, 45–60 × 12–18 μ. Flesh yellow, rufescent-lilac at apex of stem.

Shady ground. Austria.

Allied to *I. scabellus,* which differs in having smooth spores.

II. *Cystidia absent.*

ignobilis, Sacc. Syll. xi, p. 50; *Ag.* (*Heb.*) *ignobilis,* Berk., Lond. Journ. Bot., i, p. 452 (1842).

P. glabrous, silky, plane or slightly depressed at the centre, rufous-brown, 1·5–2 cm.; g. sinuate, adnexed, and with a decurrent tooth, broad, rusty; s. incurved,

equal, solid, glabrous, paler than p., 4–5 cm.; sp. irregularly oblong, nodulose, 11–12 × 8–9 μ; c. wanting.

On the ground. New Ireland.

One of the very few species having nodulose spores and no cystidia.

(Berkeley's type examined.)

margarispora, Sacc., Syll. v, p. 781; *Ag.* (*Ino.*) *margarispora,* Berk. ms. in Cke., Hdbk., ed. ii, p. 157; Cke., Ill., pl. 505.

P. campanulate, then expanded and broadly umbonate, often flexuous, silky, clad with adpressed fibrillose scales, fawn-colour or pale yellowish-brown; 3–5 cm.; g. adnexed, pallid; s. solid, equal, fibrillose, pallid; sp. subglobose, coarsely nodulose, 8–9 μ; c. absent.

On the ground. Britain.

Resembles *I. asterospora* in general appearance and in spore characters, but differs in the absence of cystidia. *I. eutheles* differs in the smooth spores.

(Type specimen examined.)

Bucknalli, Massee (sp. nov.).

P. campanulato-convex, fibrillose, with a few squamules near the disc, brownish, 1–2 cm.; g. adnexed, thick, rather distant, rusty-brown, edge minutely fimbriate; s. equal or slightly thickened at the base, slender, fibrillose, brownish, 2–4 cm.; sp. irregularly oblong, one end obliquely apiculate, rather coarsely nodulose, 15–17 × 8–9 μ; c. absent; basidia clavate, exceptionally large, 70–80 × 16–18 μ, 4-spored.

On the ground under bushes. Britain (Leigh Down, Bristol. Cedric Bucknall).

A little insignificant-looking brown Fungus, without any marked external characteristics, but at once distinguished by the size of the basidia, which are more than twice the size of those of any other known species. The spores and periphyses are also exceptionally large.

The fimbriate edge of the gills is due to the presence of numerous large thin-walled clavate cells about 75–85 × 15–20 μ. These differ in structure from the cystidia, which spring from the sides and not from the edge of the gills.

Spores rough, no knowledge of cystidia.

* *Stem whitish or pallid.*

grammata, Quél., Soc. Sci. Nat. Rouen, 1879, tab. 2, f. 8; Sacc., Syll. v, p. 781.

P. campanulate, fibrous then splitting, cream-white then bistre or buff, 5–6 cm., flesh white; s. bulbous, striate, tomentose, white, then like the flesh, assuming a rosy tint, 5–7 cm.; g. adnate, greyish then yellowish cinnamon; sp. elongated, nodulose, 10 μ long.

Sandy ground under birches. France.

albipes, Gillet, Tab. Anal. Hymen., p. 113 (1884); Sacc., Syll. v, p. 780.

P. conical then campanulate, at length nearly plane and mammilate, longitudinally fibrously cracked, dingy yellow, centre darker, edge wavy and splitting when old, 4–5 cm.; g. free, ventricose, crowded, thickish, yellowish-white then

brownish; s. entirely white, squamulose, stuffed, firm, striate, 6–8 cm.; sp. irregularly nodulose. Flesh white.

On the ground. France.

** *Stem coloured.*

asinina, Kalchbr., Icon. Hym. Hung., p. 38, pl. xxii, fig. 1 (1873); Sacc., Syll. v, p. 771; *Ag.* (*Ino.*) *asininus*, Kalchbr., in Fries, Hym. Eur., p. 230.

P. convex then plane, subgibbous, dry, adpressedly fibrillose, hoary, at length rufous-tan, 3–6 cm.; g. adnate, becoming distinctly sinuate, rather crowded, broad, yellowish-grey then dusky cinnamon, edge paler; s. solid, subventricose, or equal in small specimens, attenuated upwards, generally twisted, brownish-tan, fibrillose from the lax veil, annular zone fairly persistent, becoming umber from the spores, about 5 cm.; sp. subglobose, nodulose.

On the ground. Gregarious or subcaespitose. Hungary, Holland.

radiata, Peck, Bull. Torr. Bot. Club, xxii, p. 488 (1895); Sacc., Syll. xiv, p. 133.

P. convex or subcampanulate, distinctly umbonate, silky-fibrillose, slightly rimulose, distinctly radiately wrinkled when dry, yellowish-brown, umbo darker, 2–5 cm.; g. emarginate, rather broad, crowded, brownish becoming tawny-cinnamon, edge whitish; s. equal, solid, almost glabrous, a little paler than p., 3–5 cm.; sp. subovate, slightly nodulose or angular, 10–13 × 5–6 μ.

Open grassy ground. United States (Mass.).

The radiations of the pileus are not noticeable in the fresh plant (Peck).

I have not seen a specimen of this species, which appears to depend for its distinctness mainly on a character not present in the living plant.

B. Spores Smooth.

III. *Cystidia present.*

* *Stem whitish or pallid.*

† *Gills brownish, ochraceous, or cinnamon.*

hirtella, Bres., Fung. Trid., i, p. 52, tab. lviii, f. 1; Sacc., Syll. v, p. 770.

P. conico-campanulate, then expanded and umbonate, margin soon splitting, yellowish straw-colour, with numerous darker pilose squamules, disc glabrous, 1·5–2·5 cm.; g. adnate, rather crowded, brownish, edge white-pruinose; s. stuffed, white then tinged straw-colour, slightly narrowed downwards, white-plumulose under a lens, with a minute subterranean bulb, 2–4 cm.; sp. pip-shaped, smooth, 10–12 × 6 μ; c. fusoid, 60–70 × 12–15 μ.

On the ground. Austria.

Quélet (Fl. Myc., 105) considers this species to be a variety of *I. lucifuga*, from which it only differs in the straw-coloured pileus with darker squamules and brown gills. Spores and cystidia are the same in both.

scabra, Karst., Hattsv., p. 457 (1879); Sacc., Syll. v, p. 767; *Ag.* (*Ino.*) *scaber*, Fries, Hym. Eur., p. 228; Cke., Ill., pl. 391; *Ag. scaber*, Müll., Fl. Daw., v, fasc. xiv, p. 7, tab. 832, f. 3 (1782); Low., Eng. Fungi, pl. 207.

P. broadly conical, often subgibbous, dusky or pale yellowish tan, variegated with fibrous, adpressed darker scales, 1·5–3 cm.; g. adnexed, somewhat crowded,

pale then dusky or brownish; s. stout, short, equal, slightly thickened at the base, solid, whitish, distinctly cortinate, silky-fibrillose, 2–3 cm.; sp. pip-shaped, smooth, 9–11 × 5–6 μ; c. slightly ventricose, 65–75 × 12–16 μ, abundant. Flesh white, not changing colour.

On the ground in coniferous and mixed woods. Britain, France, Germany, Sweden, Denmark, Holland.

The above agrees with the species as understood by Fries, and represented as *I. scabra* in Roum., Fung. Gall., exs. 1902, and Rabenh., Fung. Eur. 1902.

This Fungus is much more sturdy and with a thicker stem than other allied species. The species may practically be considered as originating with Fries, for although he quoted Müller's figure and adopted his name, there is really nothing to prove that the species accepted by Fries was the plant so named by Müller, whose brief description is as follows:—

'*Agaricus* scaber *lamellis et stipile albis, pileo hemispherico fusco, squamoso.*'

Müller's figure represents a group of quite young specimens, and does not suggest anything very definite.

pyriodora, Karst., Hattsv., p. 456; Sacc., Syll. v, p. 766; Cke., Ill., pl. 472; Bres., Fung. Trid., tab. lii; *Agaricus pyriodorus,* Pers., Syn., p. 300.

P. ovate, then campanulate, at length expanded and umbonate, pale ochre, sometimes reddish when young, 4–7 cm.; g. adnate then rather rounded behind, thin, crowded, brownish, edge whitish; s. solid, nearly equal, often curved near the base, fibrillose, pallid, apex with white meal, flesh reddish when cut; sp. pip-shaped, apiculate, smooth, 9–10 × 5–6 μ; c. variable in form, ventricose or clavate, 40–50 × 15–17 μ, scattered. Smell pleasant, resembling ripe pears.

In woods. Britain, France, Germany, Austria, Sweden, Russia, Finland, United States (Waynesville, Ohio, T. G. Lea, 1844).

Odour penetrating, like that of rotten pears or *Hyacinthus racemosus* (Berk.).

The above diagnosis accords with what is universally accepted by mycologists as the species of Persoon.

rimosa, Karst., Hattsv., p. 462; Sacc., Syll. v, p. 775; *Ag. rimosus,* Bull., Champ. Fr., tab. 388; *Ag.* (*Ino.*) *rimosus,* Cke., Ill., pl. 384.

P. campanulate, sometimes subumbonate, silky-fibrous and becoming cracked from disc to margin, yellowish-brown, 2·5–5 cm.; g. almost free, somewhat crowded and ventricose, dingy tan; s. equal, firm, nearly smooth, whitish, apex mealy, 4–7 cm.; p. pip-shaped, smooth, 12–15 × 7 μ; c. ventricose, scattered, 60–65 × 15–18 μ. Smell earthy.

On the ground in woods. Britain, France, Germany, Sweden, Russia, Finland, Holland.

Differs from *I. asterospora* and *I. fastigiata* in the smooth spores. *I. eutheles* is separated by the adnate gills and umbonate pileus, and *I. pyriodora* by the strong smell.

One of the old species about the identity of which practically all are agreed. It is represented in C. Roumeg., Fung. Sel., exs. 5306; Roumeg., Fung. Gall., exs. 1302 and 3813; Sydow, Myc. March, 2609.

subochracea, Massee; *Ag.* (*Heb.*) *subochraceum,* Peck, 23 Rep. State Mus., p. 95 (1870); *Heb. subochraceum,* Sacc., Syll. v, p. 796.

P. conical or convex, sometimes expanded, generally umbonate, fibrillously squamulose, pale ochraceous, 2–3·5 cm.; g. attached, emarginate, rather broad, whitish then brownish-yellow; s. equal, whitish, slightly fibrillose, solid, 2·5–5 cm.; sp. pip-shaped, smooth, 8 × 4·5–5 μ; c. ventricose, 45–60 × 12–15 μ, fairly abundant.

On the ground in fields and by roadsides. United States (North Elba and West Albany).

(Type from Peck examined.)

cortinata, Roll., Bull. Soc. Myc., xvii, p. 117, pl. 3, f. 1 (1901).

P. campanulate with a stout umbo, pale straw-colour, umbo rusty, at first minutely fibrilloso-striate then torn and deeper coloured, up to 4 cm.; veil white, floccose, appendiculate; g. adnato-decurrent, ventricose, whitish then brownish-ochre, edge paler, floccoso-serrulate; s. stuffed, white, minutely fibrillosely striate, scurfy upwards, fragile, curved, flexuous, the imperfect ring fibrillose, white, median, cylindrical, base usually sub-bulbous, 6–8 cm.; sp. pip-shaped, smooth, 8 × 4–5 μ; c. ventricose.

Gregarious under pines. Belgium.

Differs from *I. vatricosa* in the veil not being viscid. Perhaps a cortinate form of *I. sindonia* (Rolland).

eutheles, Sacc., Syll. v, p. 776; *Ag.* (*Ino.*) *euthelus*, B. and Br., Ann. Nat. Hist., 1865, pl. viii, f. 2; Cke., Ill., pl. 386.

P. campanulate then expanded and strongly umbonate, shining, silky, rather squamulose, pale fawn-colour, 2·5–5 cm.; g. broadly and abruptly adnate, narrowish, pallid, edge whitish, denticulate; s. equal, slightly swollen at the very base, fibrous, solid, pallid or whitish, 4–8 cm.; sp. elliptical, smooth, 9–10 × 5–5·5 μ; c. fairly abundant, stout, ventricose, 60–65 × 15–20 μ. Smell mealy.

On the ground among pine leaves. Britain, France.

I. pallidipes and *I. eutheloides* are closely allied to this species, which also bears a general resemblance to *I. fastigiata*, but differs in having smooth spores. The large upper figure and section in Cooke's Ill. are copied from Berkeley's original drawing, the other figures on the plate are not authoritative.

(Berkeley's type examined.)

pallidipes, Ellis and Everh., Journ. Myc., v, p. 24 (1889); Sacc., Syll. ix, p. 96.

P. conico-campanulate, then expanded and umbonate, light brown, fibrose-squamose, disc innately scaly, margin subrimose, 2–3 cm.; g. broadly attached with a strong decurrent tooth, ascending at first then ventricose, scarcely crowded, rather broad, pale cinnamon, edge paler and fimbriate; s. white, slightly narrowed and mealy above, loosely fibrillose below, sub-bulbous and with white tomentum at the base, solid, 2·5–5 cm.; sp. pip-shaped, smooth, 8–9 × 5 μ; c. fusoid or subventricose, numerous, 40–50 × 14–18 μ.

On the ground under filbert-trees. United States (Newfield, N.Y.).

The disc of the pileus is carnose, and in wet weather rimose-squamose. Well marked by its conic-campanulate pileus and *white stem,* which remains white till the plant withers (E. and E.).

Very near to *I. eutheles*, if indeed distinct. Differing mainly in the broader gills having a strong ecurrent tooth. Also allied to *I. eutheloides*.

(Specimen in Ellis and Everh., N. Amer. Fung., ser. ii, 2102, examined.)

sambucina, Sacc., Syll. v, p. 782 ; *Ag.* (*Ino.*) *sambucinus*, Fries, Syst. Myc., i, p. 257 (1821); Fries, Icon. Sel., tab. 109, f. 2 ; Cke., Ill., pl. 399.

P. convex then expanded, obtuse or subumbonate, often wavy, silky-fibrillose, nearly glabrous and not cracking, white, often becoming tinged yellow, 5–8 cm. ; g. emarginate, slightly adnexed, broad, ventricose, whitish then dingy ochre ; s. stout, short, often curved, equal or thickened at the base, fibrillosely striate, white, solid, 2·5–3·5 cm. ; sp. elliptical, smooth, 9–12 × 6 μ; c. scattered, ventricose, 50–60 × 12–16 μ. Smell strong.

Solitary. In dry pine woods, &c. Britain, France, Germany, Sweden.

A stout Fungus, entirely white, the pileus often becoming yellowish with age. *I. sindonia* differs in the narrow gills, stuffed then hollow stem, and smaller spores.

Clarkii, Sacc., Syll. v, p. 784 ; Cke., Ill., pl. 429 B.

P. campanulate, obtuse, whitish, silky-fibrillose, 2–3 cm. ; g. adnexed, rather distant, broadish, pallid, margin white; s. equal or slightly thickened at the base, solid, white, 3–5 cm. ; sp. elliptical, smooth, 8–10 × 5–6 μ ; c. scattered, ventricose, 55–65 × 12–16 μ, some thinner.

On the ground in shady places. Britain.

Allied to *I. sindonia*, but separated by the solid stem, persistently pale gills, and larger spores.

(Type specimen examined.)

corydalina, Quél., Jur. et Vosg., iii, p. 115 ; Soc. Bot. Fr., xxiv, t. 5, f. 10 ; Sacc., Syll. v, p. 766.

P. campanulate then expanded, fibrillose, white, the prominent umbo glaucous-green, 4–6 cm. ; g. adnate, emarginate, brown, edge white ; s. fragile, white ; sp. pip-shaped, smooth, 8–10 × 5 μ ; c. ventricose, 50–60 × 12–15 μ. Smell strong, like *Corydalis cava*. Flesh white, sometimes tinged lilac.

Woods. France.

(Specimen from Quélet examined.)

Var. *roseola*, Pat., Ann. Tab. Fung., no. 553.

Pileus entirely green ; flesh tinged rosy when cut.

France.

geophylla, Karst., Hattsv., p. 464 ; Sacc., Syll. v, p. 784 ; *Ag.* (*Ino.*) *geophyllus*, Fries, Epicr., p. 176 ; Cke., Ill., pl. 401.

P. conical then expanded and umbonate, minutely fibrillose, satiny and shining, often cracking, pure white, sometimes tinged yellow when old, 1·5–3 cm. ; g. almost free, rather broad, ventricose, crowded, pale then becoming dingy clay-colour ; s. stuffed, satiny, apex minutely floccose, white, equal, base slightly thickened, often rather flexuous, 4–7 cm. ; sp. elliptical, slightly apiculate, smooth, 7–9 × 4–5 μ ; c. fairly abundant, ventricose, 45–60 × 10–16 μ. Smell earthy.

On the ground in woods, &c. Britain, Ireland, France, Germany, Sweden, Switzerland, Italy, Austria, Holland, Russia, United States.

A very distinct and well-marked species, but at the same time very variable in the colour of the pileus, which ranges from pure white, the commonest form, to yellow, lilac, violet, tawny, and brick-red. Some of these colour-forms have been quite unnecessarily considered as varieties, as var. *fulvus*, Pat., Tab. Anal., n. 544; var. *violaceus*, Pat., Tab. Anal., n. 545. The pileus is never truly squamulose.

One of the old species on which all mycologists, up to the present, are agreed. As defined above it is represented in the following exsiccati: Rab., Fung. Eur., 10; Thüm., Myc. Univ., 2001; Thüm., Fung. Austr., 1104; Desm., Crypt. France, 459; Desm., Crypt. France, ser. 2, 458; Sydow, Myc. March, 2610.

Whitei, Sacc., Syll. v, 790; *Ag.* (*Ino.*) *Whitei*, B. and Br., Ann. Nat. Hist., no. 1,527; *I. agglutinata*, Peck, 41 Rep. State Mus., p. 65; Sacc., Syll. ix, p. 98.

P. conical then convex, sometimes umbonate, fibrillose, tawny, margin whitish, then wholly pale tawny, slightly viscid, 1·5–2·5 cm.; g. adnexed, crowded, white then cinnamon; s. solid, nearly equal, base slightly thickened, whitish and pulverulent, becoming brownish downwards, 3–6 cm.; sp. pip-shaped, smooth, 9–11 × 4–5 μ; c. fairly abundant, ventricose or almost cylindrical, 50–60 × 16–20 μ.

On the ground under conifers. Britain, United States (Catskill Mts.).

Both Berkeley and Peck indicate the affinity of the present species with *I. geophylla*.

(Types from Berkeley and Peck examined.)

sindonia, Karst., Hattsv., p. 464; Sacc., Syll. v, p. 784; *Ag.* (*Ino.*) *sindonius*, Cke., Ill., pl. 400.

P. campanulato-convex, broadly umbonate, silkily downy when young, becoming almost glabrous, never fibrillose, when young the margin is appendiculate from the fibrils of the veil, white, pallid, or yellowish, 3–5 cm.; g. slightly adnexed, narrow, brownish white; s. soft, with a distinct pith then hollow, slightly fibrillose then glabrous, white, equal, 5–7 cm.; sp. pip-shaped, smooth, 8–10 × 5–6 μ; c. ventricose, 50–60 × 12–16 μ.

On the ground in damp, shady places. Britain, Germany, Sweden.

Resembling *I. geophylla* superficially; differing in the hollow stem, larger size, absence of earthy smell, &c.

descissa, Karst., Hattsv., p. 463; Sacc., Syll. v, 777; *Ag.* (*Ino.*) *descissus*, Fries, Epicr., p. 174; Cke., Ill., pl. 428.

P. conico-campanulate then expanded, edge usually slightly incurved, fibrillose, becoming radiately rimose and splitting when expanded, whitish or pale dingy brown, 1·5–2·5 cm.; g. almost free, somewhat crowded, white then brown; s. almost hollow, equal, often slightly wavy, fibrillose, white, apex with white meal, fragile, 2–3·5 cm.; sp. elliptic-oblong, sometimes slightly curved, apiculate, smooth, 8–10 × 5 μ; c. ventricose, scattered, 50–60 × 12–16 μ.

On the ground in woods. Britain, France, Sweden, Holland.

A small species somewhat resembling *I. geophylla*, differing in the colour of the pileus and absence of a strong earthy smell. I have accepted, as typical of this species, specimens determined by Berkeley and figured by Cooke (Ill., pl. 428, top figs.). The colour of the pileus is too bright in these figures.

Some authors give *Ag. auricomus*, Batsch, as a variety of this species. I have

not seen an authentic specimen, nor have I at any time collected a Fungus that seemed to be the species intended by Batsch, whose own diagnosis I give.

'*A. auricomus.* Pileo luteo, lineis fuscis radiatim piloso; stipite lineari, valido, albo; lamellis fusco-cinereis.' Batsch, Elench. Fung., 1783, p. 75, tab. v, f. 21.

Batsch's figure represents a small Fungus, pileus 1·5 cm. diam.; stem 2 cm. long, and coloured brown like the pileus, although stated to be white in the description.

cervicolor, Quél., Flor. Myc., p. 107; *Ag. cervicolor*, Pers., Icon. Pict. Rar. Fung., tab. 8, f. 4 (1803–1806).

P. campanulate, pale brown or fawn-colour, covered with brown, recurved fibrils, 3–5 cm.; g. emarginate, ventricose, distant, pale then rusty brown, margin whitish, denticulate; s. elongated, slender, firm, whitish, fibrillose with brown recurved filaments throughout its length, 6–9 cm.; spores elongate pip-shaped, smooth, 11–13 × 6–6·5; c. cylindric-fusoid, numerous, 40–50 × 12–18 μ. Flesh white, tinged purplish when cut. Smell strong, unpleasant.

Among grass in woods. Britain, France.

Quélet gives *I. Bongardi* as a synonym of the present species, which I think is not correct. *I. Bongardi* differs in the whitish mealy apex of the stem, arcuato-adnate gills, and different smell, which Weinmann says 'odor exacte ut in Pyro. com. var. "Bergamotte" dicta.' Quélet says of *I. cervicolor* 'odeur de tonneau moisi.'

(Specimen accepted as typical placed in Herb. Kew.).

deglubens, Karst., Hattsv., p. 459; Sacc., Syll. v, p. 769; *Ag. deglubens*, Fries, Epicr., p. 173; *Ag.* (*Ino.*) *deglubens*, Cke., Ill., pl. 394.

P. convex then expanded and obtusely umbonate, the cuticle becoming broken up into adpressed fibres, disc more or less squamulose, brownish bay then yellowish, the fibres and squamules darker, 1·5–2–5 cm.; g. adnate, subsinuate, ventricose, somewhat distant, greyish then cinnamon; s. solid, adpressedly fibrillose and almost glabrous, pallid, sometimes tinged lilac, apex slightly rough with brown points, 4–7 cm.; sp. 8–10 × 5–6 μ, pip-shaped, smooth; c. fairly abundant, ventricose, 50–60 × 10–15 μ. Flesh white.

On the ground in pine woods, &c. Britain, France, Germany, Sweden, Finland.

Differs from *I. lacera* in the apex of the stem being darker than the remainder, instead of being white and mealy.

Var. **trivialis**, Karst., Symb. Myc., ix, p. 43.

P. somewhat resembling in character the typical form, but up to 5 cm. across; s. fibrillose then becoming glabrous, apex whitish, somewhat mealy, then becoming darker and somewhat rufescent both outside and inside; sp. cymbiform; 10–12 × 4–5 cm.

Among grass. Finland.

infelix, Peck, 41 Rep. State Mus., p. 29; Sacc., Syll. ix, p. 96.

P. campanulate then convex or expanded and subumbonate; fibro-squamulose, greyish brown or umber, margin sometimes torn, 2·5 cm.; g. crowded, emarginate, ventricose, broadish, whitish then rusty-brown; s. equal, solid, pallid or whitish, apex white and pruinose, 2·5–5 cm.; sp. cylindric-elliptical, smooth, 11–13 × 5 μ;

c. fairly abundant, broadly ventricose, 40–50 × 14–20 μ, some narrower and almost cylindrical.

Mossy ground. United States (Indian Lake).

(Type from Peck examined.)

†† *Gills with an olive tinge.*

abjecta, Karst., Hattsv., p. 456; Sacc., Syll. v, p. 768.

P. subcampanulate or convex then expanded, sometimes subumbonate, brownish, becoming ochraceous-brown when dry, everywhere covered with white fibrils, disc with whitish subsquarrose squamules, 1–2.5 cm.; g. adnate, rather distant, broad, ventricose in front, pale cinnamon-olive, margin minutely flocculoso-crenulate at first; s. solid, equal, rather tough, flexuous, pallid, everywhere covered with white fibrous squamules, apex white-pruinose, 3–4 cm.; sp. pip-shaped, smooth, 10–14 × 5–7 μ; c. scanty, ventricose, 45–65 × 12–15 μ. Flesh persistently white.

On naked ground near paths, &c. Finland, Sweden.

(Portion of type examined.)

destricta, Karst., Hattsv., p. 462; Sacc., Syll. v, p. 777; *Ag.* (*Ino.*) *destrictus,* Fries, Epicr., p. 174; Fries, Icon. Sel., tab. 108, fig. 3; Cke., Ill., pl. 387; *Ag. Bongardi,* Kalchbr., Icon., tab. 20, f. 1.

P. convex then expanded, usually becoming depressed round the umbo, pallid then rufescent, cuticle with wide cracks showing the white under stratum, sometimes the cuticle is more or less broken up into fibrils or squamules, 3–8 cm.; g. uncinately adnate, crowded, whitish then dusky cinnamon with an olive tinge; s. nearly equal, glabrous, fibrillosely striate, whitish, becoming reddish with age, apex slightly mealy, solid, 4–5 cm.; sp. pip-shaped or elliptical, smooth, 8–9 × 5–6 μ; c. abundant, ventricose, 55–65 × 12–16 μ. Smell unpleasant.

On the ground in pine woods, &c. Britain, France, Germany, Sweden, Holland.

A large, well-marked species. The pileus becomes dark brown with age, especially the central portion. The cuticle becomes quite rigid before the pileus attains its full growth, hence during increase in size of the pileus the rigid cuticle is much cracked and torn, exposing the white flesh.

The species as understood above is represented in Roumeg., Fung. Gall. Exs., 1801.

concinna, Karst., Symb. ad Myc. Fenn., xxix, in Med. Soc. Faun. et Flor. Fenn., 1889, p. 29; Sacc., Syll. ix, p. 99.

P. convexo-plane, subumbonate, even, glabrous, innately fibrillose, rusty or pale brown, 2–3 cm.; g. sinuato-adnate, crowded, pale olive then rusty, edge paler and crenulate; s. solid, equal, flexuous, subfibrillose, pallid, apex white-pruinose, about 4 cm.; sp. pip-shaped, smooth, 8–13 × 5–6 μ; c. ventricose-fusoid, 60–65 × 14–17 μ.

In pine woods. Finland.

confusa, Karst., Symb. Myc. Fenn., xxviii, in Med. Soc. Flor. et Faun. Fenn., 1888, p. 39; Sacc., Syll. ix, p. 101.

P. conico-campanulate, then expanded and umbonate, glabrous, the cuticle breaking up into fibrils and slightly cracking, yellowish rusty or bay, up to 9 cm.; g. crowded, ventricose, yellowish then pale olive; s. solid, firm, almost

glabrous, pallid, up to 12 cm. high and 1 cm. thick, cylindrical; sp. elliptical or subreniform, ends very obtuse, smooth, 10–12 × 6 μ; c. inflato-clavate, 40 × 14–18 μ.

In mixed woods. Finland.

Karsten queries the word cystidia, and from the shape given these structures occur only along the margin of the gill, and are not spread over its surface.

Godeyi, Gillet, Champ. Fr., Hymeno., p. 517 (1874); Sacc., Syll. v, p. 778; *Ag.* (*Ino.*) *Trinii*, Pat., Tab. Anal., n. 345; and *Ag.* (*Ino.*) *Trinii*, var. *rubescens*, n. 344; *I. rubescens*, Gill., Rev. Myc., v, p. 31 (1883); and Champ. Fr. Hymen. with plate, and described in the general index (1897); Sacc., Syll. v, p. 786; *Ino. Trinii*, Bres. (non Weinm.), Fung. Trid., ii, p. 14, tab. 120; *Ino. repanda*, Quél. (non Bull.), Flor. Myc., p. 101 (1888); *Ino. hiulca*, Kalchbr., p. 33, tab. 20, f. 2; Sacc., Syll. v, p. 774.

P. campanulate, obtusely umbonate, silky-fibrillose, rimose, whitish at first then more or less suffused with a rosy tinge, which is usually accompanied by an ochraceous tinge, margin splitting, 3–5 cm.; g. narrowed behind and adnexed, almost free, somewhat crowded, whitish then dusky cinnamon, usually with an olive tinge, edge white, minutely flocculose; s. equal, slightly bulbous, colour of p., apex white-pruinose, 4–6 cm.; sp. elliptical, slightly curved or subreniform, smooth, 9–12 × 5·5–6 μ; c. ventricose, 40–65 × 15–20 μ, fairly numerous. Smell strong, unpleasant.

On the ground in woods, &c. Britain, France, Germany, Austria, Hungary.

One of the larger species of *Inocybe*, characterized by the pileus and stem being either pure white, or nearly so, and silky when young. As the Fungus advances in age rosy-red or ochraceous-rosy stains appear on the pileus and stem. These tints are also produced when the plant is bruised.

Bresadola (Fung. Trid., ii, p. 14) agrees with me in considering all the species named above to belong to one species; our only difference turns on the determination of *Ag. Trinii*, Weinm., Bresadola considering that his *I. Trinii* is Weinmann's plant, whereas under what I have considered as *Ag. Trinii* in this work the reasons are given for not coinciding with this opinion.

(Specimens of Gillet's *I. Godeyi* and Quélet's *I. repanda* examined.)

lucifuga (Fries), Karst., Hattsv., p. 465; Sacc., Syll. v, p. 783; *Agaricus lucifugus*, Fries, Obs. Myc., ii, p. 50 (1818); Cke., Ill., pl. 429 A.

P. convexo-campanulate, then expanded and more or less umbonate, longitudinally fibrillose or covered with minute adpressed scales, olive or brownish, rarely fawn-colour, often becoming pale, 1·5–2·5 cm.; flesh whitish; g. nearly free, crowded, ventricose, white then yellowish, at length dark olive; s. solid, equal, almost glabrous, often subflexuous, pallid, apex white-farinose, 3–5 cm.; sp. pip-shaped, smooth, 9–10 × 5–6 μ; c. scattered, ventricose, 60–70 × 12–14 μ. Smell strong, somewhat like radishes.

In pine woods, &c. Britain, Sweden, France, Germany, Russia, Finland.

Distinguished by the deep olive gills, almost glabrous stem, and strong smell. *I. hirtella* is probably only a variety of this species.

(Specimen from Fries examined.)

flavella, Karst., Symb. Myc. Fenn., xxix, in Med. Soc. Flor. et Faun. Fenn., 1889, p. 100; Sacc., Syll. ix, p. 100.

P. acutely conoid then expanded and acutely umbonate, innately fibrilloso-rimulose, glabrous, yellowish and somewhat shining, 2–3 cm.; g. adnexed, crowded, yellowish then olive, edge paler and crenulate; s. solid, equal, flexuous, white with a yellow tinge, apex white-flocculose, about 3 cm.; sp. oblong, ends very obtuse, almost cylindrical, smooth, 12–14 × 4–6 μ; c. fasciculate, cylindrical, and apex clavate, sometimes ventricose, 60–90 × 8–14 μ.

In pine woods. Finland.

††† *Gills tinged violet.*

violaceifolia, Sacc., Syll. ix, p. 98; *Ag.* (*Ino.*) *violaceifolius,* Peck, 41 Rep. State Mus., p. 66.

P. convex or almost plane, fibrillose, subsquamulose, grey, 1–1·5 cm.; g. crowded, adnexed, pale violet then brownish cinnamon; s. firm, solid, slender, fibrillose, whitish, 2·5 cm. long; s. smooth, elliptical, 10 × 6·5 μ; ventricose, 50–60 × 12–16 μ, fairly numerous.

Mossy ground in woods. United States (Selkirk).

Distinguished among species having the gills tinged lilac by the whitish stem.

(Peck's type examined.)

** *Stem coloured.*

† *Gills brown, ochraceous or cinnamon.*

caesariata, Karst., Hattsv., p. 459; Sacc., Syll. v, p. 783; *Ag. caesariatus,* Fries, Epicr., p. 176; *Ag.* (*Inocybe*) *caesariatus,* Cke., Ill., pl. 338.

P. convex then expanded, broadly subumbonate, tawny-ochraceous, densely covered with spreading ochraceous fibrils, which are sometimes collected into more or less concentric squarrose squamules, 2–3 cm.; g. adnexed, rounded behind, edge quite entire, pale ochraceous; s. equal, sometimes slightly wavy, solid, strongly loosely fibrillose, pale ochraceous, 4–8 cm.; sp. 8–10 × 4–5 μ, pip-shaped, smooth; c. narrowly ventricose, fairly abundant, 70–80 × 12–15 μ.

In beech woods, &c. Britain, France, Germany, Sweden.

The above diagnosis is drawn up from specimens examined by Fries in a fresh state, being sent by Berkeley. Cooke's figure in Ill., pl. 383, is copied from Berkeley's original drawing of the specimens sent to Fries. The superficial fibrils are not well shown.

(Specimens determined by Fries examined.)

obscura, Karst., Hattsv., p. 460; Sacc., Syll. v, p. 770; *Ag. obscurus,* Pers., Syn. Fung., p. 347 (1801); *Ag.* (*Heb.*) *obscurus,* Saund., Sm. and Bennet, Myc. Ill., i, pl. 21, lower fig. (1871).

P. campanulato-convex, obtuse or subumbonate, radially fibrillose, disc squamulose, brown more or less suffused with violet, 1·5–2·5 cm.; g. adnexed, uncinate, crowded, ventricose, olive then brownish; s. elongated, stuffed, often slightly wavy, fibrillose, colour of p., 4–7 cm.; sp. pip-shaped, smooth, 8–10 × 5–6 μ; c. ventricose, 65–75 × 12–16 μ, abundant. Smell strong.

Damp pine woods. Britain, France, Germany, Sweden, Finland, Russia, Holland.

The above is the typical form as described by Persoon. Pileus and stem

brown, more or less suffused with purple or lilac; gills at first olive. Flesh tinged lilac at the apex of the stem as in *I. cincinnata*. The last-named Fungus differs from *I. obscura* in the brownish-violet gills.

Var. **rufus**, Pat., Tab. Anal., no. 543. About the size of the typical form; differing in the reddish-brown, strongly umbonate pileus, violet gills, and spores much narrowed towards one end; c. as in typical form.

In woods. France.

Var. **major**, Fries, Icon. Sel., ii, p. 6, tab. 107, f. 3. This is a larger variety figured and described by Fries.

Forma major, stem 3–4 cm. long, 3–4 mm. thick; pileus more flattened when expanded, umbonate, 5 cm. broad; gills paler.

lacera, Karst., Hattsv., p. 457; Sacc., Syll. v, p. 767; *Ag.* (*Ino.*) *lacerus*, Fries, Syst. Myc., i, p. 257; Cke., Ill., pl. 583.

P. convex then expanded, often obtusely umbonate, at first smooth then scaly, the scales becoming squarrose, brownish then mouse-colour, at length pale, 2–3 cm.; g. sinuate, adnexed, ventricose, pinkish then mouse-colour; s. slender, short, covered with brown fibrillose flecks, paler than the p., apex not mealy, stuffed, flesh reddish, 3–3·5 cm.; sp. pip-shaped, smooth, 9–11 × 5–5·5 μ; c. ventricose, abundant, 55–70 × 12–16 μ.

On the ground in pine and mixed woods. Britain, France, Germany, Sweden, Russia, Finland, Holland.

Distinguished from *I. scabra* and *I. mutica* by the reddish flesh of the stem.

As understood here, *I. lacera* is represented in Syd. Myc. March, exs. 2718.

carpta, Sacc., Syll. v, p. 769; Quél., Flor. Myc., p. 104; Oudem., Rev. Champ. Pays-bas, 1892, p. 235; Mass., Brit. Fung.-Fl., ii, p. 189; *Ag.* (*Ino.*) *carptus*, Fries, Hym. Eur., p. 230; *Ag. carptus*, Scopoli, Flor. Carniol., ed. 2, vol. ii, p. 449 (1772); non Bresadola, Fung. Trid., i, p. 50, tab. 54.

P. convex then expanding until almost plane, usually at length more or less depressed at the disc, everywhere densely fibrillose, the fibrils sometimes collected into adpressed or more or less erect squamules, which are somewhat concentrically arranged in adult specimens, dusky brown, 1·5–2·5 cm.; g. adnate then seceding, or adnexed, broad, ventricose, becoming dark brown; s. hollow, somewhat narrowed downwards, covered with a spreading, fibrillose wooliness, paler than the p., 3–5 cm.; sp. pip-shaped, smooth, 8–10 × 5–6 μ; c. numerous, often slightly curved, ventricose, 60–70 × 12–15 μ.

On the ground in woods, &c. Britain, France, Germany, Sweden, Italy.

The description given above covers the species admitted by all European mycologists except Bresadola, whose description and figure quoted above may, as suggested by Saccardo (Syll. v, p. 769), represent a form of *I. maritima*.

I. umbrina, Bres., which superficially resembles *I. carpta*, differs in having rough spores.

hystrix, Karst., Hattsv., p. 453; Sacc., Syll. v, p. 762; *Agar. hystrix*, Fries, Epicr., p. 171; Fries, Icon. Sel., ii, tab. 106, f. 1.

P. convex then expanded, obtuse or slightly and obtusely umbonate, orbicular, dull brown to mouse-colour, covered with revolute, squarrose scales which become

fibrillose towards the margin, 4–9 cm.; g. adnate, slightly sinuate, crowded, broadish but not ventricose, greyish then brown; s. solid, firm, equal or often slightly narrowed downwards, or subfusiform, colour of p., with concentric squarrose and revolute floccose scales up to the distinctly marked annular zone, smooth and pallid above, 5–9 cm.; sp. pip-shaped, smooth, 11–13 × 5–6 μ; c. ventricose, fairly abundant, 70–90 × 12–17 μ. Flesh white.

On the ground in woods. Britain, France, Sweden, Germany.

The general appearance of this species suggests a small specimen of *Pholiota squarrosa*. Often smaller than the measurements given above. There is no tinge of blue nor green on the stem.

(Specimens from Sweden, determined by Dr. R. Fries, examined.)

squamosa, Bresad., Atti dell' I. R. Accad. di Sci. Agiati in Rovereto, ser. iii, vol. 3, fasc. ii, pl. 1 (1902).

P. convex then expanded, often umbonate, tawny-ochre, densely covered with similarly coloured fibrillose scales, centre somewhat smooth and often areolate, 1–1·5 cm.; g. somewhat distant, broad, sinuate, villose from the numerous cystidia, pale tawny; s. subequal, fibrillose, yellowish, stuffed then partly hollow, 1–3 cm.; flesh yellowish; sp. obovate, smooth, 9–11 × 6–7 μ; c. subclavate, 70–90 × 10–13 μ.

On the ground. Portugal.

Resembling *I. dulcamara* and *I. caesariata*, differing in the evidently scaly pileus, broader spores, and presence of numerous cystidia.

incarnata, Bres., Fung. Trid., i, pp. 49 and 102, tab. liii; Sacc., Syll. v, p. 766.

P. campanulate then expanded and broadly umbonate, fibrillose then squamulose, yellowish-red to flesh-colour, margin fimbriate, 6–8 cm.; g. crowded, slightly sinuato-adnate, broad, greyish-cinnamon then spotted with red or entirely reddish, edge paler, fimbriate; s. solid, sometimes narrowed downwards, somewhat rooting, slightly fibrillose, reddish, apex white, furfuraceous, 6–8 cm. long, 10–15 mm. thick, flesh red from the first; sp. pip-shaped, smooth, 9–10 × 6 μ; c. basidia ventricose or clavate, 55–65 × 12–16 μ. Flesh of p. white, becoming red when cut. Smell very strong, like ripe pears.

In pine and other woods. Britain, France, Austria.

Differs from *I. pyriodora* in being more robust in build, deeper red colour, and stronger odour. As defined above, this Fungus appears to be a distinct species, yet I am not certain that we are dealing with more than one species, of which *I. pyriodora* and *I. incarnata* represent the two poles. Transitional forms are not uncommon in this country which are just off one type and tending towards the other.

During the Y. N. U. Fungus Foray at Helmsley, specimens of the *I. incarnata* type were found which certainly were exaggerations of this type; pileus broken up into coarse subsquarrose scales, colour deep red everywhere, smell exceedingly strong and resembling that of hyacinth flowers.

Morphologically there is no difference between *I. pyriodora* and *I. incarnata.*

griseoscabrosa, Mass.; *Ag.* (*Heb.*) *griseoscabrosus,* Peck, 26 Rep. State Mus., p. 57 (1873); Sacc., Syll. v, p. 796.

P. hemispherical, dry, rough with adpressed fibres and scales, grey, margin whitish when young, 1–2 cm.; g. crowded, broad, whitish when young then ochre-

brown; s. firm, equal or slightly tapering downwards, solid, fibrillose or slightly scaly, nearly colour of p., 3–5 cm.; sp. smooth, elliptical, 9 × 5 μ; c. rare, subfusiform, 45–55 × 12–16 μ.

Gregarious on ground in woods. United States (Bethlehem).

(Peck's type examined.)

mutica, Karst., Hattsv., p. 459; Sacc., Syll. v, p. 769; *Ag.* (*Ino.*) *muticus*, Fries, Mon., ii, p. 346; Icon. Sel., tab. 109, f. 1; Cke., Ill., pl. 382.

P. convex then plane or slightly depressed, very obtuse, whitish or tinged straw-colour with darker adpressed squamules, 3–5 cm.; g. broadly adnate, crowded, tinged brown; s. short, 3–5 cm., rather stout, hollow, fibrillose, slightly narrowed downwards, straw-colour; sp. pip-shaped, smooth, 8–9 × 5 μ; c. abundant, ventricose, 50–60 × 14–16.

Side of paths in woods, &c. Britain, Sweden, France, Germany.

Fragments of the fibrillose veil sometimes attached to edge of pileus in young specimens. Quélet (Flor. Myc., p. 106) considers that *Ag. tomentosus*, Jungh., Linn. 1830, t. 6, f. 7, is an *Inocybe*, and has placed *I. mutica* as a variety, and *I. eutheles* as a synonym under this species. It is more than doubtful whether any other mycologist would have seen an *Inocybe* in Junghuhn's figure, which is furnished with a distinct ring on the stem.

(Specimen from Fries examined.)

eutheloides, Peck, 32 Rep. State Mus., p. 29; Sacc., Syll. ix, p. 99.

P. conical or campanulate then expanded and umbonate, silky-fibrillose, somewhat cracked, greyish fawn-colour to chestnut-brown, disc sometimes squamulose, 12–14 mm.; g. rather crowded, ventricose, broadish, narrowed behind and adnexed, whitish then rusty-brown, edge white and denticulate; s. equal, subflexuous, fibrillose, 2–2·5 cm.; sp. elliptical, smooth, 8–10 × 5–6 μ; c. fairly abundant, ventricose, 45–55 × 12–16 μ.

On the ground in woods. United States (Brewertown).

Closely allied to *I. eutheles*, differing mainly in the gills being narrowed behind and adnexed. Also allied to *I. pallidipes*.

(Type from Peck examined.)

nigrodisca, Peck, 41 Rep. State Mus., p. 67 (1888); Sacc., Syll. ix, p. 99.

P. convex then almost plane, or the centre depressed, umbonate, very minutely fibrillose, blackish-brown, margin greyish, 1·5 cm.; g. free or subadnexed, rounded behind, crowded, greyish then rusty-brown, sometimes tinged yellow; s. slender, firm, solid, flexuous, minutely pruinosely downy, reddish-brown, 2·5–3·5 cm.; sp. subelongate, smooth, 5·5–6·5 × 4·5–5 μ; c. fairly abundant, stout, slightly ventricose, 40–50 × 12–15 μ.

Under *Osmunda cinnamomea*, United States (Kasaag, Osw.).

Allied to *I. paludella*.

(Peck's type examined.)

Raveneli, Massee (sp. nov.).

P. campanulate then expanded and rather acutely umbonate, brown, silky-floccose, 1·5–2·5 cm.; g. adnate, broad, pale brown; s. 3–4·5 cm., slender, smooth, hollow, paler or same colour as pileus; sp. elliptic-oblong, obliquely apiculate,

smooth, averaging 15 × 5 μ, some reaching up to 18 μ long; c. fusoid or subventricose, thin-walled, rare and apt to be overlooked, 45–55 × 12–15 μ.

I have ventured on describing as new this Fungus, which appears to be not uncommon in the United States. The ticket accompanying Ravenel's specimen, which may be regarded as the type, bears a full description of the Fungus, accompanied by two sketches in ink. The principal features of the Fungus are the long narrow spores and acute umbo.

On the ground in damp places. United States.

It is represented in the Kew Herbarium as follows: 'Rav., 2416, April 14, 1878. On the ground in damp places, near Darien, Georgia' (as *I. maritima*). 'Maine, U.S.A.' (as *I. geophylla*). 'C. Wright, Connecticut, 5505' (as *I. dulcamara*). 'Car. Inf. no. 2821' (as *I. Bougardii*).

brunnea, Quél., Soc. Sci. nat. Rouen, 1879, tab. 2, f. 7; Flor. Myc., p. 101; Sacc., Syll. v, p. 776.

P. campanulate, umbonate, fibrillosely silky, then cracked, chestnut, 0·5 cm.; g. emarginate, uncinate, creamy then bistre, edge white and crenulate; s. solid, thickened at the base, clear brown, apex white and pruinose, 2–3 cm.; sp. pip-shaped, smooth, 9–12 × 4–5 μ; c. ventricose, scattered, 60–65 × 14–17 μ.

Grassy places in woods. France.

(Specimen from Quélet examined; Roum., Fung. Sel., exs. 5991, is also the correct species.)

haemacta, Sacc., Syll. v, p. 763; *Ag.* (*Ino.*) *haemactus,* B. and Cke., Grev., xi, p. 70; Cke., Ill., pl. 390.

P. campanulate then expanded, obtuse, umber becoming paler towards the margin, clad with long, darker fibrils, disc darkest and rather scaly, 2–3 cm.; g. slightly rounded behind, adnate, dingy tan; s. whitish above, tinged verdigris-green at the base, solid, smooth, 4–5 cm., rather stout; sp. pip-shaped, smooth, 9–11 × 5 μ; c. ventricose, 50–70 × 17–20, fairly numerous. Flesh everywhere changing to red when cut.

Among short grass. England.

The green colour of the stem extends through the flesh. Differs from *I. calamistrata* in the absence of squarrose scales.

(Type specimen examined.)

rhodiola, Bres., Fung. Trid., p. 80, tab. 87 (forma gracilis); *Ino. frumentacea,* Bres., Fung. Trid., p. 88, tab. 200 (forma typica); *Ino. jurana,* Pat., Tab. Anal., no. 551 (fide Bresadola).

P. fleshy, campanulate then expanded and umbonate, fibrillosely cracked, centre even, rufous-chestnut or fuscous flesh-colour, 4–8 cm.; g. crowded, sinuato-uncinate, almost free, edge fimbriate, white then yellowish umber, often spotted with brownish umber; s. fibrilloso-squamulose, becoming glabrous, vinous, apex pallid, subfloccose, 5–8 cm. long, 1–1·5 cm. thick, stuffed; flesh white, vinous at base of stem; spores subreniform, smooth, 10–12 × 6–8 μ, some 14–15 × 8 μ; large cells on edge of gills clavate or subfusoid, 50–60 × 12–14 μ. Smell resembling meal.

On the ground in coniferous woods. Austria, France.

Bresadola considers the present species to be the true *Ag. frumentaceus* of Bulliard, and gives the following synonymy :—

'*Inocybe frumentacea* (Bull.), Bres., Fung. Trid., p. 88, tab. 200; *Agaricus frumentaceus*, Bull., Champ. France, tab. 571, fig. 1; *Ino. jurana*, Pat., Tab. Anal., no. 551; *Ino. rhodiola*, Bres., Fung. Trid., p. 80, tab. 87 (forma gracilis).'

Bresadola is by no means the first mycologist who has essayed to define the exact species Bulliard's figure represents, and judging by the diversity of opinion expressed, the task appears to be hopeless, and one would imagine, not profitable.

Fries (Hym. Eur., 52) considers the Fungus in question to be a *Tricholoma*; Berkeley (Outl. p. 144) places it in *Entoloma*; Quélet (Flor. Myc. 262) regards it as synonymous with *Hygrophorus russula*, Schaeffer.

No type specimen of Bulliard's Fungus has been discovered, and Bresadola, like other people, has only the old figure to go by, and as Bulliard's figures were hand-coloured, and variable within fairly wide limits in different copies, I have decided not to admit Bulliard's figure, already claimed by so many mycologists, into the genus *Inocybe*, but have restored Bresadola's first name given to this Fungus, which is obviously an *Inocybe*.

fuscodisca, Mass.; *Ag.* (*Heb.*) *fuscodiscus*, Peck, 27 Rep. State Mus., p. 95, pl. 1, figs. 3–6 (1874); Sacc., Syll. v, p. 796.

P. at first subviscid, conical, covered with blackish-brown fibrils, then campanulate or expanded and umbonate, whitish, the disc remaining blackish-brown, 1·5–2·5 cm.; g. crowded, white then brownish, edge minutely fimbriate; s. equal and solid, whitish and pruinose at the apex, remainder brownish, fibrillose, 3–7 cm.; sp. pip-shaped, smooth, 8–10 × 5–5·5 μ; c. ventricose, fairly numerous, 45–55 × 12–16 μ. The odour like chestnut blossom.

In an old pasture under trees. United States (Forestburgh).

The somewhat viscid pellicle is separable.

(Peck's type examined.)

comatella, Peck, 38 Rep. State Mus., p. 87, tab. ii, f. 5–8; Sacc., Syll. v, p. 791.

P. convex or expanded, covered with whitish or greyish hairs, margin fimbriate, 4–8·5 mm.; g. adnate, rather distant, pale cinnamon; s. equal, solid, flexuous, pallid- or rufous-brown, then darker, slightly mealy or pruinosely-fibrillose, with white mycelium at the base, 2–2·5 cm.; sp. pip-shaped, smooth, 8 × 4–4·5 μ; c. strongly ventricose, 45–55 × 12–20 μ.

On trunks and branches among dead leaves. United States.

Intermediate between *I. tricholoma* and *I. strigiceps* (Peck).

(Peck's type examined.)

flocculosa, Sacc., Syll. v, p. 768; *Ag.* (*Ino.*) *flocculosus*, Berk., Engl. Fl., v, p. 97 (1836).

P. convex or subcampanulate, umbonate, silky-squamulose, brownish-fawn colour, 2·5 cm.; g. rounded behind and adnate but not broadly so, pale fawn then dull rusty, edge white; s. fibrillose, apex squamulose, brownish beneath the fibrils, 3 cm.; sp. elliptical, smooth, 8–10 × 5–6 μ; c. abundant, ventricose, 50–60 × 12–15 μ. Smell mealy but unpleasant.

On naked soil and among grass. Britain.

Amongst grass the pileus is smoother, more tawny, rimoso-sericeus; gills not arcuate behind but broadly adnate (Berk.).

Allied to *I. lanuginosa* and *I. lacera*; the former differs in the obtuse pileus with squarrose squamules at the disc, and the latter in the naked apex of the stem.

(Type specimen examined.)

conformata, Karst., Krit. Öfvers. Finl. Basid., p. 465 (1889); Sacc., Syll. ix, p. 98; *I. pusio,* Karst., Krit. Öfvers. Finl. Basid., p. 465 (1889); Sacc., Syll. ix, p. 98.

P. convex then expanded, umbonate, fibrilloso-rimose and sometimes minutely adpressed floccoso-squamulose, pale fuscous or tinged rusty, 1–3 cm.; g. adnexed, somewhat crowded, ventricose, pallid then brownish; s. solid, equal, often flexuous, minutely fibrillose, apex at first tinged violet, 3–5 cm.; sp. pip-shaped, smooth, 8–10 × 4–6 μ; c. ventricose, 70–80 × 10–15 μ, sometimes much thicker.

Mossy ground near paths. Finland.

The two forms enumerated above agree in all essential features, and cannot be separated as species; in fact Karsten states that *I. pusio* is externally exactly similar to *I. conformata*, but is distinguished by the thicker cystidia. This feature alone, however, cannot constitute a species.

(Types of *I. conformata* and *I. pusio*, from Karsten, examined).

†† *Gills tinged olive.*

dulcamara, Karst., Hattsv., p. 455; Sacc., Syll. v, p. 763; Cooke, Ill., pl. 582 B; Pat., Tab. Anal., no. 540; *Ag. dulcamarus*, A. and S., Consp. Fung., p. 171 (1805).

P. campanulate then expanded and umbonate, brownish-olive, floccosely scaly, margin more or less fimbriate and silky, 2–5 cm.; g. narrowed behind, arcuately adnexed, rounded in front, crowded, pallid, then distinctly olive; s. imperfectly hollow, fibrillose from the veil, adpressedly scaly, paler than p., apex mealy, 4–6 cm.; sp. pip-shaped, smooth, 11–13 × 5–6 μ; c. fairly abundant, ventricose, 55–65 × 15–18 μ. Flesh tinged yellow.

On the ground in pine woods, &c., gregarious. Britain, France, Germany, Sweden.

The above diagnosis agrees with the views of Patouillard and Quélet as to the plant described by Fries (Hym. Eur., p. 228) as *Ag.* (*Ino.*) *dulcamarus*, and referred by him to the Fungus described by Albertini and Schweiniz in Consp. Fung., p. 171. Why Fries connected the Fungus found by him with the plant mentioned by Albertini and Schweiniz is not quite clear, judging from the description furnished by these authors, which is as follows:—

'489. *A. G. dulcamarus.* Exempla juniora *Cortinariam* et hanc esse, velo fugaci instructam, demonstrant. *Stipes* subcavus, subfibrillosus. Sapor *Glycyrrhizae* dilutus. Varietatem hujus speciei habemus alteram *autumnalem* squamulis pilei appressis, lamellis dilutius olivascentibus; alteram *aestivalem* squamis distinctioribus subsquarrosis, lamellis saturatius olivaceis.'

relicina, Karst., Hattsv., p. 453; Sacc., Syll. v, p. 764; *Ag.* (*Ino.*) *relicinus*, Fries, Syst. Myc., i, p. 256.

P. conical then expanded, obtuse, covered everywhere with squarrose scales formed of fasciculate fibrils, dingy brown, 1·5–2·5 cm.; g. slightly adnexed, crowded,

yellow then olive; s. solid, soft, equal, fibrillosely scaly (not squarrose), apex paler, 4–5 cm., colour of p.; sp. pip-shaped, smooth, 10–12 × 7 μ; c. ventricose, scattered, 70–85 × 14–16 μ

Damp pine woods amongst *Sphagnum*, &c. Britain, Ireland, France, Sweden.

Most nearly allied to *I. dulcamara*, which differs in the umbonate pileus with an olive tinge.

(Specimen in Herb. Kew. determined by Klotzsch accepted as typical. This agrees with Quélet's conception of the species.)

Bongardi, Karst., Hattsv., p. 458; *Ag. Bongardi*, Weinm., Hymeno- et Gastero-Mycetes Imp. Rossica Obs., p. 190 (1836).

P. campanulate then expanded, obtusely umbonate, whitish with a rufescent or yellowish tinge, covered with darker fibrillose squamules, 3–7 cm.; g. arcuato-adnate, crowded, ventricose, broad, whitish then olive-cinnamon, finally dusky cinnamon, edge eroded; s. solid, equal, stuffed, straight, very tough, almost smooth, colour of p., apex with white meal, 5–8 cm.; sp. pip-shaped, smooth, 8–10 × 5–6 μ; c. ventricose, scattered, 50–65 × 12–16 μ. Flesh reddish when cut. Smell pleasant, like ripe pears.

In woods. Britain, Russia.

The above is the diagnosis given by Weinmann, so far as macroscopic structure is concerned. I have collected specimens in England agreeing admirably with the above, which differs very materially from the diagnosis given by Fries (Hym. Eur., p. 229), and also from his figures (Icon. Sel., tab. 107).

If Fries' Fungus is in reality the same species as Weinmann's, it is a marked variety, differing more especially in the following points. Pileus darker in colour and more distinctly squarrosely scaly; flexuous stem; gills not arcuate.

††† *Gills tinged violet.*

cincinnata, Karst., Hattsv., p. 456 (1879); Bres., Fung. Trid., i, p. 47, pl. 51, f. 2 (1881); Sacc., Syll. v, p. 764; *Ag. cincinnatus*, Fries, Syst. Myc., i, p. 256 (1821); *Ag.* (*Ino.*) *alienellus*, Britz., Derm., p. 154, fig. 19; *Ino. alienella*, Sacc., Syll. v, p. 764.

P. convex then expanded, obtuse or obsoletely umbonate, dusky brown, disc with more or less squarrose floccose squamules, margin fibrillose, 1·5–3 cm.; g. adnexed, seceding, crowded, ventricose, brownish-violet; s. solid, rigid, slender, fibrillosely squamulose, apex tinged violet at first, then discoloured, 3–4 cm.; sp. pip-shaped, smooth, 8–12 × 5–6 μ; c. subcylindrical or slightly ventricose, fairly abundant, 60–80 × 14–18 μ. Flesh white except apex of stem, which is lilac at first.

On the ground in woods. Britain, France, Germany, Sweden, Austria, Bavaria, Holland.

Superficially more or less resembles several species. *I. obscura* differs in the non-squamulose stem, and gills olive at first. *I. fulvella* has nodulose spores.

IV. *Cystidia absent.*

* *Stem whitish or pallid.*

† *Gills brownish, ochraceous or cinnamon.*

perlata, Sacc., Syll. v, p. 774; *Ag.* (*Ino.*) *perlatus*, Cke., Grev., xv, p. 40; Cke., Ill., pl. 960.

P. convex then expanded and broadly umbonate, fuscous, longitudinally streaked with darker fibrils, disc darker, edge paler, incurved, 6–10 cm.; g. rounded behind, adnexed, broad, pallid then pale umber; s. straight or curved, sometimes twisted, fibrously striate, pallid and mealy above, darker below, 6–10 cm. long, 1–1·5 cm. thick; flesh dingy white; sp. elliptical, smooth, 9–12 × 6–7 μ; c. absent.

Under hornbeam. Britain.

Resembling *I. fibrosa* in size, differing in the smooth spores and darker pileus.

(Type specimen examined.)

perbrevis, Karst., Hattsv., p. 462; Sacc., Syll. v, p. 777; *Ag.* (*Ino.*) *perbrevis*, Cke., Ill., pl. 519; *Ag. perbrevis*, Weinm., Hymeno- et Gastero-Mycetes Imp. Ross. Obs., p. 185 (1836).

P. convex then expanding until almost plane, obtusely umbonate, often depressed round the umbo, fibrillosely silky or minutely squamulose, rufous-brown becoming tinged yellowish, margin fibrillose and often splitting, 1·5–3 cm.; g. slightly adnexed with a slight decurrent tooth, ventricose, rather distant, pale then tan-colour; s. stuffed, often slightly narrowed downwards, pallid and white-fibrillose, 2–2·5 cm.; sp. elliptic-oblong, apiculate, smooth, 8–9 × 4·5–5 μ; c. absent.

Gregarious; on the ground in woods, &c. Britain, Russia, Germany, Sweden, United States (N. Jersey, E. & E., N. Amer. Fung. ser. ii, 1903).

A firm, compact little Fungus, recognized by the rufous-brown colour, short stem, and absence of cystidia.

fuegiana, Sacc., Syll. ix, p. 101; *Ag.* (*Ino.*) *fuegianus*, Speg., Fung. Fueg. in Bol. Acad. Nac. Cordóba, xi, p. 144, no. 25 (1887).

P. hemispherical then plano-expanded, disc almost glabrous, not at all or slightly depressed, the remainder densely papillosely villose, chestnut or smoky-brown, edge entire wavy or undulate, 6 cm.; g. sinuato-adnate, 1 cm. broad, rather distant, attenuated in front, greyish-brown, edge quite entire; s. short and thick, 5 cm. long, 1 cm. thick above, 2 cm. thick below, straight fibrillose, white then smoky-brown; sp. elliptical, smooth, ends obtuse, 10–12 × 5–6 μ.

In beech woods. Tierra del Fuego (Ushuvaia, Beagle Channel).

Solitary. Allied to *I. caesariata.*

Victoriae, Sacc., Syll. ix, p. 101; *Ag.* (*Ino.*) *Victoriae*, Cke. and Massee, Grev., xvi, p. 72 (1888).

P. convex then expanded and umbonate, whitish, viscid, glabrous, silky and shining, disc darker, 2–3 cm.; g. sinuate and adnexed then almost free, pale then umber; s. almost equal, white, glabrous, stuffed, 3–5 cm.; sp. elliptical, smooth, 11–12 × 7–8 μ; c. absent.

On grassy ground. Victoria (F. Reader, no. 26, with figs. and description).

Very near to *Hebeloma*, but the pileus is distinctly silky-fibrillose.

(Type examined.)

holophlebia, Sacc., Syll. xi, p. 52; *Agaricus* (*Ino.*) *holophlebius*, Berk., in Herb. Grev., xix, p. 104.

P. campanulate then expanded and umbonate, floccosely fibrillose, disc squamulose, fawn-colour or dull, pale yellow, 3–6 cm.; g. adnate, broad, tan then brownish;

s. cylindrical, slender, equal, whitish, 5–6 cm.; sp. broadly elliptical, smooth, 12–14 × 8 μ; c. absent.

On the ground. India (Masulipatam).

A fine, large species, superficially resembling *I. pyriodora*. The specimens, collected by Berkeley's son, are accompanied by coloured sketches.

(Type specimen examined.)

subdecurrens, Ellis and Everh., Journ. Myc., v, p. 27 (1889); Sacc., Syll. ix, p. 97; *I. tomentosa*, Ellis and Everh., Journ. Myc., v, pp. 27–28 (1889).

P. convex then plane, disc depressed and with or without a small umbo, densely adpressed pilose or tomentose, pale drab becoming yellowish, 2–5 cm.; g. adnato-decurrent, dingy cinnamon, edge serrulate; s. fibro-squamulose above, white-tomentose, hollow throughout or only upwards, 2–4 cm.; sp. elliptical, ends obtuse, often very slightly curved, smooth, 8–10 × 5–6 μ; c. absent.

On the ground under branches of Norway spruce. United States (Newfield, N.Y.).

After a very careful examination of authentic specimens from Ellis and Everhart's exs., I feel constrained to consider that but one species is present. Both were found under the same tree, and both manifest the same salient features, among which are the depressed disc, a rare feature in *Inocybe*; serrulate gills, agreement in form and size of spores, and in the absence of cystidia. In the dried condition the gills show a decided olive tinge.

Ellis however does not hold the above view, and, in a paragraph following the diagnoses of these forms, says:—

'*I. subdecurrens* is larger, with a *hollow* stem, and has the gills more crowded, nor is the margin incurved and tomentose, and it is also rather a darker shade and has the margin of the gills more strongly serrate.'

In *I. tomentosa* the margin remains incurved till the plant is nearly full grown.

In *I. subdecurrens* the margin is never incurved, even when young, nor is there any annular mark on the stem, though the fibrous veil is at first distinct.

(Specimens from Ellis and Everh., N. Amer. Fung., ser. ii, nos. 1906 and 2101 examined.)

vatricosa, Karst., Hattsv., p. 465; Sacc., Syll. v, p. 790; *Ag.* (*Ino.*) *vatricosus*, Fries, Syst. Myc., i, p. 259; Icon. Sel., ii, p. 9, tab. 110, f. 3.

P. convex then plane, obtuse or umbonate, smooth, glabrous, becoming silky towards the margin, viscid when moist, shining when dry, white, 1·5–2·5 cm. sometimes broader; g. emarginate, slightly adnexed, almost free, crowded, whitish then brown; s. fistulose, white, entirely covered with white down, not fibrillose, ascending or flexuous, about equal, 2–5 cm.; sp. elliptical, smooth, 5–6 × 3–3·5 μ; c. absent.

On the ground or on fallen chips, in damp woods. Britain, Sweden, Finland, Russia.

Very variable in size, usually small; superficially resembling *I. geophylla*, but generally smaller, and differing in absence of cystidia. Quite as much a *Hebeloma* as an *Inocybe*.

†† *Gills tinged olive.*

fibrillosa, Peck, 41 Rep. State Mus., p. 65 (1888); Sacc., Syll. ix, p. 98.

P. convex or almost plane, obtuse or subumbonate, densely fibrillose, fuscous, disc darker and with adpressed fibrillose scales, up to 3·5 cm.; g. adnate, crowded, yellowish-olive then cinnamon-brown; s. equal, hollow, fibrillosely scaly, pallid, 2·5 cm.; sp. pip-shaped, smooth, 8–10 × 5–6 μ; c. absent.

Swampy places in woods. United States (Bethlehem, Albany).

Very closely allied to *I. subtomentosa.*

(Type from Peck examined.)

** *Stem coloured.*

†† *Gills brownish, ochraceous or cinnamon.*

Cookei, Bres., Fung. Trid., p. 17, tab. cxxi; Sacc., Syll. xi, p. 52.

P. conico-campanulate then expanded and umbonate, edge at length splitting and upturned, silky-fibrillose, cracked, disc glabrous, yellowish straw-colour to lurid yellowish, 3–5 cm.; g. crowded, narrowed behind and adnexed, yellowish cinnamon, edge white, fimbriate; s. equal, solid, colour of p., base minutely marginato-bulbous, 4–7 cm. long, 5–7 mm. thick; sp. subreniform, smooth, 8–10 × 5–5·5 μ; c. absent; flesh tinged straw-colour.

Gregarious in pine woods. Austria.

Allied to *I. fastigiata.* The latter differs in having a whitish stem and olive gills.

unicolor, Peck, 50 Rep. State Mus., p. 104 (1897); Sacc., Syll. xvi, p. 134.

P. conical or very convex, then expanded or broadly convex, tomentose-squamulose, pale ochre or greyish ochre, 2 cm.; g. broad, subdistant, rather ventricose, pale ochre then tawny-brown; s. slender, equal, firm, flexuous, solid, squamulose, colour of p., 2·5–3.5 cm.; sp. elliptical, smooth, 8–10 × 5–6 μ (10–13 × 5–6 μ Peck); c. absent.

Clay soil. United States (Menands).

In dry specimens the edge of the gills is pale and minutely serrulate.

Resembles *I. ochracea,* from which it may be separated by its more highly coloured, squamulose stem and its larger spores (Peck).

(Type from Peck examined.)

mimica, Massee (sp. nov.).

P. campanulate, obtusely umbonate, fibrillose yellow-brown, everywhere covered with large, adpressed, slightly darker fibrous scales, 6–8 cm.; g. broad, deeply sinuate and attached to the stem by a very narrow portion, yellow-brown; s. solid, equal, fibrillose, paler than p., 6–8 cm. long, 1 cm. thick; sp. subcylindrical with an oblique apiculus, smooth, 14–16 × 6–8 μ; c. absent.

On the ground in woods. Britain.

This Fungus was collected in two separate localities at Castle Howard, Yorks., during the Yorks. Nat. Union Fungus foray, Sept. 1902. It was at the time referred to *Inocybe adequata,* Britz., by Dr. Cooke. It differs however very materially from that species.

The pileus exactly mimics that of *Lepiota Friesii,* as figured in Cooke's Ill., pl. 941, hence the specific name.

hirsuta, Karst., Hattsv., p. 454 (1879); Sacc., Syll. v, p. 764; Bres., Fung. Trid., i, p. 80, tab. 86, f. 2; *Ag. hirsutus,* Lasch, no. 577, in Linn., iv, p. 546 (1829);

Ag. (*Ino.*) *hirsutus*, Fries, Mon., p. 336; *I. praetermissa*, Karst., Symb. Myc. Fenn., xiii, in Med. Soc. Fauna et Flor. Fenn., 1885, p. 3; Sacc., Syll. v, p. 786.

P. conico-campanulate, then expanded and acutely or obtusely umbonate, with more or less squarrose, fibrillose squamules, edge fimbriate, brownish or ochraceous-brown, disc sometimes tinged green, 1–2 cm.; g. adnate, crowded, narrow, pale tan then dusky cinnamon, edge whitish, crenulate; s. stuffed then hollow, brownish, fibrillose, apex pale, floccose, base slightly thickened sometimes, verdigris-green, 4–7 cm.; sp. elongate pip-shaped, smooth, 11–14 × 5–5·5 μ; c. absent.

Damp places in woods. Britain, Sweden, Germany, France, Austria.

The flesh becomes faintly tinged red when cut. Closely allied to *I. calamistrata*, which differs in the squarrosely squamulose stem, strong smell, and rusty gills, and the presence of cystidia. Bresadola states (Fung. Trid., i, p. 80) that *I. haemacta*, Berk. and Cke., in Cke., Ill., pl. 390, appears to be a form of *I. hirsuta* with a glabrescent stem. This view however is not correct, as *I. haemacta* differs in possessing cystidia, smaller spores, &c. Moral: do not undertake to decide the fate of a species from an examination of pictures alone!

The above diagnosis covers the species generally accepted as *Ag. hirsutus*, Lasch.

calamistrata, Karst., Hattsv., p. 454; Sacc., Syll. v, p. 762; *Ag.* (*Ino.*) *calamistratus*, Fries, Syst. Myc., i, p. 256; Fries, Icon. Sel. Hym., tab. 106, f. 2.

P. campanulate then expanded, obtuse, dusky brown, entirely covered with rigid, recurved, squarrose scales, 2·5–6 cm.; g. adnexed, seceding, crowded, broad, white then rusty, margin whitish, minutely crenulate; s. solid, rigid, tough, equal, fuscous but dusky blue at the base, everywhere covered with minute, rigid squarrose scales, 4–6 cm.; sp. elliptic-oblong, subreniform, smooth, 11–13 × 5–6 μ; c. absent. Smell strong, not unpleasant. Flesh becoming tinged red when cut.

On the ground in pine woods. Britain, France, Sweden, Russia.

Most closely allied to *I. hirsuta*; differing in the rust-coloured gills and the squarrose scales on the stem.

(Specimen in Herb. Kew. accepted as typical.)

echinata, Sacc., Syll. v, p. 773; *Ag. echinatus*, Roth, Cat. Bot., fasc. ii, p. 255, tab. 9, f. 1 (1800); *Ag.* (*Psalliota*) *echinatus*, Fries, Hym. Eur., p. 282; *Ag.* (*Lepiota*) *haematophyllus*, Berk., Mag. Zool. and Bot., v, p. 507, tab. 15, f. 1; *Ag. fumoso-purpureus*, Lasch, in Linn., iii, p. 420 (1828); *Ag. oxyosmus*, Montag., Ann. Sci. Nat., 1836, t. 10, f. 3; *Ag.* (*Ino.*) *echinatus*, Cke., Hdbk., ed. ii, p. 154; Cke., Ill., pl. 393; *Ag. Hookeri*, Klotzsch, Engl. Fl., v, p. 97.

P. campanulate then expanded, obtuse, at first floccosely pulverulent then breaking up into scales, dusky- or sooty-brown when young, becoming dingy brownish yellow, 2–5 cm.; g. crowded, almost or quite free, pink then blood-red, finally with a brownish tinge from the spores; s. fistulose, equal, floccosely-pulverulent below the imperfect annular zone, dusky red, 3–5 cm.; sp. elliptical, smooth, yellowish-brown with a pink tinge, 4–5 × 2·5–3 μ; c. absent.

On peat and soil in gardens and conservatories. Britain, France, Germany, Sweden, United States ('3184 Car. Inf.' under *I. echinata* in Herb. Kew.), Cayenne (specimen from Montagne in Herb. Kew.).

A curious little Fungus respecting which there is much difference of opinion.

Berkeley considered it as a *Lepiota*, whereas Fries placed it in *Psalliota*, and Cooke in *Inocybe*. The spores appear to be yellowish-brown and, as it were, only stained by the red juice which permeates every part of the Fungus. *I. echinata* is probably an introduced species in Europe, never occurring in woods, &c., but only in conservatories or botanical gardens. The occurrence of specimens from Cayenne and S. Carolina suggest that it may be indigenous to the New World.

(Types of Berkeley and Klotzsch examined.)

violacea, Massee, Kew Bull., 1899, p. 169; Sacc., Syll. xvi, p. 91.

P. campanulate then expanded and broadly umbonate, squamulose, edge fimbriate, violet, paler towards the margin, 1–1·5 cm.; g. sinuato-adnate, crowded, narrow, white then tinged rosy flesh-colour, edge fimbriate; s. solid, equal, subfibrillose, rosy flesh-colour, apex white-scurfy, 2–3 cm.; sp. cylindric-ovate, apiculate, smooth, 6 × 3–3·5 μ; c. absent.

On a lawn. Perak.

Allied to *I. echinata.*

rhombospora, Massee (sp. nov.).

P. campanulate, rather acutely umbonate, fibrillose, brown, edge paler, disc squamulose, 2–3 cm.; g. adnexed, rather crowded, yellowish brown; s. fibrillose, brown, with white silky fibres up to the imperfect annular zone, 3–4 cm.; sp. rhomboidal, sometimes with a marked apical point, 6 × 5 μ, compressed laterally; c. absent.

On rotten wood. India (Nilghiris).

The specimen was collected by Berkeley's son, and placed under 'undetermined species.' It is now in the Kew Herbarium.

Readily distinguished by the peculiar spores. The basidia are also exceptional in structure, measuring 20 × 9–10 μ; the sterigmata are reduced to minute papillae.

There is an American species included in 'Rav., Fung. Amer., exs. 201, ad terram arenosam, Darien, Florida, 201,' called *Ag. Ino. maritimus.* The spores have a more or less rhomboidal form in outline, smooth, and 4·5–5 × 4 μ; c. absent. This species is certainly not *I. maritima,* and does not accord with any described species. As no diagnosis can be drawn up from the imperfectly preserved specimens, which are not accompanied by notes or sketches, it remains for American mycologists to look to this interesting form.

If the occurrence of *I. maritima* in the United States turns on this species, the name must be deleted from the list.

†† *Gills tinged olive.*

erythroxa, De Seynes, Rech. Champ. Congo Fr., i, p. 2, tab. 1, f. 1–5 (1897); Sacc., Syll. xiv, p. 133.

P. plane or subdepressed, subumbonate, margin at length irregularly reflexed, rusty, ornamented with pyramidal reddish spines, 1·5–2 cm.; flesh yellowish, tough; g. ventricose, free or slightly adnexed, distant, deep olive, edge paler; s. smooth, yellowish, base paler, becoming hollow, 1·5 cm.; sp. elliptical, smooth, 7 × 3–4 μ; c. absent.

On the ground. French Congo State.

murino-lilacina, Ellis and Everh., Journ. Myc., v, p. 25 (1889); Sacc., Syll. ix, p. 100.

P. silky-fibrillose, at length becoming squamulose around the margin, with a broad prominent disc, mouse-colour with a tinge of lilac when fresh and young, 2–4 cm.; g. adnate, rather broad, rusty with just a tinge of olive; s. fistulose and soon hollow, fibrillose, about colour of p., 2–4 cm.; sp. pip-shaped, smooth, 8–9 × 4·5–5 μ; c. absent.

On the ground in dry, bushy places. United States.

The broad, prominent disc of the pileus either has a small umbo in the centre or a slight depression, and is generally surrounded (about half-way to the margin) with a distinct ridge or zone. The margin also projects slightly, and is a little lighter coloured and, under the lens, subfimbriate (E. and E.).

(Specimen in Ellis and Everh., N. Amer. Fung., ser. ii, 1905, examined.)

subtomentosa, Peck, 48 Rep. State Mus., p. 109 (1894); Sacc., Syll. xiv, p. 134.

P. convex or plane, minutely hairy-tomentose, brownish-tawny, up to 2·5 cm.; g. adnate, slightly emarginate, crowded, whitish then tinged olive, finally tawny brown, edge whitish, crenulate; s. short, solid, slightly silky-fibrillose, coloured like p., or a little paler, 2·5 cm.; sp. elliptical, smooth, sometimes inclined to be curved, 8–10 × 5–6 μ; c. absent.

Gregarious or subcaespitose. Gravelly soil among fallen leaves. United States (Rouse's Point).

Very closely allied to *I. tomentosa*; however Peck considers the two as distinct, and distinguished as follows:—

Differs from *I. tomentosa* by its darker colour, larger spores, and the entire absence of an umbo. Its prominent features are its small size, minutely tomentose pileus, and nearly uniform brownish-tawny colour when mature. *I. fibrillosa* by its solid merely fibrillose stem, and by the absence of scales on the disc of the pileus (Peck).

(Type from Peck examined.)

fastigiata, Karst., Hattsv., p. 461 (1879); Bres., Fung. Trid., i, p. 52, tab. 57; *Ag. fastigiatus,* Schaeff., Fung. Ic., tab. 26 (1800); *Ag.* (*Ino.*) *Curreyi,* Berk., Outl., p. 155; *I. Curreyi,* Sacc., Syll. v, p. 775; *Ag.* (*Ino.*) *servatus,* Britz., Hym. Südbay., 1885, p. 52, fig. 57; *I. servata,* Sacc., Syll. xi, p. 53.

P. conico-campanulate, gibbous or obtusely umbonate, or sometimes acutely umbonate when small in size, longitudinally fibrillose and slightly cracked, the disc alone sometimes slightly squamulose, pale yellowish brown, edge sometimes slightly wavy or lobed, 3–6 cm.; g. free, ventricose, rather crowded, narrowish, yellowish then dusky olive; s. subequal, solid, minutely fibrillose, paler than p., 5–10 cm.; sp. elliptical, sometimes slightly curved, smooth, 8–11 × 6–7 μ; c. absent.

In woods, &c. Britain, France, Germany, Bavaria, Austria, Sweden, Finland, Holland.

As defined above, this Fungus is acknowledged as *Ag. fastigiatus,* Schaeff., by Quélet, Karsten, Patouillard, Gillet, Bresadola, and Oudemans. Its prominent characters are the yellowish-brown pileus, olive gills, smooth elliptical spores, and

absence of cystidia. The pileus is commonly obtusely umbonate, but in the form figured by Bresadola the umbo is acute; in other respects, however, his plant is typical.

There are, however, apparent contradictions to the above view. Fries (Hym. Eur., 232) gives a good description of Schaeffer's Fungus as defined above, and quotes Schaeffer's figure, but at the end of his remarks on the species adds 'sporae scabrae.'

Fries did not personally investigate the microscopic characters of spores, hence this statement must have been obtained from some outside source, and its value questionable.

In Cooke's Illust., pl. 383, the spores are shown to be rough; this is a mistake, however, as the specimens from which Cooke's figure was drawn, now in Herb. Kew., have smooth spores!

In Saccardo's Sylloge, v, p. 779, the Friesian description of *I. fastigiata* is copied, with an added description of rough spores and cystidia, obtained from some other source, hence valueless.

In British Fungus-Flora, ii, p. 192, not having an authentic specimen, I copied Saccardo's account of spores and cystidia, hence this cannot be urged as an argument.

(Berkeley's type of *I. Curreyi* examined.)

Spores smooth, no knowledge of cystidia.

* *Pileus dark coloured.*

mutata, Mass., *Ag.* (*Heb.*) *mutatus*, Peck, 24 Rep. State Mus., p. 69; Sacc., Syll. v, p. 799.

P. convex or broadly conical, gibbous, rough with squarrose, fasciculate, floccose scales, which at length disappear except at the disc, dark brown, 1·5–2·5 cm.; g. broad, crowded, ventricose and very deeply emarginate, dark rusty-brown, edge whitish; s. slender, equal, solid, floccosely scaly, often curved at the base, colour of p., 5–7·5 cm.; sp. elliptical, smooth, 10 μ.

Damp ground in woods. United States (Catskill Mts.).

The changed appearance produced by the disappearance of the scales suggests the specific name (Peck).

cucullata, C. Mart., Bull. Soc. Gen., vii, 1892–1894, p. 179; Sacc., Syll. xiv, p. 132.

P. variable in form, campanulate, campanulate-convex or sometimes rather irregular, tawny, scaly, those of the disc darkest, 1·5–3 cm.; g. broad, adnexed then free, rather crowded, ochre then rusty-brown, edge white, serrulate; s. equal or narrowed below, hollow, glabrous, usually curved or flexuous, 2–4 cm., paler than pileus; sp. pip-shaped, smooth. Smell like camphor.

Among grass. Switzerland.

tuberosa, Clements, Univ. Nebraska Bot. Surv., 1893, ii, p. 40; Sacc., Syll. xi, p. 52.

P. expanded, scaly, deep brown, 3 cm.; g. distant, adnexed, deep brown; s. tuberous, equal above, gilvous, 4 cm.; sp. pip-shaped, smooth, 6 × 4 μ.

United States (Sioux Co.).

violascens, Quél., Jura et Vosg., xiv Suppl., p. 4, tab. xii, f. 6; Flor. Myc., p. 103; Sacc., Syll. v, p. 766.

P. conico-campanulate, fibrillose, silky, clear buff to brown, velvety and lilac at the disc, 2·5 cm.; g. adnate, narrow, lilac then bistre; s. hollow, silky, striate and lilac under a white, silky cortina, 3–5 cm.; sp. pip-shaped, smooth, 12–15 × 6 μ.

Among grass, appearing in spring. France.

Allied to *I. corydalina*, resembles the violet form of *I. geophylla*.

tenebrosa, Quél., Assoc. Franc., 1885, t. 8, f. 8; Sacc., Syll. v, p. 775.

P. campanulate, minutely velvety, yellowish-brown, 2–3 cm.; g. adnate, narrow, ochraceous then brown; s. slender, fibrillose, striate, dusky brown or olive, apex whitish, 3–4 cm.; sp. elliptical, apiculate, sometimes slightly curved, smooth, 7–8 × 4 μ.

In woods, spring. France.

Merletii, Quél., Assoc. Franc., 1884, t. 8, f. 7; Sacc., Syll. v, p. 769.

P. convex, greyish, speckled with brownish fibrillose flecks, 3–5 cm.; g. sinuate, pale then brownish; s. whitish, streaked with yellow-brown fibrils underneath a white cobweb-like veil, 4–7 cm.; sp. elongato-elliptical, apiculate, smooth, 11–14 × 5–6 μ.

Under poplars in damp places, spring. France.

** *Pileus pale coloured.*

connexifolia, Gillet, Rev. Myc., v, p. 30 (1883); Sacc., Syll. v, p. 771; Gill., Champ. Fr., with figure.

P. conical then somewhat spreading, margin always more or less recurved, obtusely umbonate, disc with fibrous adpressed scales, fawn-colour or pale reddish, 3–4 cm.; g. crowded, narrow, uncinnately adnexed, connected by numerous veins and anastomosing, colour of p.; s. solid, equal, fibrously squamulose, whitish or tinged red, 5–7 cm.; sp. elliptical, smooth. Smell resembles fruit.

On the ground in woods. France.

Closely resembles *I. pyriodora*, differing mainly in the gills being anastomosing and conspicuously connected by ribs.

flava, Massee; *Hebeloma flavum*, Clements, Bot. Surv. Nebraska, iv, 1896, p. 22; Sacc., Syll. xiv, p. 134.

P. campanulate, viscid, 5–6 cm., edge incurved, appendiculate, pale yellow, with tawny subconcentric scales 2 mm. broad; g. somewhat sinuate, rather crowded, dingy; s. stout, solid, short, curved, yellow, densely covered except at the base with concentric, floccose, tawny scales, 3–5 cm.; sp. ovoid, smooth, 7–8 × 4 μ.

On the ground. United States (Nebraska).

maculata, Boud., Bull. Soc. Bot. Fr., xxxii, p. 283, pl. 9, f. 2; Sacc., Syll. v, p. 775.

P. campanulate then expanded and umbonate, rimose, covered with brown adpressed fibrils and ornamented with whitish adpressed squamules, mostly concentrically arranged, 3–5 cm.; almost free, broad, fawn-colour with a tinge of olive; s. solid, cylindrical, slightly thickened at the base, slightly fibrillose, colour of p., apex paler and scurfy, 3–8 cm.; sp. elliptic-oblong, smooth, 10–13 × 5–6 μ.

In woods. France.

Near to *I. rimosa*, differing in the white scales on the pileus and larger spores.

reflexa, Gillet, Champ. Fr., with figure (described in general index), 1897.

P. convex, acutely umbonate, with concentrically arranged rows of fibrils, pale yellow, apex of umbo darker, 2–2·5 cm.; g. free, ochraceous; s. solid, smooth, yellowish above, base whitish, 5–8 cm., very slightly flexuous; sp. elliptical, smooth.

On the ground. France.

According to Gillet's figure the present species has a long slender wavy stem and an acutely conical pileus having two concentric ridges, which appear to be due to the cracking and upturning of the cuticle.

squamigera, Sacc., Syll. v, p. 763; *Ag.* (*Ino.*) *squamiger,* Britz., Hym. Südbay., 153, f. 175 (1883).

P. campanulate then expanded, umbonate, covered with minute squamules, saffron or dingy yellowish-red, edge wavy, 2 cm.; g. adnate with a decurrent tooth, ventricose, brownish; s. equal, stuffed, flexuous, with rather large fibrillose scales up to the annular zone, apex smooth, colour of p., 3–5 cm.; sp. elliptical, smooth, 8 × 4 μ.

In woods. Bavaria.

Allied to *I. hirsuta.*

subgranulosa, Karst., Hedw., 1892, p. 293; Sacc., Syll. xi, p. 52.

P. convex then expanded, centre sometimes slightly depressed or obsoletely umbonate, even, pale ochraceous, with minute darker erect squamules, especially at the disc, or sometimes adpressedly squamulose, 2–4 cm.; g. adnate, seceding, crowded, greenish-cinnamon, then fuscous-cinnamon; s. stuffed then hollow, rigid, equal or narrowed below, curved or flexuous, with a minute subterranean bulb, 2–3 cm.; sp. smooth, 7–9 × 4–5 μ.

Sandy ground. Finland.

Very similar and also allied to *I. delecta,* Karst.

Imperfectly Described.

Under this heading are included those species where the general diagnosis is obviously inadequate, or where there is no mention of the spores. Such species may or may not belong to the genus *Inocybe.*

mammilaris, Sacc., Syll. v, p. 785; *Ag. mammilaris,* Passer., Fung. Parm., no. 189, p. 76.

P. white, convex, mammilose, squamulose; g. emarginately adnexed, edge white; s. white, hollow, equal, flexuous; sp. smooth.

On the ground. Italy.

grata, Karst., Hattsv., p. 463; Sacc., Syll. v, p. 777; *Ag. gratus,* Weinm., Hym. Ross., p. 185.

P. fleshy, conico-campanulate, whitish rufescent or rufescent, fibrillose, disc subsquamose; g. olive, margin white, then olive-brown, adnexed; s. equal, stuffed, fibrillose, colour of the p.

'Gregarious, somewhat fragile. Pileus at first conical or conico-campanulate, whitish-fuscous or rufescent, almost plane when adult, 1–1½′ lat. Gills 2″ lat.,

somewhat crowded. Stem 2–3′ long., 2–3″ thick. Spores pale rusty-ochre! Odour pleasant!'

The above is Weinmann's description of his species, which differs very materially from that given by Fries and copied by Saccardo.

strigiceps, Sacc., Syll. v, p. 791; *Ag.* (*Ino.*) *strigiceps*, Fries, Epicr., p. 183; *Ripartites strigiceps*, Karst., Hattsv., p. 478.

P. obtusely convex then expanded, rufescent, strigose from the presence of long fibrils, silky, edge at first involute, ciliate with long deflexed fibrils, dry, 1–2 cm.; g. adnato-decurrent, crowded, becoming brownish; s. stuffed, white, everywhere villose, 4–6 cm.

Among fallen leaves in beech woods. Sweden, Holland.

capucina, Karst., Hattsv., 458; Sacc., Syll. v, 772; *Ag.* (*Ino.*) *capucinus*, Fries, Vet. Akad. Förh., 1873, v, p. 5; Fries, Icon. Sel., tab. 108, f. 2.

P. conico-campanulate, acute but not umbonate, dusky brown, paler towards the margin, everywhere fibrillosely scaly, 2·5–5 cm.; g. sinuato-adnate, base broad and gradually narrowing towards the margin, brown; s. solid, short, equal, fibrillose, brownish but paler than p., 2·5 cm. Flesh white.

On the ground under alders. Sweden.

A section of the pileus is almost exactly an equilateral triangle.

The plant figured by Patouillard under the above name (Tab. Anal. Fung., no. 529) is obviously not the species of Fries, yet Saccardo has added to Fries' diagnosis of *I. capucina* the spore measurements of Patouillard's plant.

? pollicaris, Karsten, Symb. ad Myc. Fenn., xi, in Meddel. Soc. pro. Faun. et Flor. Fenn., ix, p. 68 (1882); Sacc., Syll. v, p. 763.

P. rather fleshy, campanulate then expanding muricate with squarrose squamules, brownish-bay, scarcely 1 cm.; g. adnexed, crowded, ventricose, brownish-cinnamon (blackish when dry); s. equal, floccosely and squarrosely squamulose, colour of p., scarcely 3 cm.; sp. broadly elliptical, brownish, 3–5 × 2–3 μ.

In a hothouse. Finland.

Karsten places this species in the genus *Inocybe* with a query, but gives no reasons for so doing.

squarrosula, Sacc., Syll. xi, p. 50; *Clypeus squarrosula*, Karst., Symb. Myc. Fenn., xxxii, p. 7.

P. convexo-plane, obtuse, fibrillose, fuscous, disc with squarrose squamules, darker, 1–2 cm.; g. crowded, subventricose, brownish, white-crenulate; s. equal, brownish squamulose, 2–3 cm.; sp. 10 × 7–8 μ.

On pine trunks. Finland.

gomphodes, Sacc., Syll. v, p. 786; *Ag.* (*Ino.*) *gomphodes*, Kalchbr., Grev., viii, p. 152, tab. 142, f. 8.

P. campanulate with a pronounced globose umbo, fibrillose, brownish, 1·5–2 cm.; g. ascending and almost free, narrow, greyish umber; s. stuffed, subequal, pallid rufous, base slightly bulbous and surrounded with white mycelium.

New South Wales (Richmond River).

Readily distinguished by the globose umbo nearly the size of a pea, perched on the top of the campanulate pileus.

delecta, Karst., Hattsv., i, p. 460; Sacc., Syll. v, p. 783; *Ag.* (*Ino.*) *caesariatus var. fibrillosus,* Fries, Icon. Sel., tab. 109, f. 3.

P. convex then plane, scarcely depressed or umbonate, fibrillosely scaly, dingy, tawny- or rufous-honey-colour, pale rufous-cinnamon when dry, about 5 cm.; g. emarginate, crowded, ventricose, pale honey-colour then with an olive tinge, finally brownish, edge paler and crenulate; s. solid, equal, slightly curved, dingy yellow or rather pallid, white fibrillose, apex nearly naked, 3–5 cm. Flesh yellowish then white.

Near paths in pine woods. Sweden, Finland.

viscosissima, Karst., Hattsv., p. 465; Sacc., Syll. v, p. 789; *Agaricus viscosissimus,* Fries, Icon. Sel., ii, p. 9, tab. 110, f. 2.

P. persistently convex, acutely umbonate, brownish umber, very glutinous then silky, 2–2·5 cm.; g. rounded behind and almost free, ventricose, rufescent; s. subfistulose, equal, glabrous, pallid, not fibrillose but usually white-pruinose, 3–4 cm.

On the ground in pine woods. Sweden.

Allied to *I. trechispora,* but differs in the colour of the pileus, and the glutinous coating, which is present in sufficient quantity to drip to the ground. Quélet (Flor. Myc. 102) gives the present species as a synonym under his *I. umbonata.* This however cannot be correct, as the last-named Fungus has the stem floccose up to a distinct ring, and is obviously a *Stropharia.*

Excluded Species.

umbonata, Quél., Bull. Soc. Bot. Fr., 1876, p. 330, pl. 2, f. 4.

This is undoubtedly a species of *Stropharia* with the stem floccosely peronate up to the ring. It was originally called *Agaricus* (*Stropharia*) *inunctus,* Fr., by Quélet (Champ. Jur. et Vosg., i, p. 110).

psamminum, Sacc., Syll. xi, p. 50 (foot-note); *Ag.* (*Heb.*) *psamminus,* Berk.

This species is a *Flammula,* and will stand as *F. psammina.*

plumosa, Karst., Hattsv., p. 455; Sacc., Syll. v, p. 763; *Ag.* (*Ino.*) *plumosus,* Fries, Mon., i, p. 337; *Ag. plumosus,* Bolton, Hist. Fung., i, p. 33, pl. 33 (1788).

On carefully going over Bolton's description of his Fungus, I can find no justification for its retention in the genus *Inocybe.* He distinctly states that the gills are *white,* both in his Latin diagnosis, and in his general description of the species. On this point Bolton can be trusted, judging from his usual accuracy in colour descriptions.

It was Fries who first suggested that Bolton's plant belonged to the section *Inocybe,* and described the gills as 'albido-fuligineis,' and most people have accepted the description given by Fries of what he supposed to be Bolton's Fungus.

No one in Britain has found an *Inocybe* corresponding to Bolton's figure and description since Bolton's time. I am inclined to think that the Fungus Bolton had in view was a species of *Collybia* of the section Vestipedes.

The following is Bolton's description of the Fungus under consideration:—

'Agaricus stipitatus, pileo hemispherico plumoso murino, lamellis trifidis albidis stipite longo plumoso.'

'The root is round, hard, the size of a pea, of a brownish-black colour, and emitting a few long hard fibres; it is not surrounded by a volva.

'The stem is hard, solid, cylindrical, often bended or waved, the thickness of a duck's quill, and about four inches high; it is closely covered with small downy or feathery tufts of a perfect mouse-colour; there is no curtain.

'The gills are in three series, deep, and terminate in a claw at the base, which just touches the top of the stem; they are numerous, soft, flexible, white, and of a dry, light substance.

'The pileus is hemispherical, an inch and a half in diameter, of a perfect mouse-colour, and like the stem, thickly covered with little tufts of a downy matter, which grow from its surface, and are of the same colour with it; there is a beautiful fringe of the same down all round the margin. The substance is thin, light, dry, and flexible; it withers in decay.'

micropyramis, Sacc., Syll. xi, p. 50 (foot-note); *Ag.* (*Heb.*) *micropyramis,* Berk.

This is a *Naucoria,* and will stand as *N. micropyrama* (Berk.).

subroindica, Bann. and Peck, 44 Rep. State Mus., p. 70; *rubroindica,* Sacc., Syll. xi, p. 52.

Only described from a sketch. No mention of spores, and no published figure.

tricholoma, Sacc., Syll. v, p. 790; *Ag. tricholoma,* Alb. and Schw., Consp., p. 188.

This has been correctly referred to *Flammula* by Karsten, on the label to 'Karst., Fung. Fenn., exs. 412.'

violaceafusca, Sacc., Syll. ix, p. 96; *Ag.* (*Ino.*) *violaceafuscus,* Cke. and Massee, Grev., xvii, p. 52; Cke., Ill., pl. 1174.

This proves to be a *Cortinarius,* and will thus become *C.* (*Dermo.*) *violaceafuscus,* Cke. and Massee.

phaeocephala, Sacc., Syll. v, p. 774; *Ag. phaeocephalus,* Bull., Champ. Fr., tab. 555, fig. 1; *Ag.* (*Ino.*) *phaeocephalus,* Fries, Hym. Eur., p. 231; Cke., Hdbk., ed. ii, p. 155; Cke., Ill., pl. 396.

I can find no justification for the retention of this species in *Inocybe.* It was first placed there by Fries, who never saw what he considered to be that species, but drew up his diagnosis from Bulliard's figure, adding a rider to the effect that its position is doubtful. Cooke's description with a 'smooth pileus' and 'bright ferruginous spores' certainly does not suggest *Inocybe.*

schista, Sacc., Syll. v, p. 774; *Ag.* (*Ino.*) *schistus,* Cke. and Sm., in Cke., Hdbk., ed. ii, p. 154; Cke., Ill., pl. 504.

P. campanulate, broadly subumbonate, cracking longitudinally, rather fibrillose, bay-brown, 5–8 cm.; flesh thin, equal, dingy like that of the s.; g. adnate with a decurrent tooth, rather ventricose, broad, tawny-rufous at maturity, edge pale, serrulate; s. solid, equal, twisted, paler than p., 6–8 cm.

Among short grass. Britain.

A species founded entirely on a sketch, which may or may not have been accurately done in the first instance.

tomentella, Sacc., Syll. v, p. 783; Quél., Fl. Myc., p. 106; *Ag.* (*Ino.*) *tomentellus,* Fries, Epicr., p. 176; *Ag. tomentosus,* Jungh., Linn., 1830, p. 403, tab. v, f. 7; (not Bull. nor Bolton).

This does not belong to the genus *Inocybe.* It may be an *Hebeloma.* Fries, who

first placed it in *Inocybe*, did not see a specimen, but simply copied Junghuhn's description, says:—'facie Hebelomatis insignis.'

imbecillis, Sacc., Syll. v, p. 790; *Ag.* (*Ino.*) *imbecillis*, Fries, Hym. Eur., p. 236; *Ag. imbecillis*, Passer., Fung. Parm., p. 76.

Too imperfectly described to be referred to any genus except as a pure speculation.

DESCRIPTION OF FIGURES IN PLATE XXXII.

Illustrating Mr. Massee's monograph of *Inocybe*.

Fig. 1. Section through portion of gill of *Inocybe geophylla*, Karst. *a*, *a*, basidia bearing spores in different stages of development. *b*, *b*, basidia. *c*, paraphyses. *d*, subhymenial hyphae. *e*, *e*, hyphae of trama. × 500.

Fig. 2. *Inocybe rhombospora*, Massee. Nat. size.

Fig. 3. Spores of same. × 400.

Fig. 4. Spores of same, very highly magnified. One spore shows surface and the other lateral view.

Fig. 5. *Inocybe Bucknalli*, Massee. Nat. size.

Fig. 6. Basidia and spores of same. × 500.

Fig. 7. Cystidium of *Inocybe geophylla*, Karst., showing sphere of mucilage extended from its apex. × 500.

Fig. 8. Apex of cystidium after the sphere of mucilage has contracted and formed a brownish mass of crystalloids. × 500.

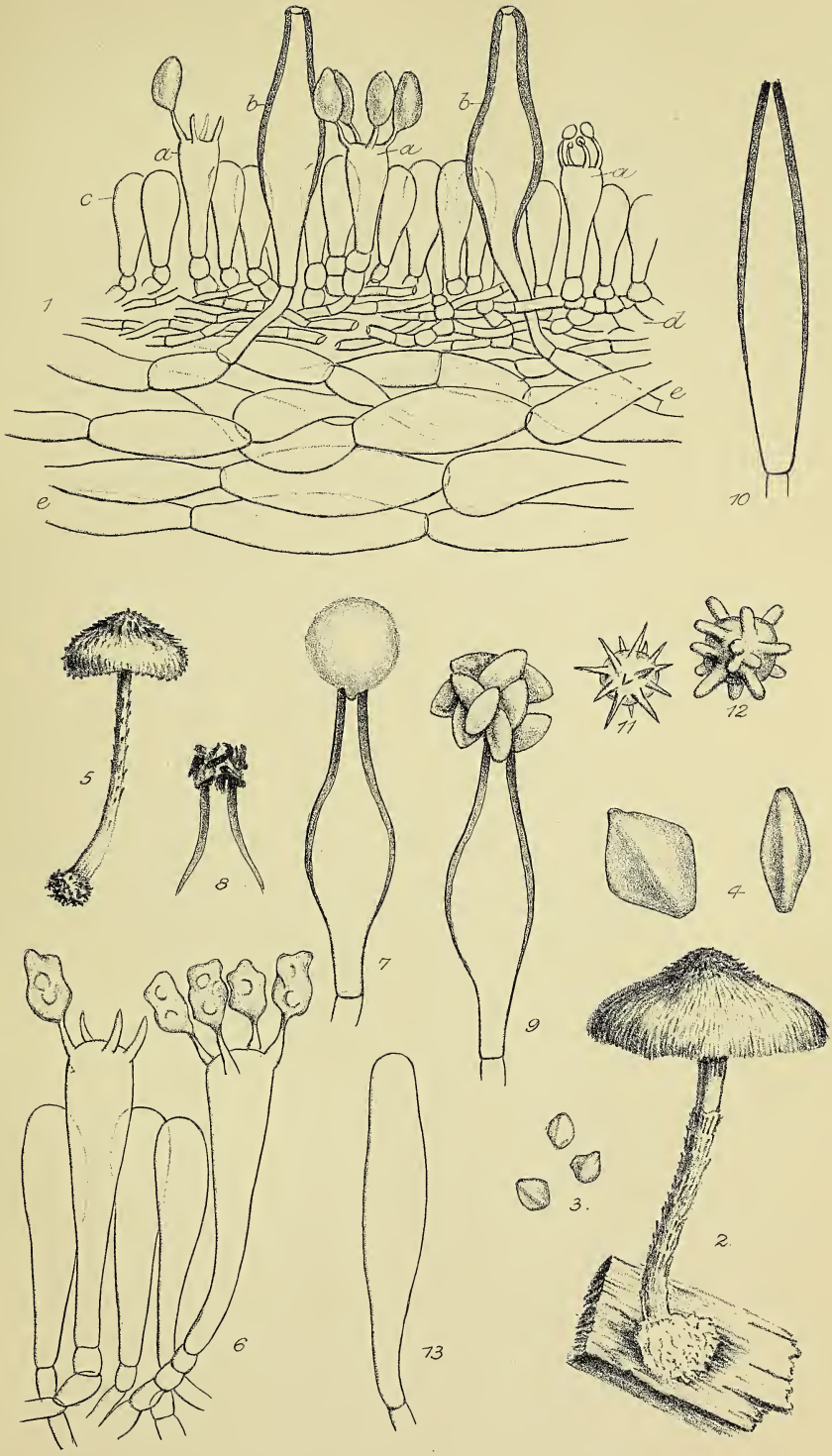
Fig. 9. Cystidium showing a dense mass of spores held together by the sphere of mucilage extended from its apex. × 500.

Fig. 10. Fusiform or fusoid type of cystidium. × 500.

Fig. 11. *Inocybe Gaillardi*, Gillet, single spore of. × 500.

Fig. 12. *Inocybe calospora*, Quél., single spore of. × 500.

Fig. 13. Thin-walled cell from margin of gill of *Inocybe Bucknalli*, Massee. × 500.



G. Massee, del. Highley, Lith. et Imp.

MASSEE. *Characters of hymenium in Inocybe.*

Index.

On the Occurrence of Secondary Xylem in Psilotum[1].

BY

L. A. BOODLE, F.L.S.

With Plate XXXIII and seven Figures in the Text.

AN examination of the structure of *Psilotum* and *Tmesipteris* was begun some years ago, at the suggestion of Dr. D. H. Scott, F.R.S., in connexion with the hypothesis put forward by him regarding these genera, viz. that of their affinity with the Sphenophylleae[2] among fossil plants; the object being to learn what additional evidence a re-examination of the anatomy might afford in relation to this view. It was intended to publish the results in a joint paper, but, as this has been postponed, some observations on *Psilotum triquetrum*, Sw., have been completed and are described separately in the present paper[3].

A few remarks must first be made on the external characters of the stem of *Psilotum triquetrum*. There are no roots, but the different branches of the stem may be classed under aerial parts and subterranean parts[4]. The aerial shoots are upright, repeatedly forked, and bear scale-leaves, and, in the upper region, sporophylls with synangia; most of the subterranean branches have no scale-leaves, and are densely covered with rhizoids. Among the subterranean branches three types have been distinguished[5], viz. (1) those completely covered with rhizoids and with only a terminal growing apex; (2) those similar to (1) except that they have laterally placed arrested apices besides the terminal growing-point, the existence of these apices being revealed by the absence of rhizoids on their surfaces[6]; (3) those in which the rhizoid-covering is reduced, and, on parts formed by further growth, is entirely absent, lateral apices being also present on these branches as well as small scale-leaves, and the direction of

[1] From the Jodrell Laboratory, Royal Botanic Gardens, Kew.

[2] Scott ('00), p. 499.

[3] A preliminary account has been given in the New Phytologist, vol. iii, 1904, p. 48.

[4] This is only a rough criterion. There appears not to be a constant relation between the level of the soil and a definite structural region of the stem.

[5] See Solms-Laubach ('84, p. 156), Nägeli and Leitgeb ('68, p. 147), and Pritzel ('00, p. 612).

[6] Later they may grow out as branches.

[Annals of Botany, Vol. XVIII. No. LXXI. July, 1904.]

growth being obliquely ascending. In the later growth of these branches the tips become vertical, and on leaving the soil they continue their growth as aerial shoots.

In branches of type (2) Bertrand ('81, p. 265) regards each lateral apex as being an arrested and laterally displaced branch of a dichotomy, the other branch having continued its growth and appearing like the direct continuation of the parent branch [1]. The growth is therefore described by Bertrand as sympodial. He also ('81, p. 274, &c.) regards some parts of the plant, e. g. certain aerial shoots and branches of category (3), as being 'cladodes,' produced by a kind of cohesion or fasciation of ordinary branches, founding this view partly on the structure of the stele. These views are referred to by Solms-Laubach ('84, p. 157) and need not be entered into further here.

For the purpose of the present description it will be sufficient to distinguish between the different parts as follows: (*a*) aerial stem; (*b*) ordinary rhizome, including branches of types (1) and (2); (*c*) type (3) described above, having characters transitional between rhizome and aerial stem; this may be called the stock of the aerial stem. The nature of the transition makes it impossible in some cases to draw any sharp limit between the stock of the aerial stem and the ordinary rhizome, or even to classify some of the older branches [2].

In Bertrand's work the results are given of an elaborate investigation of the structure of *Psilotum*. The figures illustrating this work may be referred to for the primary structure of the stele. Thus Fig. 134 (p. 298) shows the stele of some very delicate branches of the rhizome, having only a small number of tracheides in the xylem, namely four, three, two and one respectively.

A transverse section of a branch of the rhizome having only three tracheides is shown in Fig. 45 in the text in the present paper. The stele of a stouter branch of the rhizome is reproduced in Fig. 1, Pl. XXXIII; here nine tracheides are seen [3]. In the ordinary rhizome the number of tracheides in the stele varies greatly with the diameter of the organ. When only a few tracheides are present, there may be no clearly marked protoxylem, but when the tracheides form a larger group the xylem often takes the form of a diarch plate [4]. Fig. 46 in the text shows an incompletely differentiated diarch plate [5] of eleven elements, of which five tracheides only are lignified. Here, and in other cases (e. g. at the base of the aerial shoot),

[1] Solms-Laubach ('84, p. 158) however found that true lateral branching occurred in addition to this type of growth.

[2] In doubtful cases the stumps of rhizoids, which have fallen off, give some clue by their abundance or otherwise.

[3] Seven are distinct, and there are two pale ones on the left.

[4] See Bertrand ('81), Figs. 135 and 136.

[5] Differentiation appeared to have been arrested.

where the protoxylem is clear, it is evident that the differentiation of the primary xylem is centripetal in the rhizome and in the stock of the aerial stem.

At the base of the stock of an aerial branch the xylem is often diarch, as in many of the larger branches of the rhizome, but when traced upwards (acropetally) the stele gradually increases in size and becomes triarch; above the soil the exarch stele continues to enlarge, becoming successively tetrarch, pentarch, and so on, up to e. g. octarch, but, during this enlargement of the xylem, sclerenchyma (preceded by a little parenchyma) has appeared within it in a central position, and has increased to a large central group, so that the tracheides now form a hollow many-rayed exarch star enclosing this group of sclerotic tissue[1]. Figures illustrating the form or structure of the stele of the aerial stem are given by Brongniart ('37, Pl. XI, Fig. 1), Link ('42, Fasc. IV, Taf. V, Fig. 1), Nägeli ('58, Pl. I, Fig. 3), Bertrand ('81, Fig. 174, &c.), and Pritzel ('00, Fig. 383 A).

So far all that has been said refers to the primary structure of the stem, and nothing besides this is to be seen either in Bertrand's figures of the rhizome and aerial stem, or in the illustrations of the other authors referred to. In certain parts of the plant however, when examined at a sufficiently late stage, additional tracheides are found to be present. They will be described below as secondary, and the reasons for regarding them as such will be given later.

In Fig. 1, Pl. XXXIII, which is a transverse section of the rhizome with primary structure only, one sees a zone of 'parenchyma[2]' often three to four cells thick between the centrally placed group of xylem-elements and the zone of sieve-tubes[3], the position of the latter being given at *s. t.* A similar zone of parenchyma is found in a corresponding position in the stock of the aerial shoot and also in the aerial shoot itself (though here often reduced opposite the protoxylem groups). The primary xylem is generally composed of a solid mass of tracheides, not interrupted by parenchyma, though this is not without exception, and the development is centripetal.

An examination of the stock, of the lower part of the aerial shoot, and of some parts of the ordinary rhizome (in each case when old) showed additional

[1] Reduction of the stele through somewhat similar steps accompanies its decrease in size through successive branches of the aerial stem.

[2] Thin-walled considerably elongated elements.

[3] A careful examination of these elements was not made, but it may be mentioned that, in the presence of numerous granules clinging to the walls, they resemble the Fern-type, that they are present in the rhizome as well as in the aerial stem, although De Bary ('77, p. 349) failed to find them in the former. In the rhizome and stock of the aerial stem they form an interrupted ring, single sieve-tubes and also tangential rows of two or three sieve-tubes being separated by parenchyma. In the aerial stem they may form a thicker zone. There are no special gaps opposite the protoxylems; see Russow ('75, p. 40), where he corrects the mistake as to their distribution shown by his earlier figure ('72, Taf. XI, Fig. 30).

tracheides, to be regarded as secondary, scattered in the zone of parenchyma referred to above [1], and hence outside the mass of tracheides forming the primary xylem. These additional tracheides are mostly separated by at least one layer of parenchyma from the central group, but some of them may be in contact with it. They are only to be found in old parts of the plant, where they may show all stages of differentiation, but in the oldest parts examined [2] all the secondary tracheides present were completely lignified.

Figs. 2–6 in Pl. XXXIII show secondary tracheides in the tissue surrounding the primary xylem. Text-Fig. 44 is an external view of the stem from which these sections were cut. In this figure, *e*, *f*, and *g* are three aerial shoots, and it is clear from the disposition of the parts that *e* must have been the first-formed aerial shoot, and that from its lower region an obliquely ascending branch (*c*, *d*) grew out on the right and gave rise to the two other aerial shoots *f* and *g*.

The whole of the branch *c*, *d* should perhaps be classed as the stock of the aerial shoots, the lower part certainly so, but, on the right beyond the point of insertion of *f*, it becomes rhizome-like again, as evidenced by reduced diameter, remains of rather numerous rhizoids, and by the character of the stele. A transverse section from this region, cut at the level of *d* in Text-Fig. 44, is represented in Fig. 2, Pl. XXXIII. It will be seen that there is a centrally placed group of tracheides (*p*), probably diarch, which is comparable with the primary group in Fig. 1, Pl. XXXIII, though the size and number of the elements differ. In Fig. 2 the central group of primary tracheides (*p*) is surrounded by a considerable number of smaller tracheides, which are to be regarded as of secondary origin. Several of these elements have much darker walls than the tracheides of the primary group. This is due to the colour-differentiation of the stain employed (methyl-green and eosine), all incompletely lignified walls having taken up eosine as well as methyl-green, the combination of the two stains producing a purple colour, which is in strong contrast to the green or blue of the fully lignified elements. Watery methyl-green followed by alcoholic eosine proved a very satisfactory stain for differentiating such partially lignified elements [3]. Phloroglucin with hydrochloric acid was used for comparison; two consecutive sections were chosen, and one was stained with methyl-green and eosine, the other with phloroglucin. A close correspondence was shown; the elements which stained purple or purplish black with the double stain being just those which only stained faintly with phloroglucin, while all the

[1] Or largely replacing it.

[2] Probably one year or more old. Proportional age may be estimated by the amount of the well-known brown substance, which encroaches on and gradually fills up the cavities of many of the cells of the inner cortex, and by the hardness of the specimen. An old branch of the rhizome showed seven primary and twenty secondary tracheides, all mature.

[3] This stain was used for the same purpose in *Ophioglossum* (Boodle, '99, p. 386).

FIG. 44.

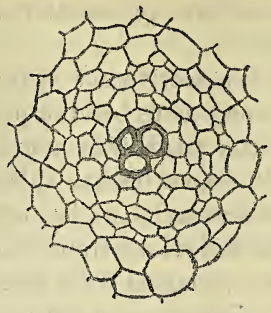


FIG. 45.

FIG. 46.

FIG. 47.

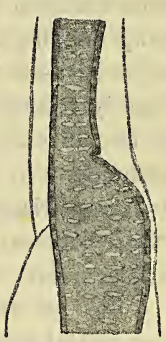


FIG. 48.

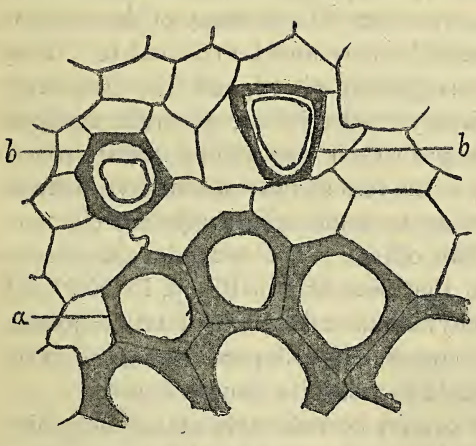


FIG. 49.

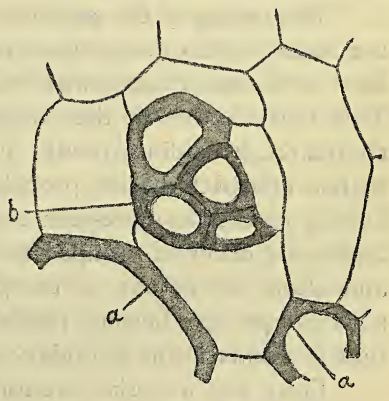


FIG. 50.

FIG. 44. Specimen from which numerous sections were cut. *e*, *f*, and *g*, three aerial branches. About natural size. FIG. 45. Stele of small branch of rhizome in transverse section. There are only three tracheides; sieve-tubes not specially indicated. × 95. FIG. 46. Diarch xylem of stele of a rhizome, arrested in development. × 95. FIG. 47. Part of a secondary tracheide showing a constriction. × 390. FIG. 48. Scalariform secondary tracheide showing sinuous course. × 95. FIG. 49. From a transverse section through the basal part of the aerial branch *g*, in Fig. 44. *a*, primary tracheides; *b*, *b*, secondary tracheides, incompletely lignified and containing a protoplasmic lining. × 390. FIG. 50. Group of four secondary tracheides (*b*), with parts of two primary tracheides (*a*) and adjacent parenchyma. This group is seen at *s*, in Fig. 3 in the plate. × 390.

blue or green walls in the first preparation showed full coloration with phloroglucin in the other.

Fig. 3, Pl. XXXIII, is a section cut at the point marked *c* in Fig. 44 in the text. It shows a conspicuous band of primary tracheides and a large number of smaller secondary tracheides, among which one group of four, marked *s* in this figure, is drawn further enlarged in Text-Fig. 50. The three elements with the darker walls are incompletely lignified.

Fig. 4, 5, and 6 in Pl. XXXIII are transverse sections of the stock of the aerial branch *e* in Text-Fig. 44, cut at three levels in ascending order. Their positions are indicated by letters: *a* is the level of Fig. 4, *b* that of Fig. 6, and about half-way between *a* and *b* is the position of Fig. 5. In these three sections numerous secondary tracheides are present, many of them being incompletely lignified. In Fig. 4 the primary xylem can be recognized as a broad plate of tracheides. In Fig. 5 the xylem has become roughly triangular, and at *r* a parenchymatous ray is seen running inwards as far as one of the protoxylem-groups, in which there is a crushed tracheide. In Fig. 6 the primary xylem-group is considerably larger than in Fig. 5, triangular and doubtless triarch. At several points outside the primary xylem, e. g. at *r*, radial arrangement is found among the parenchyma and secondary tracheides.

Fig. 49 in the text is from a transverse section through the lower part of an aerial shoot. It shows some primary tracheides (*a*), and two secondary tracheides (*b*, *b*). The latter are incompletely lignified, and still contain a protoplasmic lining. Similar cases were met with in many of the sections.

The nature of the secondary tracheides may now be referred to. They are scalariform, or sometimes rather irregularly pitted, and they frequently have a sinuous course, while the primary tracheides are generally straight. This must be due to their having to push their way among mature parenchyma, &c., by sliding growth. Fig. 47 in the text shows a secondary tracheide with a constricted part, probably owing to some of the adjacent elements having resisted compression more than others. The secondary tracheides frequently occur in groups similar to that seen at *s* in Fig. 3, Pl. XXXIII, and often one or two of the parenchymatous cells immediately adjoining such groups have become partially or completely collapsed [1]. Fig. 48 in the text is a scalariform secondary tracheide showing its sinuous course.

From the material examined it cannot be definitely stated how high up in the aerial stem secondary tracheides may occur, but in one specimen several were present in a stele containing a fair-sized central group of sclerenchyma.

The examples of the occurrence of secondary tracheides chosen for illustration in Pl. XXXIII do not include any case in which it was perfectly clear that the organ in which they were present should be classed as

[1] The rigidity of the cortex preventing expansion of the stele.

ordinary rhizome, since the nature of the branch, shown in section in Fig. 2, might be disputed. Examples were found, however, in which true rhizome branches attached to the stock of an aerial shoot contained a few secondary tracheides[1].

We must now discuss the reasons for regarding the outer tracheides as secondary. An important point is the late differentiation of the earliest of these elements. If one takes the case of the stock of an aerial shoot with diarch structure, the xylem differentiates centripetally until complete, and there appears to be a considerable pause before the outer tracheides arise. In this respect the fact is significant that Bertrand does not appear to have seen secondary tracheides in any part of the plant. This appeared so remarkable that the question suggested itself whether the production of these elements might not be exceptional and dependent on special conditions of growth, or perhaps shown only by one variety of the species. This however is improbable, because I learn from Miss S. O. Ford and from Miss E. N. Thomas that they also have found these elements. Hence it is probable that Bertrand did not examine material of suitable age. At what distance from the apex they first begin to develop could not be determined.

Another fact suggestive of secondary growth is that successive differentiation of these tracheides is still proceeding in quite old parts of the subterranean stem, a long way back from the aerial branch. Thus at *a* in Fig. 44 in the text, differentiation is still going on, and the basipetal distance of this from the cut end of the aerial branch *g* (where 'primary' development is complete) amounts to about 10 cm.[2] The stem at *a* is probably several months old. There is nothing to suggest that the apparently developing tracheides are permanently arrested immature elements, since the outer tracheides in the oldest stems examined were all mature, and true arrest in the metaxylem of one branch showed quite different characters.

No definite cambium is present, but radial arrangement is often shown to a slight extent. In Fig. 6, Pl. XXXIII, *r* marks a radial row of five elements, all parenchymatous except the outermost one, which is a young tracheide containing protoplasm. In a few cases parenchymatous rays occur opposite the protoxylem-groups; a rather good case is seen at *r* in Fig. 5. These last two features, though not particularly well marked here, are familiar characters of secondary xylem.

Taking all these facts into consideration one is justified in assuming that the late-formed tracheides represent a comparatively slight amount of secondary xylem, and that this is probably a reduction from more normal secondary thickening is suggested by the fact that in *Psilotum* the leaves

[1] In other cases, where the branches were older and their rhizomic nature less certain, numerous secondary tracheides were present.

[2] Several centimeters of branched stem had been cut away above *g*.

have been reduced to scales, whereby reduction of transpiration must have been achieved.

The mode of occurrence of the secondary tracheides appears to point to the plant having retained the power of forming such additional elements in consequence of the necessity for an adequate water-conduction or water-storage capacity in branches, on which the water-supply of well-developed aerial shoots devolves. The stimulus, which leads to the differentiation of the secondary tracheides, may perhaps be either some secondary effect of increased transference of water caused by the transpiration of the aerial shoots, or the backward conduction of carbohydrates from the latter.

The specimen shown in Fig. 44 in the text presents some instructive features in relation to this. Secondary tracheides are present in the base of the aerial shoot *g*, throughout the branch (*d*, *c*) bearing it (though decreasing between *d* and the insertion of *f*), and downwards in the parent stem to the base of *b*, *a*. This may well be due to a backward stimulus from the shoots *f* and *g*. The other aerial shoot (*e*) had evidently been cut or broken off at *e* when young. In its upper region primary differentiation had been arrested before completion, but in its lower region, though primary differentiation was complete, there were no secondary tracheides. In tracing the structure upwards in the stock of this branch from *b* towards the point where *c*, *d* was given off, it was interesting to find the secondary tracheides gradually becoming restricted to one side of the stele, so that when the branching took place they all accompanied the stele supplying *d*, *c*. This disposition certainly favours the supposition of a basipetal stimulus.

If one adopts the view that the outer tracheides represent reduced secondary xylem, the correspondence between the stem-structure of some parts of the plant in *Psilotum* and that of *Sphenophyllum* becomes rather striking. Setting minor details aside, the triarch xylem-mass surrounded by a small amount of secondary xylem in a young stem of *Sphenophyllum* (Scott, '00, Fig. 35, p. 85, and Williamson, '74, Pl. I, Figs. 2–4) compares well with the structure of the stock of an aerial stem of *Psilotum* seen in Fig. 6, Pl. XXXIII. The structure of the aerial stem of *Psilotum* with its stellate xylem bears a strong resemblance to that of the axis of *Cheirostrobus*[1], but there is the difference that in *Cheirostrobus* the tracheides form a solid star, while in *Psilotum* the more central part of the xylem has been replaced by sclerotic tissue. Comparison of rhizomes cannot well be made, as an organ of this kind is not known with certainty in *Sphenophyllum*, and the rhizome of *Psilotum* may perhaps have been greatly modified by

[1] See Scott, '97, Pl. 1, Phot. 3. *Cheirostrobus* most resembles *Sphenophyllum*, though it shares certain characters with Lycopods and Calamarians. See Scott, '97, p. 26, &c.: 'We may hazard the inference that *Cheirostrobus* as well as *Sphenophyllum* sprang from a very old stock, which existed prior to the divergence of the Lycopods and Calamarians.'

reduction in consequence of its saprophytism. Dr. Scott, however, kindly gives me the information that organs with indeterminate xylem are sometimes associated with specimens of *Sphenophyllum*, and may possibly prove to be the rhizome of that plant.

Thus in structure the base of the stem of *Psilotum* recalls the stem of *Sphenophyllum*, while the upper part of the stem resembles the axis of the cone of *Cheirostrobus*, a plant with some relationship to the Sphenophylleae.

This amount of structural agreement [1] owes its value to the fact that the sporophylls of the plants concerned show distinct relationship of type. The correspondence of the typical synangium of the Psiloteae (*Tmesipteris* and *Psilotum*), together with its pedicel, to the sporangiophore of the Sphenophylleae was pointed out by Scott ('97, p. 27, and '00, p. 499), and this correspondence was found by Thomas ('02) to become, on the whole, still closer in the case of variations of the sporophylls of *Tmesipteris* to a presumably more primitive type [2]. The whorled arrangement of the leaves in the Sphenophylleae is a marked point of distinction from the Psiloteae. This and the nature of the sporophylls and the other characters of these two orders are discussed by Bower ('03, p. 227 et seq.), who adopts the hypothesis of the relationship, and further agrees with Thomas that the Psilotaceae should be included in the group Sphenophyllales.

It must be mentioned here that Lignier ('03, p. 106, &c.) holds the view that the Tmesipterideae or analogous types are among the ancestors of the Sphenophyllales, but he intercalates a Filicinean type between the two. Lignier ('03, p. 105) also assumes as probable the presence of secondary xylem and phloem in the ancient Filicinean types, so that on this supposition the presence of secondary thickening in living Psilotaceae and the deduction of its existence in their remote ancestors would not be discordant with Lignier's hypothesis of the phylogeny.

[1] In view of the fact that modifications of vascular structure can readily take place, the value of such resemblances between a recent plant and an ancient type may appear very small, but that well-defined details of *primary* structure may be retained for long ages is generally admitted and is well illustrated by the case of *Equisetum* and the Calamarieae. The value of *secondary* xylem as a character must be admitted to be much less, especially as secondary thickening appears to have been so general among vascular plants in the coal-measures. In a case of this kind, however, every additional point of agreement has some value, and, further, the presence of secondary growth may be taken as suggesting affinity with ancient types of vascular cryptogams. As a parallel case *Isoetes* may be quoted, in which the presence of secondary thickening helps the view of affinity with the Lepidodendreae. In a genus like *Isoetes*, in which a large number of species are submerged aquatics, the presence of secondary thickening is rather surprising, and the same is the case in a less degree for *Psilotum* with its reduced transpiring surface. That is to say, in both cases there is a probability against the secondary growth being a recent acquirement.

[2] Namely branched sporophylls and formation of extra sporangiophores. Bower ('03, pp. 228, 229) regards these as not reversional, but as cases of increased complexity due to favourable nutrition as the determining factor (in this respect agreeing with Thomas's view), but corresponding to 'morphological possibilities of further amplification.'

A short comparison of the structure of *Psilotum* and *Tmesipteris* is called for here. No secondary growth is known in *Tmesipteris*. It is possible that it may yet be found in old parts of luxuriant plants, or on the other hand it may have been quite lost by reduction. In *Tmesipteris* the lower part of the stem, which is covered with rhizoids, may be called rhizome; the structure exhibits much the same type as the primary structure of the same organ in *Psilotum*. The xylem of the stele consists of a solid group of tracheides, either with no distinct protoxylem in the case of small steles, or exarch and diarch in larger ones[1]. At the base of the 'aerial' stem of *Tmesipteris* the stele has a solid triangular group of xylem with three protoxylem-groups (see Dangeard, '91, Pl. XII, Fig. 10), and agrees with that of *Psilotum* (in the same region), except that the xylem is mesarch in *Tmesipteris*.

In the aerial stem also *Tmesipteris* has mesarch structure. A less important point of difference is that the xylem takes the form of a number of separate strands surrounding a sclerotic pith. Such a structure might easily be attained in *Psilotum* by the suppression of the later-formed part of the metaxylem, and this had actually taken place by arrest in the upper part of the aerial branch *e* (Fig. 44 in the text), there being five separate groups of xylem, each with its protoxylem.

The other distinction between the two genera, viz. the absence of mesarch structure in *Psilotum*, is apparently not absolute, though further investigation is desirable on this subject. Cases of apparent mesarch structure were certainly met with locally in the lower part of the aerial stem[2], a small number of scalariform tracheides being found on the outer side of one or more of the protoxylem-groups. The difficulty here is to prove that these elements are not secondary, and the material was not quite sufficient for the purpose. It may be noted, however, that these elements were mostly just opposite the protoxylem-groups, usually in contact with them (i. e. without the intervention of a layer of parenchyma), and that none of them were found in an immature condition. It is also important that, internal to the peripheral scalariform element of one of these apparently mesarch groups, there may be one or more tracheides with rather dense spiral thickening before one reaches the smallest tracheide characterized by a loose spiral, and even a form of thickening transitional between scalariform and spiral may be met with between the tracheides with these two types of thickening. Thus if one disregards the scalariform tracheides (as being possibly secondary) the evidently primary elements outside the protoxylem indicate mesarch differentiation.

[1] Bertrand ('81), Fig. 208 on p. 482, Dangeard ('91), Pl. IX, Fig. 4 and Pl. XII, Fig. 7, for diarch structure. Dangeard ('91, p. 206) states that in the ramifications of the rhizome 'la stèle binaire peut perdre son caractère de détermination.'

[2] In the region where five or more xylem-rays are present.

Hence, though examination of young shoots showing a suitable stage of differentiation at this level is required, one may provisionally regard local mesarch structure as established for *Psilotum*, and the most natural conclusion is that the structure of its aerial stem has been reduced from the mesarch to the exarch type in connexion with the disappearance of the leaf-traces. *Psilotum* and *Tmesipteris* might then be referred to a common parent-form, in which the aerial stem had a rayed mesarch xylem-mass, the suppression of leaf-traces having caused the loss of centrifugal wood in the one genus, and the influence of the leaf-traces in the other genus having broken up the xylem into distinct bundles. If we make a comparison once more with *Cheirostrobus*, it is interesting that in that plant there are indications of mesarch structure [1] (see Scott, '97, p. 9, footnote, and Pl. IV, Fig. 1), and that towards the centre of the stele a considerable amount of parenchyma is found separating the tracheides (Scott, '97, Pl. I, Phot. 3). This parenchyma might easily become converted into sclerotic tissue in response to mechanical requirements, the intervening tracheides becoming replaced by similar elements [2].

The material of *Psilotum triquetrum*, which was chiefly used for this investigation, was a plant which had lain in spirit for several years, and was probably grown at Kew, though its origin had not been recorded. For comparison fresh specimens of one or two old aerial shoots with adjacent subterranean parts were obtained from a plant grown at Kew, and these showed the same structural characters.

Summary.

The solid mass of tracheides described and figured by Bertrand in the subterranean parts of *Psilotum triquetrum* does not represent the whole of the xylem present in parts of the specimens examined, but additional tracheides are found outside it but internal to the ring of sieve-tubes.

These tracheides are formed considerably later than those of the central mass, and show successive and somewhat irregular development. Certain of them are to be found still incompletely differentiated in quite old parts

[1] And that secondary xylem is present in the peduncle of the cone (Scott, '97, p. 15, and Pl. VI, Fig. 21).

[2] It may be mentioned here that in a large aerial branch of *Psilotum*, preparation for the first dichotomy by the appearance of one tracheide in the middle of the central sclerotic tissue of the stele occurred at four inches below the fork. At two and a half inches below the latter there were eight tracheides in this position, and the group then increased in size to form a bridge across the sclerenchyma, afterwards splitting for the dichotomy of the stele. This differs from Bertrand's description ('81, p. 405 et seq. and Fig. 179) and may be exceptional. In *Tmesipteris* Bertrand ('81, p. 494 and Fig. 215) mentions that occasionally certain of the xylem-strands are interior with regard to the rest, and figures a small strand of five tracheides placed roughly at the centre of the pith, which is surrounded by a ring of six xylem-groups. It is possible that this central group may have some such connexion with the branching of the stem, as in the case just referred to in *Psilotum*.

of the stem. They are to be regarded as reduced secondary xylem, and they are present in the aerial as well as subterranean stem.

There is no definite cambial layer, but radial arrangement among the parenchyma and tracheides is often found, and parenchymatous rays opposite the protoxylems are sometimes present.

The secondary tracheides are scalariform or irregularly pitted, and often have a sinuous course.

The presence of secondary tracheides around a triarch primary xylem, such as occurs in some parts of the stem, gives a close approximation to the structure of the stem of *Sphenophyllum*.

In the lower region of the aerial stem a few cases of apparent mesarch structure were observed. If this should be verified, an important distinction between *Psilotum* and *Tmesipteris* would break down, and a further agreement between the aerial stem of *Psilotum* and the axis of *Cheirostrobus* (already rather striking) would be established.

Thus the new facts derived from a study of the vegetative anatomy strengthen the hypothesis of the affinity of the Psilotaceae with the Sphenophyllales. This view, put forward by Scott and adopted by Thomas and Bower, was founded partly on the vegetative anatomy, but more especially on the characters of the sporophylls.

The production of secondary tracheides in subterranean parts is probably dependent on the development of aerial shoots, and appears to be due to a basipetal stimulus from the latter.

In conclusion I wish to express my thanks to Dr. D. H. Scott, F.R.S., for valuable suggestions and criticism and for information regarding Sphenophylleae.

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EXPLANATION OF FIGURES IN PLATE XXXIII.

Illustrating Mr. Boodle's paper on *Psilotum*.

Figs. 1–6 are photographs from sections of *Psilotum triquetrum* stained with methyl-green and eosine; they are all × 90.

Fig. 1. Transverse section of rhizome, showing primary structure only. The xylem consists of a solid group of nine tracheides (seven quite distinct and two small paler ones on the left). *e*, endodermis; *s. t.*, sieve-tubes.

In Figs. 2–6 numerous secondary tracheides are present, many of them being incompletely lignified and therefore stained dark.

Fig. 2. Transverse section of the stem, cut at *d* in Text-Fig. 44. The large pale-walled primary tracheides (*p*) are surrounded by a large number of secondary tracheides.

Fig. 3. Transverse section of the stem, cut at *c* in Text-Fig. 44. *s*, a group of secondary tracheides. Primary xylem band-shaped and probably diarch.

Fig. 4. Transverse section of the stem, cut at *a* in Text-Fig. 44. Primary xylem band-shaped.

Fig. 5. Transverse section of the stem cut half-way between *a* and *b* in Text-Fig. 44. Primary xylem triarch. *r*, parenchymatous ray opposite one of the protoxylem-groups.

Fig. 6. Transverse section of the stem, cut at *b* in Text-Fig. 44. Xylem triarch. Radial arrangement of elements shown at *r*.

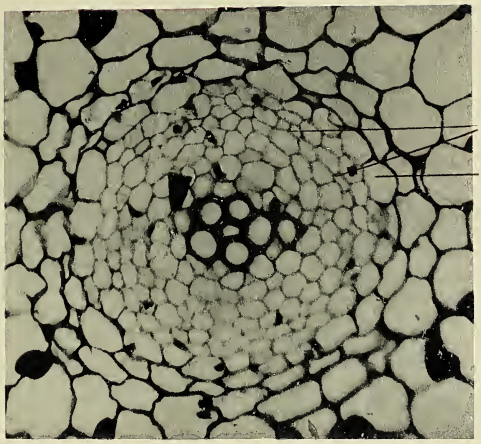

Fig. 1.

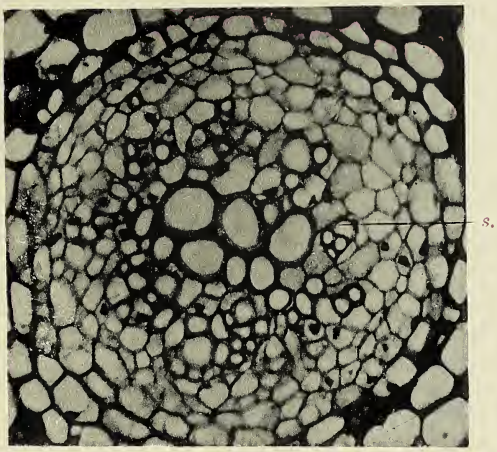

Fig. 3.

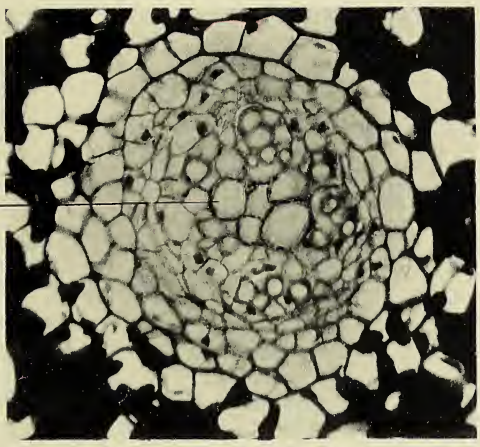

Fig. 2.

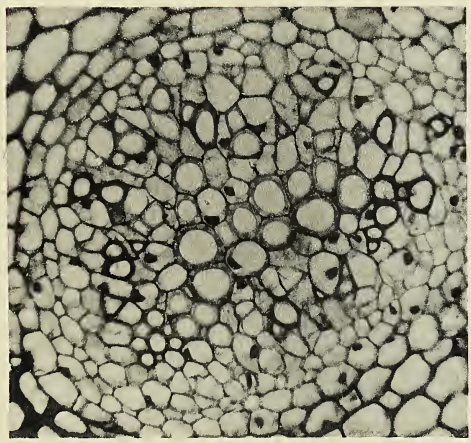

Fig. 4.

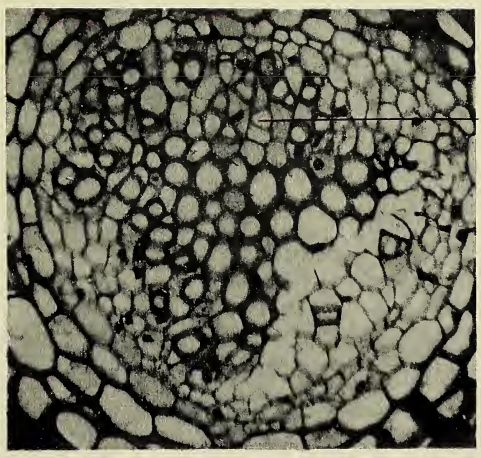

Fig. 6.

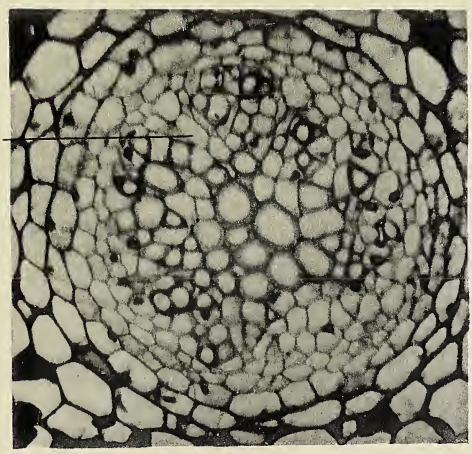

Fig. 5.

NOTE.

ON THE OCCURRENCE OF SIGILLARIOPSIS IN THE LOWER COAL-MEASURES OF BRITAIN.—In 1879, M. Renault, in his classical work 'Structure comparée de quelques tiges de la flore carbonifère,' established the genus *Sigillariopsis* for a small silicified stem, with leaves attached, from the Permian of Autun; he named the species *S. Decaisnei*[1]. The general character of the fossil is Lycopodiaceous; the stem resembles *Sigillaria Menardi* in structure, but has the peculiarity that the outer tracheides of the secondary wood are pitted and not scalariform, a character which seems to be unknown elsewhere among Palaeozoic Lycopods. The leaves are even more remarkable, for each leaf contains two parallel vascular bundles, except towards the apex where they unite into one. No member of the Lycopodiales, recent or fossil, is known to present this character. Yet the details of structure described by M. Renault agree very closely with those of a Sigillarian or Lepidodendroid leaf, except for the presence, here as in the stem, of pitted tracheides in addition to those of the usual scalariform type. M. Renault regarded his genus as establishing a bond of union between the smooth-barked Sigillariae and the Cordaiteae, a view which is connected with his general theory of the origin of the Gymnosperms, and which it would take too long to discuss in this preliminary note.

Hitherto no other fossil referable to the genus *Sigillariopsis* has been described. Recently, however, two specimens have come under my observation which agree with M. Renault's genus in so far as they are Lycopodiaceous leaves with two vascular bundles. Both specimens occur in calcareous nodules from the Lower Coal-Measures of Lancashire; the one, which I received about three years ago, came from the well-known locality at Dulesgate, while the other, which only reached me this spring, is derived from a new source, lately opened up, at Shore Littleborough. The material in which the leaves occur was in each case collected and prepared by Mr. J. Lomax.

The Shore Littleborough specimen is better preserved than the other and may therefore be first described. Two leaves are shown, both in transverse section. The larger is about 4 mm. wide by 1·4 mm. in maximum thickness. The lower surface is strongly convex, the upper more or less flat, but with a shallow median depression. The leaf thins out rapidly towards its edges, and on each side is a deep and narrow furrow, on the sides of which the stomata appear to have been placed. Thus the form of the section is that characteristic of the leaves of *Lepidodendron* and *Sigillaria*[2].

The mesophyll, which is almost perfectly preserved, has a well-marked palisade-layer on the upper side, interrupted about the median line. In the narrow wings of the leaf, and extending round the lateral furrows, characteristic spongy parenchyma is present. A sclerotic hypoderma extends all round the leaf, except at the lateral

[1] l. c. p. 270, Pl. XII, Fig. 15–19; Pl. XIII, Figs. 1–4.

[2] Renault, Flore fossile d'Autun et d'Épinac, Part II, Atlas, Pl. 34 and 41; Scott, Studies in Fossil Botany, Fig. 59.

furrows, but is thicker on the lower side; otherwise the mesophyll is made up of rather large, isodiametric cells.

The vascular tissue lies within a definite central region about 960 μ wide by 480 μ deep, which is well defined, but not marked off by any evident sheath. There are two vascular bundles with their xylem-groups widely separated, the distance between them being 280 μ, while the maximum diameter of each xylem-strand is under 200 μ. The xylem-strands are embedded in thin-walled tissue, in which the limits of the phloem cannot be distinguished in transverse section. Below each bundle is a broad band of dark, apparently sclerotic tissue, perhaps identical with the 'gaîne' of M. Renault's description. In the median line of the leaf and below the level of the bundles is a gap in the tissue, exactly agreeing in position with the lacuna or strand of delicate tissue figured by M. Renault in the leaf-traces and leaves of *Sigillaria*[1].

The transfusion-tissue ('tissu vasiforme' of M. Renault), consisting of reticulate tracheides, is extremely well developed, and forms a horseshoe, embracing the whole lower side of the central region, and approaching the bundles at its two upper extremities. Thus the whole structure of the leaf, apart from the presence of two bundles instead of one, is altogether that of a leaf of one of the Lepidodendreae; the analogy with certain Coniferous leaves is striking, though probably quite unconnected with affinity.

The other leaf in the Shore slide was evidently cut near its tip. The width is 1·7 mm. and the maximum thickness only 190 μ. The lateral furrows are scarcely indicated; it is sufficient to say that here also the two vascular bundles are quite separate, with a group of thick-walled tissue between them.

Of the Dulesgate specimen there are four successive sections, passing through the same leaf (Nos. 1166–1169 in my collection), but there is little change of structure throughout the series. The leaf has a different sectional form from that of the Shore specimen, for the upper surface is markedly concave, giving some of the sections a U-shaped outline. A sharp median depression is present on the upper side and a narrow dorsal rib on the opposite surface. There is very little trace of lateral furrows, but the hypoderma is interrupted at points corresponding to them. Here also palisade-tissue is present towards the upper surface. The central region is less well defined than in the Shore leaf; the two vascular bundles are quite separate, though not so far apart as in that specimen; the space between the two xylem-groups is about 140 μ wide, the xylem-groups themselves having a maximum diameter of about 260 μ. Transfusion-tissue is present, chiefly towards the lower side, as in the first specimen.

Another leaf is cut in obliquely longitudinal section; I have not yet been able to make out any pitted elements for certain.

The Dulesgate specimen makes the impression of being less highly differentiated than that from Shore; it is possible that all the sections of the former were cut from the apical part of a large leaf, but of course it is quite doubtful whether the two specimens belonged to the same species.

[1] Tiges de la flore carbonifère, Pl. 12, Fig. 1; Flore foss. d'Autun, &c., Pt. 2, Pl. XLI, Fig. 7, *e*; Figs. 12–14; Figs. 18 and 19, *r*.

It appeared desirable to place these specimens on record as the first of the kind obtained in Britain, or from so ancient an horizon as the Lower Coal-Measures. At present no stem is known with which these leaves can be correlated. While they appear to find their natural place in M. Renault's genus, it is evident that they are specifically different from the form described by him, as shown for example by the marked lateral furrows of the Shore leaf, while they are described as entirely absent from the leaf of *S. Decaisnei*, even in its widest part. Neither is there any trace of centrifugal wood, as in the latter species, in the British examples.

The name *Sigillariopsis sulcata* may conveniently be given to the leaf described above, the specific designation referring to its characteristic lateral furrows. The new species must be regarded as founded on the Shore specimen, with which the Dulesgate leaf may or may not prove to be identical.

There is every prospect that additional specimens will be recognized on further search, and we may hope to identify the stem; in any case it is proposed to give a fuller account of these fossils, with illustrations, on another occasion.

D. H. SCOTT, Kew.

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Plants of the Northern Temperate Zone in their Transition to the High Mountains of Tropical Africa.

BY

A. ENGLER.

IN building up the theories of the evolution of species, those plants which are either fully identical or appear as closely allied forms in widely separated localities, have always received special attention. It is known that it is not unusual to find one and the same species or nearly related ones in the northern as well as in the southern extra-tropical regions. Further, it is well known that these facts may be accounted for by the similar climates in similar latitudes, by the changing of area of hekisto-thermic or mesothermic plants during the glacial period or after it, by the wholesale extinction of species during that period owing to lack of resistance or their inability to adapt themselves to the new conditions of life.

Still, there remains a large number of cases of species or nearly allied ones which are disjointedly distributed in meridional direction across the equator. Sir Joseph Hooker, in his memorable Introductory Essay to the Flora of Tasmania, was the first to call attention to the species occurring at the south corner of America which are identical with those of the arctic or northern temperate zone. The same author was the first to give a list of the so-called European types on Cameroon Peak.

Later on, species of the same character were found on the Kilimanjaro and other high mountains of tropical East Africa.

As a result of the botanical investigation of Africa, a considerable number of highland forms have been recorded whose nearest relatives are to be looked for partly in the boreal region, partly in other widely separated countries. Also at lower altitudes several species are to be found which appear in regions far distant from Africa.

In regarding those plants, the following questions must always be borne in mind :

1. Are they identical with the forms living in other latitudes, or do they show any small variation from them?

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2. Is there any possibility of their having originated from a species which was once distributed throughout the intermediate area between the present localities, or in the lower regions, and having developed themselves into identical or convergent highland-forms in the higher regions? Or is it only to be supposed that the seeds have been brought by birds or wind across so many degrees of latitude?

3. What are the means of transportation of seeds and fruits?

4. What is the power of germination? especially, how long are the seeds able to keep it?

5. How do the plants cultivated in Europe from tropical seeds compare with their closest relatives which are indigenous to Europe?

No experiments in answer to questions 4 and 5 have yet been instituted. But whatever the results may be, they will not be able to unsettle the assumption of the close affinity of an African plant to a European one when based upon morphological comparison.

If of two nearly allied forms, *Ae* in Europe and *Aa* in Africa, the seeds of *Aa* when cultivated in Europe give the form *Ae*, it is proved that the evolution of *Ae* is only due to climatic conditions. But if the seeds of *Aa* again give *Aa*, the assertion of *Aa* having once originated from *Ae* is not by any means refuted: for the transformation of *Aa* into *Ae* may well have become fixed during a long period. However, it is much to be recommended that many such experiments of cultivation should be made.

Regarding question 2, the answer is given for those plants which are isolated in the high mountains of Africa, whereas there are many closely allied forms in Europe. This answer is definitely settled in the case of those species whose European forms belong to a larger group of related plants developed in Europe or in the northern temperate zone generally.

From these, I shall select for discussion principally such forms as, not being represented in Egypt, are to be found only in Abyssinia or further south.

Since the first travels of Schimper in Abyssinia, we are acquainted with a species of *Luzula*, growing at 3,600 m. above sea-level, which was named *L. spicata*, (L.) DC., var. *simensis*, Hochst. This plant was found, later on, on the Kilimanjaro also. On the same mountain, another one was collected by Prof. Volkens and described as *L. Volkensii* by Prof. Buchenau. This *Luzula* seemed to be worthy of more careful examination, since any forms at all closely related to *L. spicata*, (L.) DC., are absent from tropical Africa (as well as from Southern Africa). *Luzula spicata* itself is an arctic-alpine plant, and arctic-alpine plants are not observed elsewhere in tropical Africa.

When visiting the Kilimanjaro in October 1902, I had the first opportunity of observing these plants on a meadow below the Muë River, at about 1,900–2,000 m., in company of other plants more frequently to be met

with in the grass-region above 2,900 m. Of a *Luzula* about 25–60 cm. high, I saw here smaller specimens with leaves 3 mm. wide, which are very much like a *Luzula spicata*, (L.) DC., such as is found on the Schneekoppe, Riesen-Gebirge—the more so when exceptionally the inflorescence happens to be not erect but slightly pendulous. Afterwards, in the same meadow as well as on the grassy slopes above 2,900 m. up to 3,100 m., I found other forms with taller stems (up to 70 cm.) and wider leaves (5–10 mm.). Between these extremes noted there exist all intermediate forms, just as one may find specimens of *Luzula* of 5–40 cm. in height and with leaves from 1–3 mm. wide growing closely together in the same range of the Alps.

Great variation is shown also in the foliation of the stem by the *Luzula* of the Kilimanjaro. Some of them have the uppermost leaf about 15–20 cm. below the inflorescence, this leaf being narrow-linear, only about 5 cm. long by 1 mm. wide; in other cases, the uppermost leaf is 2–3 mm. wide, up to 10 cm. long, closely beset with long hairs at the lower margin, and also borne nearer to the inflorescence: such specimens being very similar to *L. spicata*, (L.) DC., var. *simensis*, Hochst.

Besides, there are to be found specimens about 15–20 cm. high not yet fully developed. Their stem is completely covered by the sheathing leaves which, together with the bracts, overtop the crowded inflorescence, still about 3 cm. long; these leaves being long-pilose at the edge, the inflorescence is completely enclosed by the spreading hairs. Such specimens had been described as *L. Volkensii* by Prof. Buchenau. By studying the specimens collected by myself and Prof. Volkens and comparing them with *L. spicata*, (L.) DC., observed by me repeatedly in Northern and Southern Europe, I obtained the following results:

Luzula spicata is to be found in the whole arctic and sub-arctic belt, in Scotland, the Riesen-Gebirge, the Jura, the Auvergne, the Cevennes, from the Pyrenees through the Alps to the Carpathian Mountains; also in the Sierra Nevada, Corsica, Sardinia, Albania, the Pindus, Balkan Mountains, Thracia, on Mount Olympus, Bithynia, and the mountains of Pontus, Mount Ida, Mount Argaeus, Cappadocia, at 3,200 m., moreover in the Altai and Alatan Mountains, in Turkestan, the Cashmere at 4,200 m. In North America beyond the arctic region, it is found on the White Mountains, on the Rocky Mountains of Montana at 3,000 m., and of Colorado at 4,000 m. By comparing specimens from these localities, it is evident that the same forms of *Luzula spicata* are produced in far distant localities, and that the same region produces very different forms. I have myself collected forms with leaves 2–3 cm. long and 1 mm. wide, and with inflorescences 6–8 mm. long, at the North Cape as well as in Vallée d'Eynes, Pyrenees.

But in the same locality in the Pyrenees specimens may also be seen

with leaves 10–12 cm. long by 1–2·5 mm. wide and inflorescences 3 cm. long. I have seen specimens with leaves 3 mm. wide from several localities of the Alps, of the Sudetic Chain, and from Colorado. Here and there, the tepals are pale-brown, but as a rule only forms with dark brown tepals are to be found in all the different districts of the area.

Very low forms with a short, compact inflorescence are to be seen in the Alps, the Altai mountains, and in Cashmere side by side with other forms. The inflorescence is always bent to one side, the growth is always densely tufted, the upper cauline leaves are acute, but the basal leaves obtuse. The plant never occurs below the subalpine region except in the arctic belt. No allied plant is to be found in the plains throughout the temperate zone of the northern hemisphere.

From these facts, the supposition presents itself that the species originated in the arctics, where it has developed the physiological quality of growing only in regions with long periods of vegetative rest. On the other hand, its occurrence in distant mountains of the Mediterranean region not having been connected with the Pyrenees, Alps, or Carpathian Mountains, even during the glacial period, appears to suggest the assumption that the seeds have been transported over long distances by some means or other. They are too large for distribution by wind ; there remains, then, distribution by birds. This supposition is only hypothetical for the present, but is supported by other species of *Luzula* growing often in localities far distant from the main area of the species.

It seems, then, quite safe to accept the view expressed by Buchenau (in Engler's Botan. Jahrb., xii, 130) that the Abyssinian plant belongs as a variety, *simensis*, Hochst., to *L. spicata*. Further, if the Abyssinian plant belongs to this species as a variety, it is obvious that *L. Volkensii* belongs to it also.

All these African forms, however, differ from all the other forms of *L. spicata* in having stolons, in their obtuse leaves and bracts, and in the inflorescence being erect not pendulous. I hold the opinion, then, that the African plant must be contrasted, under the name of *L. abyssinica*, Parlat., with the widely distributed *L. spicata*. To this species are to be reckoned as varieties, the var. *kilimandscharica*, Engl., a tall plant of 60 cm. which ascends up to 3,600 m., and the var. *Volkensii*, (Buchenau) Engl., which grows at 3,700–3,900 m.

There is no doubt that this variety is produced by the climatic conditions prevailing in the upper regions of the Kilimanjaro, which retard the prolific development of stolons.

L. abyssinica appears never to have advanced further south. In fact, there is no country in the Eastern hemisphere, except New Zealand, where the type of *L. spicata* has developed itself further.

It is otherwise in America. Here we observe *Luzula spicata* spread as

far as the Rocky Mountains of Colorado. Again, in the mountains of Mexico we find *L. racemosa*, growing always at the considerable altitude of 3,000–4,500 m. In its narrow leaves, the acute cauline leaves and pendulous inflorescences, this plant appears even more similar to the *L. spicata* than *L. abyssinica*, Parlat.; it grows also tufted just in the same way as *L. spicata*. But it has mostly rigid leaves, revolute at the edge, and three stamens only.

The facts of distribution and the relations of affinity of the species allied to *L. spicata* lead me to the hypothesis that *L. spicata*, after having originated in the Northern hemisphere, was widely distributed in the mountainous parts of it as well as throughout the arctic circle; that it advanced along the Andes of North America as far as Mexico, where it was transformed into *L. racemosa*, and further that from this other peculiar species have branched off. It is not only on the mountains connected by an arctic-alpine flora during the glacial period that *L. spicata* was distributed, but also on the mountains further south, isolated from the continuous arctic-alpine flora. To the east, it appears to have not advanced beyond the Himalaya Mountains. When passing over to Abyssinia, only few transformations took place: the inflorescence became erect, the basal axillary shoots were prolonged like stolons, and the cauline leaves became obtuse like the basal leaves: from these characters, Buchenau is inclined to believe *L. abyssinica*, Parlat. (*L. spicata* var. *simensis*), an hybrid of *L. spicata* and *campestris* (cf. Engler's Botan. Jahrb., xii, 130). From Abyssinia to Kilimanjaro our plant had to travel a long distance; but it is not impossible that it either still exists or has existed previously on a few of the high mountains between Abyssinia and Kenia, from which, having advanced to the Kilimanjaro, it again produced new forms not much differentiated up to the present. At any rate, it is impossible to do without distribution of seeds of alpine plants by air-currents or by birds from one mountain to the other in explaining the history of distribution.

I wish only briefly to mention the two other types of *Luzula* which have reached tropical Africa. *L. campestris*, (L.) DC., var. *Mannii*, Buchenau, on Fernando Po, at 2,700 m., and on Cameroon Peak, at 3,000–4,300 m., shows deviations from the Euro-asiatic forms of *L. campestris*, somewhat in the same direction as *L. abyssinica* from *L. spicata*. The growth is taller and the leaves are more vigorous.

L. Johnstonii, Buchenau, also holds about the same relation to *L. Forsteri* as *L. abyssinica* does to *L. spicata*; it has stolons, *L. Forsteri* not; its inflorescence is richer than ordinarily in *L. Forsteri*; but specimens from Florence and from Tenerife are fully as many-flowered as *L. Johnstonii*, Buchenau, of the Kilimanjaro, where it occurs in the uppermost region of the forest-belt and in the upward extensions of forest between 2,500 and 2,900 m.

Of the genus *Anthoxanthum* two plants have been found lately in the mountains of East Africa. The first one, collected by Prof. Volkens near streamlets of the Kilimanjaro at 2,700–2,730 m., was described as *A. nivale*, K. Schum. The other one, growing on Lukwangula Plateau, Uluguru Mountains, at 2,300 m., has been designated as *A. monticola*, K. Schum. in MS. Dr. Pilger of the Berlin Botanic Museum has carefully compared both these plants with *A. odoratum*, L., which is distributed from Europe to Algeria, but is not recorded from Abyssinia so far. He arrives at the conclusion that the Uluguru plant belongs to *A. odoratum*. He finds the European forms of *A. odoratum* having smaller spikelets, shorter awn, and the second and third palea more hirsute as a rule, but he has found a specimen collected near Dürkheim, Palatinate, by A. Braun having spikelets and awns just as large as those of the Uluguru plant. On the other hand, *Anthoxanthum nivale*, K. Schum., although very different from *A. odoratum*, is more nearly related to this species than to any other. It is necessary to consider separately the specimens collected at 2,700 m. from those grown at 3,700–3,900 m. Both of them are perennial, having rhizomes and short stolons. The specimens grown at 2,700 m. have sheaths 15 cm. long, thickened upwards and pilose, and leaves of the same length and 5–7 mm. wide, as is found in *Anthoxanthum odoratum* only very rarely. The panicle is similar to that of the European plant in a young stage, but is elongated afterwards to 9–12 cm., at the same time becoming looser and developing distant lateral branches. The first glume is rather larger than usual, reaching as it does beyond the middle of the spikelet. Several of the differences stated are to be found occasionally in European forms also, more particularly in f. *umbrosum*, Bluff; but the perennial growth and the longer outer glume are entirely peculiar to *A. nivale*.

The specimens grown near melting snow at 3,700–3,900 m. have the sheaths shorter and still wider, the lamina up to 1 cm. wide, the panicles elongated to 15 cm. and loose below, the glumes still darker coloured; the rhizome is also more robust.

We have, then, a well-distinguished species whose ancestors could spread to the high mountains of Africa from the northern temperate zone only, but have taken a peculiar course of development under the climatic conditions of the upper Kilimanjaro.

Another grass which has spread from the north temperate zone to Africa is *Koeleria cristata*, (L.) Pers. It is to be found in Abyssinia, the Kilimanjaro, and Cameroon Peak. This plant is absent from the lower regions in the same manner as *Anthoxanthum*. Specimens collected in Abyssinia on the slopes of Mount Silke may be named as var. *convoluta* (Hochst.), but it seems to be impossible to separate them from our *Koeleria cristata*. The young leaves are flat, only the older ones becoming convolute. The panicles are sometimes loose like those of *K. cristata* var.

lobata, Marss. In higher altitudes at the summit of M. Silke, M. Bachit, M. Dedjen at 3,000 m. to about 4,500 m., the plant does not grow taller than to 10–20 cm., the panicle being more contracted. It is the species of grass which ascends to the highest altitudes. On the Kilimanjaro, from 2,700–3,200 m., we find a robust form, 50–70 cm. high, with a many-flowered panicle 10 cm. long. At the altitude of 3,600 m., in the uppermost region of *Ericinella*, we find specimens of nearly the same height, with a pubescent rhachis and a long but condensed panicle, which, shading into violet, recalls *Anthoxanthum nivale*, K. Schum., to some extent. Very similar, somewhat smaller, specimens were collected in Abyssinia at Guna at 3,900 m. Finally, on the Kilimanjaro, being the last grass on lava-fields at 4,500 m., a variety 15–18 cm. high makes its appearance and is called var. *supina* by Dr. Pilger. This variety forms thick, unusually dense tufts, having rigid, much convolute basal leaves, flatter cauline leaves, and a narrow panicle 5–7 cm. long, with frequently glabrous rhachis. On the Cameroon Peak, only tall forms of *Koeleria cristata* have been collected so far, which have rather loose or more contracted panicles and flat or convolute leaves. In conclusion, it may be mentioned that a form of *K. cristata*, (L.) Pers., somewhat different from the above, having leaves partly flat, partly convolute, and a contracted panicle, var. *gracilis*, Hack., grows on Devil's Peak, near Capetown. We see, then, again a plant, which, having reached Africa from Europe, appears in varieties and forms somewhat different from those produced in Europe.

Among the species which came from the northern temperate zone to the mountains of Africa, *Arabis albida*, Stev. (1812), being known also as *A. caucasica*, Willd.[1] (1813), a later synonym, is a very remarkable instance. While holding this species sufficiently distinct from *A. alpina*, I have no doubt that the forms attributed as varieties to *A. albida* by Boissier in Flor. Orient., i, p. 174, are only local modifications. *A. albida*, being widely distributed in the vertical direction from the forest-region upwards nearly to the uppermost limit of siphonogamic vegetation, presents quite a number of varieties completely corresponding to the conditions of localities: longer internodia, larger leaves, reduced hairiness in the woods, deep-reaching roots, much-branched rhizomes, shorter, mostly very hairy, often tomentose leaves, also pale pink petals in the débris of the mountain-slopes. Under similar conditions, the same forms appear even at far separated localities. Specimens gathered by me in Algeria in the forest of cedar near Teniet el Haad at 1,400 m. completely agree with plants from the Curral grande, Madeira. Abyssinian specimens from the Hedscha at 3,000–4,000 m. approach very much the var. *Billardieri* of Cyprus. On the other hand,

[1] Halácsy in his Flora Graeca has preferred *A. caucasica*, Willd., quoting Willd. Enum. Suppl., 1809; but this Supplement of the Enumeratio of Willdenow was issued not earlier than 1813, viz. one year after the publication of *A. albida*, Stev.

forms with very tall (up to 1 m.) stems, as found by me on the Kilimanjaro in the upper forest at 2,600–2,900 m., have the same long-cuneate sinuate-dentate leaves as specimens from the island Palma, Canary Islands. Other specimens 0·5 m. high, collected by Volkens below the Mawensi at 2,700 m. in a clump of woods, may be reckoned undoubtedly to var. *umbrosa*, Boiss., being very like specimens of the sub-alpine region of M. Olympus, Bithynia. Other specimens, similar to those of Palma, were found by Dr. Ellenbeck in the Upper Galla country, in the territory of the Arussi Galla, and on the Gara Mulata near Harar at 2,500 m. Agreeing in the general shape of the leaves with the above, but distinct by its numerous smaller teeth, is forma *meruensis*, Engl., 3–5 dm. alta; foliis cuneiformibus, dense breviter dentatis 3–4 cm. longis, 6 mm. latis.

This form, collected on the Meru at 3,500–3,600 m. by Prof. Uhlig, has sometimes pods 4–5 cm. long (as in the majority of the forms of our polymorphous type), sometimes only 1·5 to 2 cm. long. Specimens grown in moist crevices of the summit of the Meru (4,700 m.) have also pods only 1·5 to 2 cm. long. At the eastern base of the Kibo summit, at 4,800 m., Prof. Uhlig gathered specimens both with long and with short pods, the first belonging to a form intermediate between *meruensis*, and another one which I call *kiboensis*. This last one—forma *kiboensis*, Engl., 1–3 dm. alta, foliis inferioribus anguste cuneatis 2–4 cm. longis, 3–5 mm. latis, subintegris—shows the greatest differences from all the other forms. In fact, it would easily pass as a distinct species if there were not so many intermediate forms of the polymorphous type. It was collected also at a lower altitude (3,300 m.) by Prof. Hans Meyer and Prof. Volkens, also above Kibosho at 3,600 m., being one of the last flowering-plants.

Arabis albida, then, is a polymorphous type originally descended from the same protype which *A. alpina* sprang from. Its various forms are to be found from the upper forest-region to the uppermost limit of flowering-plants throughout the Mediterranean mountains, from Persia through the Caucasus Mountains to the Crimea and through the mountains of Asia Minor via Cyprus and the Greek mountains to Sicily, from there to the Atlas Mountains of Algeria and Morocco, to Madeira and the Canary Islands. The light narrow-winged seeds, being apparently suitable to be transported easily by air currents, have reached far distant summits. The seeds have found their way to Abyssinia also (for Arabia, the plant is not yet recorded up to the present); from Abyssinia, it was distributed to Galla Highland and to Kilimanjaro, where, besides the typical forms, the tall sylvatic form of 1 m. in height, and, on the other hand, the narrow-leaved forms only 10–15 cm. high, were developed by the influence of changed conditions of climate. These new forms are therefore of a special interest, because undoubtedly they have been formed only by the influence of new conditions of life, without any intervention of allied forms. The

same holds good for *Anthoxanthum nivale*, K. Schum., and *Koeleria cristata*, var. *supina*, Pilger.

I will mention two more Cruciferae which have apparently travelled from Europe to the mountains of East Africa. *Subularia monticola*, A. Br., which grows in large tufts on the Dedjen, Abyssinia, at 4,000 m., in swampy and at the same time stony places, was collected again by Prof. Volkens, and later on again by Prof. Uhlig on the Kilimanjaro, close to melting snow at 3,750 m. There is only one species resembling it, viz. *Subularia aquatica*, L., having a very sporadic distribution in Europe from England to southern Russia at the bottom and near the edge of lakes. Hiltner, in a careful study on these plants (Engler's Botan. Jahrb., vii, p. 264), after having compared the external and internal structure both of the aquatic and terrestrial forms of *S. aquatica* from various localities, with each other and with *S. monticola* of Abyssinia, sums up that *S. monticola* is not more different from the terrestrial form of *S. aquatica* than this from its aquatic form. Therefrom it is not improbable that birds of passage brought seeds of *S. aquatica* from Europe to the higher mountains of Abyssinia, where a somewhat different variety was developed. It should be borne in mind, however, that the genus *Subularia*, though classified near *Lepidium* as a rule, has a very isolated position. Its distribution may have been wider in former times.

A Cruciferous plant, very generally diffused in Europe, *Stenophragma Thalianum*, (L.) Cel., is to be found in Abyssinia and on the Kilimanjaro at the considerable altitude of 3,300–3,500 and 3,600 m. respectively. In Abyssinia, on the summit of M. Bachit and M. Silke at 4,000 m., a dwarf form occurs which produces flowers and fruits when only 2–3 cm. in height. It was named *Cardamine pusilla* by Hochstetter, *Sisymbrium pumilio* by Oliver. In the 'Hochgebirgsflora des tropischen Afrika,' I classified it as a variety; at present, I should rather believe it to be only a local modification. The seeds of the *Stenophragma Thalianum*, common throughout the northern temperate zone, are so minute and light, that they may be spread very far by winds. The species growing also on cultivated ground, it may even have been introduced into Abyssinia with seeds of cultivated plants. Even in this case it remains a remarkable feature, that it reaches to the sub-alpine region only in Europe, whereas it produces a dwarf form on the lofty summits of Abyssinia.

Modifications of growth resembling those of *A. albida* are to be seen in *Cerastium caespitosum*, Gilib. (*C. vulgatum*, auct., *C. triviale*, Link). In every herbarium containing a large number of specimens from numerous localities, it is found that this species, while varying only little in hairiness and juncture of the sepals, has the cauline leaves now more acute, now more obtuse, varying, at the same time, from 0·5–5 cm. in length, and from 0·2–1·2 cm. in width. Broad-leaved forms are especially numerous from

Southern Europe; but also in examining Central European specimens, one sees var. *elatius*, Peterm., or var. *nemorale*, Uechtritz, having long internodes and leaves sometimes 1·5–2 cm. wide; further var. *fontanum*, (Baumg.) Gürke (=var. *alpinum*, Mert. et Koch=var. *macrocarpum*, Fenzl=*longirostre*, Wichura). Var. *elatius* occurs also in Japan.

The Berlin Herbarium contains several specimens from the Himalaya, India, Ceylon, and Java. These specimens, having been collected in mountainous regions (some of them by Prof. Warburg), more or less match the *C. vulgatum* as figured by Wight, Icones, 948/153; that is, they have the leaves not only broad but also short, so that the length is only one and a half or twice the width. For this plant, belonging undoubtedly, as far as I can see, to *caespitosum*, yet differing in various directions, I propose the name var. *Wightii*, Engl., foliis ovalibus vel late ellipticis 2–2·5 cm. longis, 1–1·2 cm. latis.

A dwarfed form of this variety, collected on Merapi, Java, by Warburg, is only 3 cm. in height.

C. caespitosum, Gilib., is another frequent plant of the mountains of Abyssinia, where it presents three varieties. The first one, var. *octandrum*, (Hochst.) Engl., is superficially almost identical with acute-leaved forms of the European *C. caespitosum*; but the flowers are almost always tetramerous! This plant was gathered by Schimper near Amogai, at 2,200 m., in fields and along road-sides, near Adoa, near Gaffat at 2,600 m. in fields and meadows, also near Debra Eski at 3,000 m. In this variety the petals are constantly a little shorter than the sepals.

Flowers identical but 5-merous are to be seen in a plant distributed in moist woods of Galla Highland, and collected by Dr. Ellenbeck and O. Neumann. It corresponds somewhat to var. *elatius*, Peterm., of the European forests, but has always acute leaves, as shown only rarely by the European form; the inflorescences are also more developed, being provided, at the same time, with longer internodes than those of var. *elatius*. I name this plant var. *scandens*, Engl., caulibus scandentibus usque 5 dm. longis, foliis oblongo-ellipticis acutis; inflorescentia elongata 5–12 cm. longa multiflora.

Hab. in the country of the Arussi Galla at the high plateau near Jidah at 2,600 m. a. s. l. (Ellenbeck) and in Sidamo, near Awara, on meadows close to the bamboo-forest, at 3,100 m. (O. Neumann).

A third variety, *simense*, (Hochst.) Engl., having elliptical acute leaves and looser or more contracted inflorescences, is hardly to be distinguished from certain European plants. It grows on the Bachit, Abyssinia (Schimper, It. Abyss., Sect. II. 756). On the Dedjen, at about the same elevation, it passes gradually into a dwarf form of 2–5 cm. in height only, having short internodes and crowded flowers, sometimes also petals a little

larger, *humile*, A. Br. (without diagnosis in Schweinfurth, Beitr. Fl. Aethiop., p. 58). This I can consider to be only forma *humile*, A. Br., not even as a variety.

At the Kilimanjaro, the var. *simense* occurs in forms of 3–10 cm. in height. It was collected near the eastern source of Garanga River at 3,700 m. by Prof. Uhlig. Forma *humile* of the same variety was collected also on Cameroon Peak at 4,000 m., being the last flowering plant there.

At the Kilimanjaro, in the upper Ericinella region, at 3,300 m. (Uhlig, n. 628), and on the grass-plains of 3,500–4,000 m. (H. Meyer, n. 8), there is another form which I have seen from these localities only. It has somewhat thick, narrow-elliptical, acute leaves, very glandular inflorescences, larger petals (1½ the length of the sepals), and long horizontally spreading capsule twice the length of the sepals. I name this var. *kilimandscharicum*, Engl.; ramulis decumbentibus vel erectis superne cum pedicellis, bracteis et sepalis fere omnino viridibus densius glanduloso-pilosis ; foliis crassiusculis ellipticis acutis ; petalis quam sepala 1½-plo longioribus ; capsula quam sepala duplo longiore.

Specimens of *C. caespitosum*, Gilib., sent from Cape Colony are exactly like the ordinary European ones. The same applies to the specimens collected in the Transvaal by Dr. Wilms. Nor can I see any difference between the European plant and the specimens gathered by Moseley on the Challenger Expedition in the most western island of Tristan d'Acunha. Whereas specimens (without flowers) collected by Dr. Naumann, on the Gazelle Expedition on Green Mountain, Ascension, are very remarkable in having slender stems covered below with rudiments of decayed leaves and with crowded upper leaves of the same form as those of the ordinary European plant. I do not want to dwell upon the forms occurring in America and the Antarctic regions ; generally, it is only to be remarked that numerous specimens gathered at the Strait of Magellan and in the Kerguelen are completely identical with European ones. Some of them show a vigorous growth.

Against all these varieties, the peculiar *C. africanum*, (Hook. f.) Oliv., stands apart as a species of its own, which I observed in woods and clearings of Usambara at 1,250–1,400 m., and on Kilimanjaro at 1,200–2,900 m. Its stem ascending between shrubs and brake is often more than 1 m. high. The petals are one and a half times or twice the length of the sepals, the oblong-lanceolate, upwards more distinctly narrowed leaves are acute, often attenuated into a distinct point. This species, which was collected in the country of the Arussi Galla near Ladjo by Dr. Ellenbeck, which occurs also in Uluguru, on Kirunga, Ruwenzori, also on Cameroon Peak up to 3,000 m., may possibly be a descendant of *C. caespitosum*, Gilib. The *C. caespitosum*, Gilib., var. *kilimandscharicum*, Engl., above mentioned holds, indeed, about an intermediate position

between the ordinary *C. caespitosum* and *C. africanum*, (Hook. f.) Oliv., having green sepals, larger petals, and acute leaves.

All these species mentioned so far are closely allied to plants widely distributed in Europe or more generally throughout the northern temperate zone of the Old World, growing there in the lower as well as the upper regions, whereas, in Africa, they are to be found only in the upper or uppermost belts. For several of them, their seeds being small and light, it may be assumed that they have been distributed by heavy gales; still stronger seems the argument that the first transport from Europe or Western Asia to the upper treeless regions of Africa has been effected by birds of passage, the seeds adhering to their feet with soil or by hairs and bristles to the plumage, and that they were dispersed afterwards from mountain to mountain by winds. It is not impossible that in this manner seeds are reaching Africa from the northern temperate zone even at the present time. But most of these species are not only to be found now on several of the high mountains of Africa, but they appear there in forms constantly distinct from the European ones. It seems reasonable, then, to assume that immigration took place at some earlier date. On the other hand, from our experiences of alpine plants cultivated in the lowlands and growing there more vigorously, I am led to the opinion that the forms modified by a longer period of vegetation and by higher temperature have arisen in a relatively shorter length of time. The principal reason for my supposing that immigration took place at some earlier date is the probable state of external conditions during that pluvial epoch as assumed by geologists, which must have been more favourable for any immigration of forms of the temperate zone than the conditions prevailing at the present time. Extensive alluvial deposits of a recent date, traces of a formerly larger amount of water in the present lakes and rivers, traces of a wider extension of African glaciers in the past (Hans Meyer on Kilimanjaro, in Zeitschr. Gesellsch. Erdkunde, Berlin, 1904, p. 193) serve as a proof of this pluvial epoch. During this period the forests must have extended further down, the treeless tracts spread to lower regions. At this time, then, the areas suitable for plants of more temperate zones were larger and approaching each other more, though not really continuous. There has always been also a large region between Abyssinia and Southern Europe or Western Asia which has never been suited to harbour the highland plants mentioned by me.

All these highland forms have a common feature in being systematically isolated in Tropical Africa, whereas there is quite a number of allied species in the temperate zone. This is easily to be accounted for, because, in the present time as well as during the pluvial epoch, only the loftiest mountains of Tropical Africa afforded conditions which

enabled any seed carried from the northern temperate zone to this continent to germinate and to grow.

The differences to be seen in most of these highland forms, as compared with their relatives of the northern temperate zone, are always in harmony with the different climatic conditions. The temperature of the alpine region of Kilimanjaro or Ruwenzori may be somewhat like that of our high Alps during the summer time; but there is this great difference that, in the snow-region of Africa, the ground is free from snow several months longer than in the Alps, and that, during the dry period, the strong insolation, even when acting only a few hours of the day, dries up the soil very much. Only in crevices and small ravines more favourable conditions exist for the development of turf-forming hygrophilous plants such as are so abundant on our alpine meadows. The variety of such plants is at the present time (and has been also during the pluvial period) much inferior to that on the mountains of the northern temperate zone, where large continental areas allowed a rich development of the plants wanting only little warmth for existence, and where repeatedly climatic changes were responsible for far-reaching migration of the highland forms originally evolved in the various centres of evolution.

There is also very little in common between the alpine flora of the lofty mountains of Africa and the highland flora of the Mediterranean mountains, where the arctic-alpine plants are wanting or very scarcely represented. A number of genera, it is true, are to be found also on the mountains of Tropical Africa, viz. *Trifolium*, *Scabiosa*, *Cephalaria*, *Campanula*, *Crepis*, *Hypericum*, *Micromeria*, *Anemone*, *Carduus*, *Centaurea*, *Helichrysum*, and a few others. Of *Helichrysum*, a few species are marked even by their woolly tomentum. But we do not in the least find the extraordinary amount of woolly or tomentose and spinous perennials so conspicuous on the mountains of Asia Minor and Central Asia, on the mountains of Greece, and even on the Sierra Nevada; nor are the leafless broom-like shrubs prevailing in the hilly parts of the Mediterranean countries to be seen on the mountains of Tropical Africa. It is obvious that this feature is explained by the much more intense dryness of the atmosphere and of the soil during the Mediterranean summer. From the same cause, only a few types of the steppe may be seen ascending to the alpine region in Tropical Africa, although access seems easy enough. How very different in the high mountains of Asia Minor, where the types of the steppe (*Astragalus*, *Cousinia*, *Artemisia*, *Statice*, Labiatae, Boraginaceae, Cruciferae, Umbelliferae, bulbous plants) prevail to an astonishing extent! In Tropical Africa we find only grasses of the steppe ascend to higher altitudes. Also Ericaceae and small-leaved shrubs of a similar habit are more abundant, but these belong to types more or less developed in Southern Africa.

After all, the highland flora of Tropical Africa is not very rich in peculiar components derived from types of the lower regions. This, again, is the cause of the enormous extension of a few species and the amount of yet tenantless ground in the upper regions. Such ground has been always at the disposal of any seed brought from anywhere by wind or birds as long as it kept its power of germination. Further, I may be allowed to refer to some species of the forest-region of Tropical Africa which, being likewise nearly allied to such of the temperate zone, have undoubtedly not reached Tropical Africa by man's intervention.

Such a European type widely distributed in the forests of Africa is *Sanicula europaea*. When observing it in Usambara and on Kilimanjaro, I tried to find out if there were any differences between the African plants and the European ones. The specimens not uncommonly attain a height of about 60 cm.; they frequently possess a leafy stem, they have sometimes lateral branches, and the leaves have always segments much narrowed from the base towards the apex; the flowers are mostly purplish-brown, the lateral branches of the inflorescence often much elongated. The same characters are to be found in Abyssinia, on Ruwenzori, Cameroon Peak, in the mountains of Nyassaland, Natal, and Cape Colony, at the Comoro Islands and in Madagascar. But the purplish colour of the petals is not constant. And some specimens collected by Medley Wood in Natal at about 1,000 m. a. s. l. show only one leaf on the simple stem. On the other hand, even in Germany (near Eisleben, Lagow, Rastatt—Herb. Berlin), there are vigorous specimens with several cauline leaves and the same cutting of the leaves which is the general rule in the African specimens. Some specimens from Daghestan and the mountains of Pontus approach even more the African plant, and, at the same time, the *S. elata*, Hamilt., of India. This is simply brought under *S. europaea* by C. B. Clarke (in Hooker, Flora of Brit. India, ii, 670), while Hooker fil. called it *S. europaea*, var. *elata* on the labels of his Himalayan collections. I incline to adopt Hooker's terminology for the African plant also. This was named var. *capensis* by Chamisso and Schlechtendal in Linnaea I. (1826) 352. But the name of *Sanicula elata*, Hamilt., being published as early as 1825, is to be recommended, so much the more as it is indicative of the habit and, at the same time, does not favour any of the many countries where the variety occurs. In Asia this variety is to be found in the mountains of Pontus and Daghestan, Himalaya, British India, and Ceylon, in Java and Sumatra (*S. javanica*, Bl., *S. montana*, Reinw.), in Central China and Japan; it seems to be very difficult to classify any subvarieties.

All the differences of these *Saniculae* from the European form seem obviously to be called forth by the longer vegetation-period as afforded

in the mountain-woods of the tropical and sub-tropical countries. The wide distribution is accounted for by the fruits being densely covered with hooked bristles adhering to the plumage of birds.

Sambucus, as well as other Caprifoliaceae, has long been unknown from Tropical Africa. Therefore, when examining the valuable collection of Fischer collected at Abori, Kikuyu, it was very surprising to me to find a species of *Sambucus* exactly coinciding with *S. ebulus* in habit, but different by unusually elongated nearly cylindrical fruits and by yellow anthers. Last year I received the same plant from Mr. C. F. Elliott, the Director of the Forest Department in the Uganda Protectorate; he collected it in the hills north of Nairobi. There is no doubt of its close affinity to *Sambucus ebulus*, which is rather an isolated type of the genus; at the same time it is quite certain to be indigenous in these regions of Africa, because no European had settled at Kikuyu at the time of Fischer's travels. Further, the idea must be refuted that this African *Sambucus* is a relict form. There is no doubt that it came to the mountains of Equatorial Africa from its northern and Mediterranean area (extending from England and Gotland via Central and Southern Europe as far as Asia Minor and Persia and Algeria) quite in the same manner as it reached Madeira and the North-western Himalaya.

In passing over to the Himalayan region, as well as in some localities of Asia Minor, the species has developed yellow anthers instead of the violet ones as shown by the ordinary forms. Our African specimens have the inflorescences densely clothed by rusty hairs; they have narrow linear bracts and also yellowish anthers. Furthermore, the oblong-ovoid green fruits afford a striking character: in all the specimens, however, both Fischer's and those collected by Elliott seventeen years later, these fruits contain only unfertilized ovules. Still, even leaving out of consideration these abnormally developed fruits, we may well consider the plant as a variety more recently evolved, var. *africanus*, Engl.; foliis robustis, foliolis elongato-lanceolatis acuminatis; inflorescentia dense ferrugineo-pilosa, bracteolis ramulos fulcrantibus lineari-lanceolatis, antheris flavis.

English East Africa: Abori (Fischer, n. 327); Kikuyu (C. F. Elliott, n. 12, 177).

Veronica chamaedrys, L., widely distributed in Europe and occurring in Asia Minor also, shows only slight variations of hairiness and cutting of the leaf-margin throughout these countries. Also *V. chamaedryoides*, Bory et Chaub., a native of the fir-region of M. Parnassus, M. Taygetus, of Thasus, of Northern Laconia, Asia Minor, differs from the common form by denser pubescence only. Against it, *Veronica micrantha*, Hffgg. et Link, found in Portugal, near Beira, must rather be kept as a distinct species derived from *V. chamaedrys*, owing to the short peduncles, otherwise not seen in European forms, and to the smaller flowers. Both these characters,

combined with erect habit, freer branching, and more scanty pubescence of the leaves, are to be seen on a plant collected first by Volkens on the Kilimanjaro, afterwards above Sakare, Western Usambara, by myself, and in Coromma Valley, Galla Country, by Riva on Ruspoli's expedition. Being unable to acknowledge the specific rank of *V. chamaedryoides*, Bory et Chaub., I had named this plant *V. chamaedryoides*, Engl. (in Pflanzenwelt Ostafrikas, C, 358). Still, in order to avoid any possible confusion, I would rather drop this name now and substitute *afrochamaedrys*, Engl., in its place. We do not know any species in Tropical Africa which could possibly be compared with it or considered to be its ancestor. For *Veronica abyssinica*, Fresen., a frequent herb in the woods of Abyssinia, Gallaland, East Africa, and Cameroon, belongs to another affinity (that of the Europaeo-mediterranean *V. montana*, L.). The flat seeds of *V. afrochamaedrys*, being still somewhat smaller than the 1 mm. long seeds of *V. chamaedrys*, may easily be carried away on the feet of birds. Therefore it is no bold speculation to assume that *V. chamaedrys* from the Mediterranean transported to Gallaland (probably to Abyssinia before that time) developed itself to *V. afrochamaedrys*. On this occasion, *V. filiformis* may be mentioned, which occurs in Abyssinia Highland, in America, and the Caucasus Mountains, and also *V. violifolia*, Hochst., allied to *V. agrestis*, L.

Further, I should like to bring forward the interesting fact, reported by me in Sitzber. k. Preuss. Akad. Wiss., February 18, 1904, that the well-known *Populus euphratica*, Olivier, of the Mediterranean region (in the broadest sense) has nearly reached the equator (at Korokoro, Upper Tane River), and has produced there a peculiar large-fruited form named by me sub-sp. *Denhardtiorum* (in Notizbl. k. Bot. Gart. Berlin, II (1898), 218). *Populus euphratica*, Olivier, is distributed from Songaria to Palestine and to Western Tibet; further, it is found on the Morocco-Algerian border, and has been recorded from the Libyan Desert by Ascherson, 1877. It is likely to be found also in Arabia on further investigation in that country. But at any rate there remains a wide gap of about twenty-two to twenty-three degrees of latitude from there to the African stations of *P. euphratica*. The African sub-species approaches somewhat the Indian specimens in having a long petiole and ovate remotely sinuate leaves; it differs, however, most decidedly in having short (not more than 3 cm. long) fruiting-spikes and capsules nearly 1 cm. long, 6–7 mm. thick. It is another instance of a species having been modified by advancing from the temperate zone to its new situation. The wider expansion of the steppes in Africa being the result of more modern geological changes, it is safe to assume that sub-sp. *Denhardtiorum* is a younger type than *P. euphratica* of the northern temperate zone.

In conclusion, I should like to add to the above-mentioned cases

a few more cases of disjointed distribution which seem to be the result of some other evolution than that of the species considered so far.

One of the most prominent instances of disjointed range is presented by the genus *Canarina*. For a long time, only one species was known, *C. campanula*, Lam., a plant very characteristic by its large, bell-shaped, orange-coloured flowers. On the Canary Islands, it is very frequent in some places; it is often cultivated in greenhouses. It was considered to be an isolated type of the Campanulaceae, an endemic product of the Canary Islands. But quite recently, a second species was discovered by Dr. Stuhlmann on the Emin Pasha expedition: *C. Eminii*, Ascherson (in Sitzber. naturf. Freunde, Berlin, 1892), which was found in the forest-region of M. Ruwenzori, at 2,500 m. a. s. l. A third species, *C. abyssinica*, more allied to the second one, was collected by Dr. Ellenbeck, who accompanied as the physician the expedition of Baron Carl von Erlanger to Somaliland and Gallaland. It was found in Galla Highland, west of Lake Abbaya, at 2,000–2,500 m. a. s. l., later on also more towards the west by O. Neumann. These plants, having berries eaten by men on the Canary Islands and likely to be consumed by birds also, may be assumed to have been spread from mountain to mountain by birds. The rather striking differences, however, between the Canarian species and the two species of the African continent seem to indicate that *Canarina* is an older genus whose species, having travelled in more remote periods, may have had wider areas formerly. It is not impossible that this genus was indigenous even to the Mediterranean region at some distant period. I am inclined to the consideration of this hypothesis by the distribution of *Sempervivum arboreum*, which is spread throughout the southern Mediterranean countries, from Portugal and Spain as far as Cyprus. By natural affinity it is connected with *S. chrysanthum*, Hochst., a native of Abyssinia, and at the same time with a number of species (viz. *S. canariense*, L., *S. urbicum*, Chr. Smith, *S. palmense*, (Webb) Christ, *S. cuneatum*, (Webb) Christ, *S. ciliatum*, Willd., *S. percarneum*, Murray, *S. tabulaeforme*, Haw.) which are indigenous to the Canary Islands or (*S. glandulosum*, Ait., *S. glutinosum*, Ait.) which are natives of Madeira.

With regard to the species or varieties I have enumerated above (*Canarina* excluded), I may be allowed to make some general remarks. We may call such modifications CLIMATICAL ADAPTATIONS, but only in this sense, that this adaptation is a passive one, caused by the physical conditions of the climate, not an active one, which would correspond to the views of Lamarckians.

The first condition for such modifications is that the fruits or seeds are such that, without losing the faculty of germination, they can be easily transported to regions removed from the locality where they were indigenous before.

This will in many cases happen accidentally. It is also only accidental,

when the seeds find place and conditions suited for germination and climatic conditions for their development, which are not too different from those of their former native country.

I am convinced that in such cases the somewhat different climate is the cause of all or at least of a part of the modifications. Sometimes in connexion with these new variations are also to be observed (cf. *Cerastium caespitosum*), which may become the beginning of other new forms. The constancy of such climatical adaptations may be a different one and often become fixed through a geological period. I may add that systematic studies have also convinced me that many of the xerophytes must have originated from mesophytes, and that a good deal (I do not say all) of the qualities of xerophytes, which are usually called adaptations for protection against a dry climate, are caused by this climate itself (cf. A. Engler, Pflanzenwelt von Deutsch-Ostafrika, A, p. 25).

On Fertilization in the Saprolegnieae.

BY

A. H. TROW.

With Plates XXXIV–XXXVI.

TEN years ago, at the suggestion of Prof. Oltmanns of Freiburg, and stimulated by the publication of Humphrey's ('92) admirable monograph on the Saprolegniaceae of the United States, I commenced a study of the cytology of the sexual organs in the genus *Saprolegnia*.

In the years 1894–5, as one of the results of a very long series of observations, the conclusion was reached that fertilization takes place invariably in *Saprolegnia dioica* and at least occasionally in *S. mixta*. The evidence, so far as it concerns the first of these two species, was exceptionally simple, clear, and convincing, but it apparently proved unacceptable to a considerable number of botanists. The evidence rests on three distinct, and in the main, easily verifiable observations, viz. (1) the invariably uninucleate character of the oosphere-origins and oospheres, (2) the invariably binucleate character of the young oospores, and (3) the uninucleate character of the ripe oospores, a feature however which was not so definitely established as was obviously desirable. Special attention should be paid to this statement, for it has been recently suggested by Davis ('03) that my view as to the occurrence of fertilization in the Saprolegnieae rested on other and altogether insufficient grounds, viz. the occasional occurrence of binucleate oospheres (eggs). He says that I have found binucleate eggs (!) in *Saprolegnia* and 'attached much significance to them as evidence of sexuality.' It is unfortunate that Davis uses the term eggs very loosely as synonymous with oospheres and oospores—indeed, as he does not figure the oospore membranes, it becomes difficult to ascertain whether he paid any attention at all to that critical period in development when the oosphere is converted into an oospore. I have seen, in *S. dioica*, on one or two occasions, binucleate oospheres and have of course regarded them as anomalies, even as monstrosities. Certainly, no theories were

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based on such isolated observations. Statistically considered they were so rare as to be without significance. The change from oosphere to oospore, even in apandrous forms such as Davis worked with, is easily recognizable and should have been specially noted.

It was, no doubt, a mistake on my part to present the results concerning fertilization in the two species *S. dioica* and *S. mixta*, side by side, as the additional complexity made it difficult for the reader to realize the exact state of affairs without careful study. I ventured, moreover, to suggest that the binucleate oospores observed by Humphrey ('92) in *Achlya americana*, and mistaken by him for oospheres, furnished sufficient evidence for the view that another species of Saprolegnieae was also functionally sexual.

There was good ground for this suggestion in the case of Humphrey's work, for he had examined sections and preparations somewhat similar to my own. The endeavour, however, to explain Hartog's ('89, '91) results on the same lines may have been somewhat rash and certainly gave birth to a discussion of a controversial character. The interpretation given of Hartog's observations, however, turns out, so far as it has been tested by new observations, to have been justified.

The view, thus promulgated, that certain species of Saprolegnieae were functionally sexual and that the antheridia and fertilization-tubes were not degenerate organs, being obviously incapable of assimilation to that proposed by Hartog, was at once attacked by him. He, however, had no new observations to bring forward in support of his contentions, and as it was obviously impossible to make any real progress in a controversy of this kind, in the absence of fresh evidence, I resolved to continue my work and investigate the genus *Achlya* for myself. It seemed to me at that time, and the progress of research has only served to strengthen the conviction, that the normal occurrence of two nuclei in the young oospores was good presumptive evidence for the occurrence of fertilization. The real value of such evidence depends entirely on the previous demonstration, in my own preparations, of the uninucleate character of the oospheres.

In the year 1897 Miss Dawson collected and cultivated a species of *Achlya*, which, on examination, turned out to be a variety of *Achlya americana*. This form was examined by me in great detail in the years 1897–8, with the result that I ('99) was able to prove that in this species (1) the oospheres are invariably uninucleate, (2) the young oospores are binucleate (in one case out of hundreds three nuclei were observed), and (3) the old oospores are invariably uninucleate. It cannot be too strongly insisted upon that these observations, in the case of this species of *Achlya*, are relatively simple ones, sufficiently so indeed as to form no more than moderately difficult exercises for advanced students. The real difficulties raised by the investigation of the cytological problems of the Saprolegnieae

do not arise in connexion with the rigid determination of these relatively simple points. What then was the evidence for the existence of fertilization in the Saprolegnieae in 1898?

In the three species most fully investigated by me, and excluding the admittedly apogamous *S. ferax*, the results were definite enough.

In *Achlya americana cambrica* a practically invariable succession had been proved; the uninucleate oospheres become binucleate oospores, which as they grow older return to the uninucleate condition. This succession, especially so far as concerns the number of the nuclei, may be expressed as a formula:—1, 2, 1.

In *Saprolegnia dioica* the same succession had been shown to occur, but the fusion of the gameto-nuclei being long delayed and difficult to demonstrate, the last stage in the process still required some corroboration. The formula of succession was found to be 1, 2, 1?

In *Saprolegnia mixta* (apparently not the same species as Davis's *S. mixta*) it had been shown that (1) the oospheres were uninucleate, (2) the young oospores either uninucleate or binucleate, and (3) the old oospores invariably uninucleate. The formula of succession was 1, 1 or 2, 1. The formula suggests that parthenogenesis and functional sex exist side by side in this species.

In *Achlya americana*, investigated by Humphrey, it had been shown that the young oospores were binucleate, but there was no evidence as to the number of nuclei in the real oospheres. The old oospores were found to be uninucleate. The formula in this case would be x, 2, 1. The balance of probability is in favour of the view that $x=1$.

It is worth noting that in another species of *Achlya* investigated by Hartog, according to his reiterated statements ('95, '96, '99) the formula would be x, 2, 1 where x is greater than 2. This, if verified, would make the process of fertilization in this case of quite exceptional interest. How far Hartog's views are in accord with the actual facts can only be determined by further investigation. Davis ('03) refuses, as we shall see, to accept Hartog's theory as to the reduction in the number of the nuclei in the oogonium by a process of wholesale nuclear fusions. My observations lead me to conclude that such discrepancies as exist between Hartog's account and those of other botanists must be due either to errors of observation or to such differences in the cytology of the Saprolegnieae as have been shown to exist in the single genus *Albugo* (Cystopus). Further investigations by unbiased observers must be made before the question can now be finally settled. Taking all the evidence into consideration, the view propounded by me in 1899 that 'fertilization is now known to take place in four distinct forms of Saprolegnieae, viz. *Saprolegnia dioica*, *S. mixta*, *Achlya americana*, and *Achlya americana cambrica*,' may be regarded as strictly in accordance with the facts in the case of the three

species examined by me and as very probably correct in the case of the remaining one examined by Humphrey.

To those who are still sceptical as to the occurrence of fertilization in the Saprolegnieae two questions may be put. If this remarkable succession concerning the number of nuclei, viz. 1, 2, 1, is not evidence of normal fertilization, what can be the meaning of it? Are botanists prepared to reject all the other very numerous cases of fertilization which are founded on exactly analogous evidence? Most of the proofs concerning fertilization in the Peronosporeae—to cite one series of cases only out of many—are of this character. King ('03) concludes that fertilization takes place in *Araiospora* on the basis of the demonstration of the formula 1, 2, 1.

But the strength of the case for the occurrence of fertilization in the Saprolegnieae by no means rests on this demonstration of succession in the number of nuclei in the oospheres and oospores. It was proved, at any rate to my own satisfaction, that (1) the increase from one to two nuclei at the point of time when fertilization would naturally take place did not arise by division of the original single nucleus, but, on the contrary, that (2) the second nucleus was first noticeable at the periphery of the oosphere in the immediate vicinity of a fertilization-tube. Indeed, in *Achlya americana cambrica* the tip of the fertilization-tube was traced to a point inside the oosphere (not the oospore) and shown to contain a single nucleus. These additional observations published in 1899 have not met with universal acceptance—indeed they have been subjected to considerable criticism by both Hartog ('99) and Davis ('03).

The criticism, so far as it is relevant to the question of fertilization, is no doubt traceable to three sources:—(1) the incompleteness of the work, (2) the character of the drawings used to illustrate the papers, and (3) the influence of De Bary's views as to the apogamy of the Saprolegnieae.

Concerning the first point, nothing need be said. My only concern is that the work may go on towards completion—that progress may be made. With respect to the second, some explanation is perhaps advisable. At the present day it scarcely needs pointing out that every drawing, especially of protoplasmic structures, is of necessity more or less diagrammatic, and that every observer, consciously or unconsciously, decides for himself what conventional forms he shall adopt to convey to others the picture he has himself seen. The convention deliberately adopted by me was to draw every nucleus present in the actual section figured, and to take one optical section, out of six or seven possible ones, as a rough guide for filling in the cytoplasm. In the endeavour to be as realistic as possible the nuclei in the original drawings were not drawn boldly enough. The fine shades of difference in form and colour, perceptible readily enough under the microscope to the trained eye, when realistically copied in black and white, did not show sufficient contrast. The lithographer, with the original draw-

ings before him, had a difficulty in interpreting the nuclear figures correctly, and readers of the paper have, at any rate in some cases, been led completely astray. Davis ('03) and Hartog ('99) have even gone so far as to find in these imperfect conventional figures—separated, that is to say, by two removes from the actual facts—structures which the author of the paper was not able to see in the original preparations. An expert observer is always able to see more than he can reproduce in a figure, and every piece of original work must, therefore, be finally judged by the verdict of his fellow workers as based on collateral or confirmatory original observations. If special reference to the illustrations which have been used as the vehicle to communicate the result to others is resorted to, it must be done with due caution. If the author has been simply unfortunate in his methods of presentation, the verdict in his favour may be delayed, but it will not be permanently withheld. But there is a real value in the criticism as to the want of detail in the drawings, in so far as that depends on the imperfection of the preparations themselves. The material used in both researches had been fixed by means of mercuric chloride. I am convinced that, so far as concerns the finer details of the structure of the nucleus, this method of fixation is quite unsatisfactory. Nevertheless the better methods used by me recently, and to be described hereafter, have not led to results in any sense contradictory of the earlier ones. The new results, indeed, confirm the old in every essential particular. Concerning De Bary's influence much might be said if it were not already fairly well known. It is interesting, however, to contrast his mental attitude with that of Davis. De Bary ('87) says, referring to Pringsheim's work:—'If there is really an open communication in these species between the antheridium and oosphere . . . we must admit a fertilization in their case.' Davis ('03) says, at p. 246:—'The writer cannot better sum up his attitude towards Trow's opinions on sexuality in the Saprolegniales than by defining them as *not proven* and *improbable* in the face of the mass of observations upon which botanists have generally agreed that the group is apogamous,' and again, at p. 236:—'The writer cannot justify Trow's conclusions in this matter, believing them to be premature as to evidence and illogical as to probabilities.' One is tempted to ask what is meant by premature evidence and illogical probabilities. Further comment is needless. The greatest student of the Saprolegnieae, had he been able to pronounce judgement on the same facts, would, I imagine, have expressed himself differently and somewhat as follows:—If this remarkable succession in the number of the nuclei be confirmed, and there be no question of a nuclear division, we must at length admit that fertilization takes place in three species of Saprolegnieae—the view concerning the species destitute of antheridia, however, of course, remains unaltered.

It must be admitted that the most difficult problem in the cytology of

the sexual organs, that of the fate of the supernumerary nuclei in the oogonium, had not been very successfully attacked during the course of these investigations. My view that the supernumerary nuclei disappeared by a process of digestion and absorption was distinctly opposed to that held by both Hartog and Humphrey.

The difficulty of following the process of degeneration of the nuclei in detail was so great that I determined to study a member of the Peronosporeae with a view to getting some practice in following the degeneration of indubitable superfluous nuclei. With this end in view a special study of a species of *Pythium* was made in the years 1899 and 1900. It was very gratifying to find that with the same methods as had been employed in the case of the Saprolegnieae, practically the same results were obtained; in particular it was easy to trace exactly the same succession as regards the number of nuclei in the reproductive organs. Moreover, it became apparent that chromacetic acid was an excellent fixing reagent for use in this group of plants.

With the knowledge gained by these investigations a fresh start was made in the study of the Saprolegnieae in 1902. The first suitable species isolated, which was collected for me by one of my pupils, Mr. Pole-Evans, proved to be *Achlya polyandra*, Hildebrand. I had already spent twelve months upon the investigation of this and practically completed my work when Davis ('03) published his interesting observations on the cytology of the oogonia in an apandrous species of *Saprolegnia*. This well-known cytologist fully confirms the observations already made by me in that genus, and delineates with characteristic care and diagrammatic clearness the details of nuclear structure. The great merit of the research from the cytological point of view rests on the discovery of a structure which the author classes as a 'coenocentrum,' but which has all the appearances of a centrosome and its astrosphere. Although this structure has not, in my opinion, the significance which Davis attaches to it, yet it may be conceded that its presence materially helps the observer to clear up the difficulties inherent to the study of the nuclei in these sexual organs. Unfortunately, its discoverer made comparatively little use of his opportunity, although he seems to have been fully aware of it. Davis's paper is mainly occupied with theoretical discussions of a highly controversial character. The author rejects Hartog's observations as to wholesale fusions of nuclei, and adopts the theory first promulgated by me, that the supernumerary nuclei are digested and absorbed by the ooplasm. As already noted above, however, he refuses, with very marked emphasis, to accept my observations and conclusions as to fertilization, although, strangely enough, they are, so far as the observations are concerned, perfectly consistent with his own. His reason for this somewhat singular attitude of mind appears to be

based on the fact that he attaches extreme importance to the occasional occurrence of binucleate and trinucleate oospores in the apandrous species examined by him, a phenomenon which appears from its infrequency and the author's drawings and methods of culture to be in all probability of a pathological character. It is simply unfortunate that Davis should have ventured to enter upon such a controversy upon the basis of the examination of an obviously apogamous form; especially as it is clear that his methods would, if diligently followed out on appropriate material, have inevitably led him to the perception of the truth as to fertilization. There can, indeed, be no doubt at all that if he had examined a typical species—one i. e. with antheridia—he would have observed the succession in the number of the nuclei which has been already described, and have been compelled to modify his views accordingly. While his material was admirably suited to enable him to study the development of the oospheres and oospores, untrammelled by the difficulties associated with the presence of antheridia and fertilization-tubes, it is almost inconceivable that any one should choose it to enable him to take part in the settlement of those controversial questions which have been raised by the recent study of species of *Achlya* and *Saprolegnia*. Such an apogamous form as was examined by Davis is exceptional in the genus *Saprolegnia*. I have not yet succeeded in my endeavours to collect one in the genus *Achlya*. In cytology, as elsewhere, it is best and safest to proceed from the rule to the exception and not from the exception to the rule. These observations of Davis, however, impelled me to seek for another species of Saprolegnieae in the hope of verifying his observations on the so-called 'coenocentrum.'

With the assistance of Mr. Pole-Evans, *Achlya De Baryana*, Humphrey, was obtained, and proved in every way a most desirable subject for experiment and observation. The communications to be made on these two species of *Achlya* will, it is to be hoped, dispose finally of the vexed question as to the occurrence of fertilization in the Saprolegnieae.

Methods.

It is very desirable that the work already done on the Saprolegnieae should be extended and amplified as well as confirmed. A short account of the methods used by me, the result of the experience of the last ten years, may be of service to those who wish to specialize in this promising field of research. It has been pointed out in earlier papers that the Saprolegnieae may be easily collected and cultivated. Fine cultures of single well-identified species are highly desirable for cytological work, and they are readily obtainable if attention be paid to a few simple particulars. Excellent nutrient material for the cultures is provided by house-flies, which should be killed by chloroform, placed in plugged test-tubes and thoroughly sterilized. These flies will, if properly prepared, keep in good condition for several

years—a point of no small importance. The best and most convenient culture vessels are Petri dishes, and these too should be sterilized. Sterilized tap water is suitable where the water is sufficiently free from lime. If lime is precipitated on sterilization, distilled water or rain water should be used. On no account should cultures be attempted on white of egg, chopped beef, or other similar substrata, unless the inoculating material has been entirely freed from bacteria and other micro-organisms. The flies, having been transferred to Petri dishes half filled with water, are inoculated by cuttings of hyphae from healthy cultures, a single filament being transferred by means of a sterilized needle. When this process is repeated with the requisite care for a few generations, micro-organisms are practically eliminated, and if an even temperature be maintained the sexual organs appear on the cultures in great abundance with clock-work regularity a few days (generally about five) after inoculation. I have kept such cultures going for twenty to thirty generations in perfect health and vigour, and such as these were alone used by me for fixing. The first essential in cytological work is thoroughly healthy and vigorous material. The time and labour spent in the preliminary study of the living plant meets with its due reward in the greater constancy and accuracy of the final results. All sorts of anomalies occur when the conditions of life are unfavourable, as the Saprolegnieae are exceptionally susceptible to changes in their environment. Both the species of *Achlya* collected for me by Mr. Pole-Evans were found in the hard waters which everywhere flow from the lias in the Vale of Glamorgan. They frequently occur in a curious stunted, coral-like form which is almost, if not quite, sterile. Such cultures, if transferred to distilled water, develop normal growths at once.

The best fixative is a solution of chromic and acetic acids in water in such proportions that the percentages of the two acids are respectively 0·7 and 0·3. The much weaker solution used by Davis was tried, but the results in *A. De Baryana* were distinctly bad. The solution should be allowed to act for twenty-four hours and the material should then be washed in running water for another twenty-four hours. The transference to absolute alcohol is effected very readily and safely by the use of the diffusion shells of Schleicher and Schüll. All danger of shrinkage is avoided by this method and it has the merits of being easy to use and certain in its effect. The transition from absolute alcohol to paraffin may be successfully made by means of xylol, chloroform, or cedar-wood oil, but care must be taken to avoid sudden transitions at every stage of the process. The material itself should never be handled either with lifters, forceps, or other tools, at any time, but rather floated gently from one vessel to another when changes are necessary. The two essentials for success are undoubtedly fine healthy cultures free from micro-organisms and an appropriate fixative. The method of applying the fixative is important. Two cultures grown

side by side in the same Petri dish, apparently exactly alike, fixed simultaneously with chromacetic acid of the usual strength, and subsequently treated alike in every respect, yielded different results. One yielded me my finest preparations, the other provided only moderately good material.

Sections 7·5 μ thick are sufficiently thin to show nearly all the details perfectly, but I have relied entirely on sections 5 μ thick for the work described below. Sections which are thinner than this are more difficult to work with and there is as a rule no special advantage to be gained by their use.

Most of the nuclear stains give good results, but gentian-violet is undoubtedly the best for general work. This was used, according to Gram's method, with or without a second stain such as eosin. Very fine preparations were obtained with both gentian-violet and fuchsin when differentiation was carried out by chromic acid or picric acid solutions. Flemming's triple stain proved to be an excellent one for most purposes, but was less constant in its action than the others. Haematoxylin stains are the least suitable, for the microsomata in the protoplasm—the vibrioid organoids of Swingle ('98)—take the stain so greedily as to unduly mask the nuclear structures. With sections 1 μ thick, however, this stain would be the most satisfactory of all. The demonstration of critical and difficult points of structure may be carried out with any one of these stains, provided the material has been properly selected and prepared.

Note on Nomenclature.

The synonymy of the two species of *Achlya* with which we are concerned is somewhat confusing, both species having received the same name, *Achlya polyandra*. Humphrey ('92) recognized the confusion when preparing his monograph on the group and cleared up the difficulty satisfactorily. Fischer's ('92) view is in practical agreement with that of Humphrey, although he left the synonymy in its original confusion. I have only to add that I accept the conclusions of the American monographer. It will suffice to point out here that the first species examined by me should be named *Achlya polyandra*, Hildebrand ; it is *Achlya gracilipes*, De Bary. The second species should be named *Achlya De Baryana*, Humphrey ; it is *Achlya polyandra*, De Bary.

Observations on *Achlya polyandra*, Hildebrand.

The examination of this species occupied about eighteen months and many interesting facts came to light in addition to those with which we are at present concerned. I shall restrict myself to indicating the evidence procured as to the occurrence of fertilization, and such cytological phenomena as are associated therewith. Observations on the living

material revealed no new facts of interest. The examination of sections showed almost at once that the cytology of this species of *Achlya* corresponded closely with that of other species of Saprolegnieae which possess perfect sexual organs.

There is no necessity for a detailed description of the observations. The essential points can readily be appreciated by reference to Figs. 1 to 13. The oogonia and antheridia are multinucleate (Figs. 1, 2, and 3). The nuclei, although already much more numerous than is necessary to meet all the requirements of the sexual process, undergo an indirect division in both oogonia and antheridia (Figs. 1, 2, and 3). The oogonial nuclei are reduced in number by the degeneration and absorption of the excess before the oospheres are fully formed, and each oosphere as it rounds itself off is provided with a single, centrally-placed nucleus (Figs. 4, 5, and 6). The fertilization-tubes grow into the cavity of the oogonium, and when they reach the oospheres, each of these acquires a cell wall and a second nucleus (Fig. 7). The oospheres become, in fact, young oospores. That the entry of the second nucleus could not be traced seems to be of relatively small importance so far as the demonstration of the occurrence of fertilization is concerned. The two nuclei of the young oospore approach each other, but the actual fusion is delayed until the oospore wall has acquired a considerable thickness (Figs. 8, 9, and 10). Ultimately the nuclei invariably fuse, as is proved by the fact that the old oospores are always uninucleate. The superfluous nuclei and protoplasm of the antheridia undergo very slow degeneration and eventually disappear. In the case of this species no single exception as to the number of nuclei has been discovered;—the formula of succession, based on the examination of hundreds of normally developed sexual organs, is 1, 2, 1, and absolutely invariable.

Several points in the cytology, however, deserve special attention. In the anaphases of some of the nuclei in the case of a series of sections through one oogonium, appearances were noted such as are represented in Fig. 1, *a* and *b*, which suggested that a second mitosis sometimes took place immediately after the first without the normal intervening resting stage. I spent much time and labour in following up this clue, but failed to secure any better results in this species. It was partly this fact that impelled me to seek an additional species for further investigation.

The degeneration of the nuclei during the formation of the oospheres was also very difficult to follow; the experience gained in the study of degenerate nuclei in *Pythium* was of very little assistance. It was certainly not difficult to distinguish a single, central, deeply stained nuclear structure in each 'origin' and oosphere, but after Davis had published his paper I was obliged to admit that there would be ample justification for the criticism that in this case the 'coenocentrum,' if present, would be grouped

with its accompanying nucleus and be regarded as one body. In fact, it is extremely likely that during imperfect fixation of the nuclei and 'coenocentrum' a fusion of these very distinct bodies actually takes place—a result, however, of no special significance so far as concerns the demonstration of the occurrence of fertilization. The nuclear membrane of the centrally placed nucleus of the 'origin' is very difficult to demonstrate. I frequently could not satisfy myself as to its presence at this stage. It is probable that the nuclear membranes of the nuclei of the oospheres make their appearance just at this point.

The representation of a fertilization-tube in direct, open communication with a young oospore in Fig. 11 is noteworthy. It might well be accepted as a positive proof of fertilization. It is, however, an anomaly; the tubular neck has a relatively thick wall, a feature invariably absent from normal fertilization-tubes; and the adjacent section, represented in Fig. 12, shows a fertilization-tube already attached to the oogonium and delimited from it in the usual manner by a definite transverse membrane. There can be little doubt that the normal fertilization was effected by the second of these tubes and that the first one, notwithstanding its clearness, is anomalous. It is the only one of this type seen, and yet hundreds, if not thousands, of fertilization-tubes have been examined in normal contact with the young oospores. Such an anomaly, though obviously pathological, or at any rate very exceptional, is however not without its significance. Special reference should be made to Fig. 13, showing a typical fertilization-tube in direct communication with the oosphere or oospore. In this case the staining was so dense that the nuclei could not be seen at all, but an area of protoplasm in the oosphere, just opposite to the fertilization-tube, was stained in exactly the same way as the protoplasm of the tube itself, and the direct continuity of the protoplasm of the two organs was, without difficulty, established. This preparation, though useless for the study of karyology, sufficed to settle the question as to the existence of an open communication between the fertilization-tube and the oosphere, and furnishes some evidence for the view that protoplasm passes over from the fertilization-tube to the oosphere. It is interesting to note the way in which the gameto-nuclei approach each other, as shown in Fig. 8. The nuclei are oval and possess deeply stained granules at the somewhat pointed anterior ends. These granules lie apparently inside the nuclear membrane. They are typical, not exceptional, structures in this species, at this stage of development—at any rate with the methods of preparation employed by me—and are probably to be connected with the existence of centrosome-like structures which have fused more or less completely with the nucleus. However, the newer observations made on the second species—the details of nuclear structure being worked out more thoroughly in that case—will throw additional light on this phenomenon.

It is clear that the work on *Achlya polyandra*, Hildebrand, suffices to enable us to add another species to the list of the Saprolegnieae in which functional sex has now been established.

Observations on *Achlya De Baryana*, Humphrey.

Figs. 14 to 34 serve to illustrate all the fundamental features of the cytology of this species. These may be compared with the corresponding figures published by me in '95 and '99, as well as with Figs. 1 to 13. Novel or controversial points will alone be discussed in this paper.

The structure of the nucleus in the Saprolegnieae and the first mitosis in the oogonium. The resting nucleus of the Saprolegnieae has, in common with many other fungal nuclei, been frequently figured as consisting of a central chromatic body separated from the investing nuclear membrane by an achromatic perfectly hyaline substance. Davis ('03) occasionally represents the nucleus as of this type, as e. g. in Figs. 31 to 35 of his recent paper. However, Davis's description, with which most of his figures agree, gives a correct account of the actual appearances. He says, 'There is a nuclear membrane enclosing a well-differentiated nucleolus, prominent by its size and staining qualities. Much less conspicuous, but readily demonstrated in well-fixed material, is a loose linin network which contains the chromatic material.' The observations summed up in this statement agree with my own recent ones; they do not differ, indeed, in point of fact from those I published in '99. Still, I must object to the view that the central body has been proved to be a nucleolus. This is not so much a question of the relative value of preparations, concerning which every botanist may be allowed to have his own opinions, but rather of the interpretation of observations. If this conspicuous structure is a nucleolus, it is a giant of its kind, and such nucleoli in the higher plants would excite considerable interest. I have looked in vain for figures of such. My present conception of this problematic body, which I provisionally regarded as a 'chromosome' in '95, may be best realized if one imagines the nucleolar matter in the nucleus of a lily to increase in amount to such an extent as to half fill the nuclear cavity, and in doing so, to enclose, not displace, a large portion of the pre-existing linin network and the associated chromatin. This view, first promulgated in '99, I am not yet prepared to discard. It is based mainly on observations made on the central body during the prophases of karyokinesis. It is in accordance both with Davis's figures and the descriptions of his observations, but is in conflict with his interpretation of these; whether in the light of the recent researches of Wisselingh ('00) this view finds corroboration or not, seems to me of little importance. According to Wisselingh, the so-called nucleoli of *Spirogyra* give rise during karyokinesis to distinct chromosomes. The nuclei of the Saprolegnieae are not very suitable objects for the elucidation

of this rather difficult, and in the present connexion somewhat irrelevant, problem. A comparative critical study of nucleoli is obviously a desideratum. The recent work of Wager ('04) and Williams ('04) make it clear that the current conceptions of the structure of the nucleus and of its behaviour during mitosis will have to be modified in several respects.

My observations, so far as they concern other features of the karyology, agree with those of Davis in the following features:—(1) the intranuclear character of the spindle, (2) the persistence of the nucleolus (as a much smaller body than the original central mass) up to the metaphase stage in mitosis, and (3) the absence of centrosomes and astrospheres. It is clear to me, however, that the number of chromosomes in the nuclei of the species is certainly greater than four and probably eight, and that the nuclear figures are not nearly so regular as those figured by Davis. Moreover, the divisions of the nuclei are not so exactly synchronous as in the cases figured by him. The arrangement of the daughter-chromosomes as in Fig. 16 *a* suggests that a second mitosis is to take place, at any rate in some cases. The conditions seem to be more complex in this species than in Davis's apandrous *S. mixta.*

Whilst the first mitosis is in progress the layer of protoplasm lining the oogonium gets thinner and thinner and it attains its minimum thickness before the second mitosis is initiated. It is at this stage that the real cytological difficulties have to be faced. It is noteworthy that up to this point in the development of the oogonia, *Achlya De Baryana* does not differ in any fundamental feature of its cytology from the apandrous species investigated by Davis. Such slight differences as are noticeable are probably traceable either to specific variations in the plants themselves or to differences in the methods employed in their examination.

The second mitosis. Davis recognized the difficulties which naturally arise at this stage, but does not appear to have succeeded in solving the main problem. He seems to have overlooked the fact that the stage in the development of the oogonium immediately preceding that of 'balling' is readily distinguished in sections by the thinness of the parietal layer of protoplasm. His figures appear to be founded on the examination of tangential sections alone. Making all allowance for possible errors of interpretation, I am unable to reconcile his views with the observations made by me in *Achlya De Baryana.* Future investigations must decide to what extent these differences are founded on errors of observation and judgement, or on differences of fact.

When the anaphases of the first mitosis are over a single row of nuclei may be observed in the thin parietal layer of protoplasm (Fig. 17). The nuclei are not so numerous as they ought to be, and as some of them—marked *k*, *l*, *m*—stain so badly as to leave little doubt that they are undergoing dissolution we may infer that the absorption of the superfluous

nuclei has already commenced. The most distinct and deeply stained nuclei are in the spirem stage (Fig. 17 *a, b.*) Later prophases are to be seen (Fig. 17 *c*). Some of the nuclei, e.g. those marked *e, f, g, h,* are provided with deeply stained granules attached to the outside of the nuclear membrane. In one case, marked *j,* three granules were seen in this position, with protoplasmic threads radiating outwards from them. Such an assemblage of nuclei is of course very puzzling, and as this and the following stages are difficult to fix properly, there is room for errors in interpretation. Contrary to Davis's experience, the staining is not exceptionally difficult, the detail comes out perfectly with all the methods employed. The most natural inference to be drawn from these observations seems to be, that a certain number of nuclei are selected to undergo a second mitosis, and that the remainder are doomed to degeneration.

The further study of the fate of these dividing nuclei is very difficult, and, but for the successful fixation of one culture, might have proved barren of real result. One oogonium, thoroughly examined in a series of seventeen sections—a week's work—furnished examples of the metaphases and anaphases of this second mitosis. Some of these sections are represented, wholly or in part, in Fig. 18. It will be observed that the dividing nuclei are associated with small resting nuclei which still retain their normal characters and with degenerate nuclei of various types. The nuclei do not divide synchronously. In the late metaphases, as shown at *a, b, c, d,* a small elongated spindle is seen. It is noteworthy that this appears to consist always of three threads. Whether the two outer lines represent spindle threads or the optical sections of the nuclear membrane may be a matter of controversy, but an examination of the nuclear figure at *a* leads to the belief that the nuclear membrane is absent and that the chromosomes pass polewards along all these threads in a less regular manner than is the case during the first mitosis. This division, indeed, resembles in many of its features the corresponding division in *Albugo* described by Stevens ('99) and Ruhland ('03). At the poles of the spindles there can generally be detected a small deeply stained granule, which, as it is the centre of a system of polar radiations, must be regarded as a centrosome, the radiating threads constituting its astrosphere or aster. The rays of the astrosphere can be traced without any break of continuity right up to the centrosome. Late anaphases are seen in *e, f, g, h, j.* The two nuclei at *e* are daughter-nuclei, the connecting threads of the spindle being no longer continuous. One of the daughter-nuclei has turned through an angle of nearly 90°. The new nuclear membrane makes its appearance at this point, but is at first not readily demonstrable. At *f, g,* the centrosomes somewhat obscure their nuclei. These, although demonstrable, have been omitted from the drawings for the sake of clearness. The nucleus figured at *g* had neither centro-

some nor astrosphere. Cases such as *g* and *h* require attention. In *h* the association of the centrosome and astrosphere with its nucleus is clear. The centrosome is in close contact with the nuclear membrane. From a study of *g* an observer might conclude that the centrosome was attracting to it the adjacent nucleus. Considerations such as these, combined perhaps with preconceived ideas as to the distribution and physiological significance of 'coenocentra,' may have led Davis astray when working at this very complex problem.

Against the adequacy of the proof furnished here of a second mitosis taking place, it may be urged that the peculiar appearance of elongated spindles may be explained more easily and naturally on the assumption that we have before us a case of the division of the centrosome and the formation of an extra-nuclear spindle, the nucleus itself remaining undivided or its division being overlooked. Such a contention must not be lightly dismissed. Certain observations, relatively few in number serve, however, to dispose of it effectually. Nuclei in the nuclear-plate stage are occasionally seen as at *k* and *l*—the latter, it is well to note, in an antheridium. These nuclei are provided with a nuclear membrane, and are certainly undergoing a second mitosis. A comparison of the earlier stage in the development of the oogonium represented in Fig. 17, where all the nuclei have completed the first division, and of the still earlier stages represented in Figs. 15 and 16, where all the nuclei are undergoing division, with this one, must convince any unprejudiced observer that there can be no question here of the inadmissibility of the explanation that these early metaphases may belong to a deferred first mitosis. The dissimilar elongated nuclei seen in late metaphases and anaphases are, no doubt, derived from the earlier metaphases by the loss of the nuclear membrane and the elongation of the spindle. Harper ('99) has figured a similar elongation of the spindle in the nuclear divisions in the ascus of *Lachnea*. The apparent absence of the astrosphere in early metaphase, when at best it must be very feebly developed, is of little importance. It is only in very late anaphases that it makes itself really conspicuous.

Degeneration of the supernumerary nuclei. There are one or two points which must be clearly borne in mind when endeavouring to clear up the difficulties associated with the actual observation of the degeneration of the nuclei. A functional nucleus is only definitely recognizable by its structure, and in general that structure is only demonstrable in well-stained preparations. Degeneration is associated with loss of structure. Degenerate nuclei must therefore soon become unrecognizable. The degeneration and digestion of the nuclei is very rapid in *Achlya*, indeed much more so than in *Pythium*. Badly-stained nuclei associated in the same section with well-stained ones must be regarded as degenerate. We have to try and arrange these in a consecutive series to form some idea of the process of

degeneration. At one end of such a series the nuclei would with difficulty be recognized as degenerate at all—at the other end it would in most cases be impossible to feel sure that they really were nuclei. Notwithstanding much study it has proved impossible to give any definite account of the various stages in degeneration which the nuclei pass through. Degeneration may apparently commence either when the nuclei are in the resting condition, or when in the prophases or even the metaphases of mitosis. The nuclear membrane seems to disappear, the surrounding protoplasm to suffer a change which causes it to take up stains more readily, and the central mass—the doubtful nucleolus—to break up into smaller portions or remain intact, according to circumstances (Fig. 18, *m*, *n*, *o*, *p*). A blurred astrosphere-like arrangement of rather thick but indefinite protoplasmic filaments is sometimes associated with these degenerate nuclei, as seen in Fig. 18, *q*, *r*, *s*. I would suggest as a provisional explanation of this rather curious feature that they are the daughter-nuclei of the second mitosis undergoing degeneration along with their associated astrospheres. This observation is difficult to reconcile with Davis's contention that his 'coenocentra' select and nourish the functional nuclei.

In the last phases of degeneration, after the supernumerary nuclei have entirely vanished as such, large deeply-stained granules are to be found in the ooplasm, as shown in Fig. 19. These may best be regarded as certain by-products of the degeneration process. They correspond somewhat to the similar structures figured by Davis, but are much fewer and larger.

Development of the astrospheres and ovocentra. Before all the superfluous nuclei are quite destroyed, the process of the formation of the oospheres (balling) begins. The astrospheres grow rapidly in size. The protoplasm associated with them loses its larger vacuoles and becomes fine grained. The nucleus remains in close contact with the centrosome—Fig. 19, *a*—and its chromatin seems to concentrate itself in the immediate neighbourhood of that body. As Davis has already pointed out, it would be very easy to mistake this dual structure for a single one, even in fine preparations. The centrosome absorbs nuclear stains with the same facility as the chromatin of the nucleus. In good preparations the centrosome, like the nucleus itself, is readily demonstrable by its structure. It always appears, when well developed, as a single granule, from which there radiates outwards in all directions a relatively small number of protoplasmic filaments. These filaments are traceable right up to the centrosome and are continuous with it. Centrosome and astrosphere correspond very closely with those in *Pellia*, figured and described by Farmer and Reeves ('94). The nucleus can be recognized with certainty even when it lies immediately under the centrosome. Nuclei occupying this position have not been inserted in the drawings for obvious reasons. The nuclei are not shown consequently in Fig. 19, *b*, *c*, *d*. In one case—Fig. 19, *d*—I

observed a well-developed nucleus, its centrosome and astrosphere, in close association with a recognizable nucleus bearing at one end a few astral rays. Some botanists might be prepared to argue from this isolated occurrence that a fusion of nuclei takes place at the commencement of 'balling.' The more reasonable explanation is, I think, that this solitary supernumerary nucleus was the last recognizable survivor of the superfluous nuclei, and destined to disappear by degeneration in due course, like its fellows. In fact, it would probably have been overlooked had it not had associated with it a few astral rays. In the same section two small indefinite astrospheres with the nuclei no longer definitely demonstrable were noted, viz. at *e* and *f*. As 'balling' proceeds the astrosphere continues to increase in size until it is a very conspicuous structure indeed. It reaches its maximum development just before the separation of the 'origins' in the process of the formation of the oospheres (Figs. 20 and 21). The rays of the astrosphere, it will be noted, are mainly directed towards the oogonial walls in radial sections. The nucleus has a well-defined nuclear membrane, and the chromatin is aggregated at the end nex the centrosome, forming there, as it were, a deposit on the inside of the nuclear membrane. The nucleus thus acquires at this stage a distinct vesicular character—one specially remarkable from the absence of the usual central body. *At this stage the 'origins' are invariably uninucleate.* The absolute determination of this point is necessary in order to prove fertilization by means of the order of succession in the number of nuclei in the oospheres and oospores. Eleven oogonia were examined critically. These contained respectively 5, 13, 11, 10, 8, 8, 9, 11, 4, 6, and 14 'origins.' Ninety-nine origins were thus examined. All were uninucleate. Many other oogonia were casually examined in the course of the investigation, and in no case were two nuclei observed in one 'origin.' At about this time the fine-grained protoplasm surrounding the nuclei in the centre of the 'origins' begins to stain more deeply and thus to acquire a certain appearance of individuality. I propose to call this central mass of material, for convenience in description among other reasons, the *ovocentrum*. I do not regard this ovocentrum as a morphological unit in any sense, but rather as a transitory appearance depending primarily upon the forces acting in the oosphere. The materials —whether living protoplasm or reserve food products—by virtue of which the acting forces put themselves in evidence, play apparently a passive part; to use a hackneyed simile, they behave as iron filings do to a magnet. It is to this body that I have hitherto applied in other species of plants the term 'coenocentrum.' It is clear that the term 'coenocentrum,' first proposed by Stevens ('99), and used afterwards by Davis ('00, '03), Ruhland ('03), myself, and others, has been applied to more than one type of object. The use of the term 'coenocentrum' has no doubt facilitated the study of the sexual organs of the Phycomycetes, but it can scarcely be regarded as

a happy one, for the structure in question is actually at the centre of an ovum. The term in question is not unsuitable for the case in which it was first used by Stevens—the oospheres of *Albugo*—for the numerous nuclei in these can be considered as having a common centre. Ovocentrum appears to be a more suitable term for general use, and will be used by me to denote the aggregation of granular protoplasm about the astrosphere and nucleus in the oospheres of this *Achlya* and the other members of the Phycomycetes investigated by me. Ruhland criticizes my comparison of such an ovocentrum to a 'whirlpool in a river.' Now, as the simile was used to call attention to the view that the constancy of form was maintained rather by a constant force than by a constant substance, there seems no real difference of opinion between us except as to choice of illustration. Surely, though, a whirlpool whose relatively permanent form depends primarily on the force of the rushing water, and whose actual particles, whether of swirling water or floating foam, are continually entering and leaving it, furnishes a not inappropriate simile. When the ovocentrum takes the form of a single well-defined granule or globule, with a definite limit, perhaps even a limiting membrane, as figured by Davis ('00) and Stevens ('99) for *Albugo*, the simile is no doubt an inadequate one. The fact, however, remains, that even here we are dealing primarily with force rather than with substance—the acting force is regular and necessary, the substance is more or less irregular and accidental. Figs. 22, 24, 25, 26, 28, 29, and 34 lead one to infer that the ovocentrum is the result of forces emanating from the region of the centrosome. The question of the function of the centrosomes themselves has been the cause of much speculation, but it is one that can scarcely be attacked profitably in this connexion. We require more detailed observations on their origin and distribution in plants. My observations, indeed, lead me to doubt whether the centrosomes and astrospheres are themselves anything more than manifestations of the forces acting at certain points in the oospheres, or better, at points in the immediate vicinity of the nuclei. To build up theories of chemiotaxis on such slender foundations as the juxtaposition of centrosome and nucleus, as has been done by Davis, appears to me to lead to no result of real value.

The entry of the sperm nucleus into the egg. Fertilization. Three oogonia, containing respectively 4, 17, and 11 oospheres, were critically examined. Each oosphere had the structure represented in Fig. 22. It was provided with a single nucleus in the resting condition, a centrosome and astrosphere, and an ovocentrum of dense protoplasm. Many other oospheres were casually examined, and if fertilization-tubes had not reached them, they always proved to be uninucleate, and to possess the structure shown in Fig. 22. The accurate demonstration of the entry of the sperm-nucleus into the egg is excessively difficult, and has only been satisfactorily carried out on one culture fixed in chromacetic acid. The fertilization-tubes, as

in all the other sexual species, can be traced up to the oospheres. With these they come into close contact—one which, once established, persists for a considerable time, and can be recognized even in fairly old oospores. It was the nature of this contact (see Figs. 26, 28, and 29), combined with the variation in the number of the nuclei, that led me, years ago, to look carefully for proofs of the entry of a sperm-nucleus. The fertilization-tube, after coming into close contact with one oosphere, generally sends out a lateral branch which may, and frequently does, come into contact with another oosphere. This fact, together with the failure to note the constant adhesion of the fertilization-tubes to the young oospore membranes, apparently led Hartog to regard the fertilization-tubes as functionless organs. A study of Figs. 23, 24, and 25 will suffice to show that the apex of the fertilization-tube not only indents the oosphere, but really penetrates into the ooplasm, and by virtue of the solution of its membrane allows a direct protoplasmic continuity to be set up between the fertilization-tube and the oosphere. The passage of a nucleus from the one organ to the other may be traced (Figs. 23, 24, 25, and 26). An admirable proof of the continuity of the protoplasm in both organs is furnished by Fig. 23, which may be interpreted as meaning that the nucleus is accompanied by an appreciable quantity of protoplasm—a gonoplasm is indeed demonstrated. Such a figure reminds one very much of the nuclei squeezing themselves through the sterigmata of the Basidiomycetes, as figured by Ruhland ('01) in *Hypholoma.* Attention may be directed to one or two other points of interest. The protoplasm in the oosphere in the neighbourhood of the fertilization-tube becomes more granular, stains more deeply, and acquires the character of a receptive spot. An elongated vacuole is often associated with the sperm-nucleus. In Fig. 25 this could be traced back into the fertilization-tube outside the oosphere. In Fig. 26, a case in which the fixing was not quite satisfactory, the vacuole-like structure occupies a tangential, not a radial, position. The limits of the cellulose cell-wall of the fertilization-tube at this time were not determinable with any degree of accuracy. The cellulose wall seems to stop short at the periphery of the egg, but the boundary between the ooplasm and the contents of the fertilization-tube is as represented in the figures, and gives the impression of a tube which penetrates some distance into the egg. Just at the time when the wall of the young oospore makes its first appearance, one frequently finds what appears as a prolongation of the fertilization-tube into the oospore in the form of a thimble-like vacuole (Fig. 28).

These facts, taken by themselves, might still fail to satisfy sceptics as to the existence of fertilization in the Saprolegnieae. Fortunately, the close study given to the sperm-nuclei resulted in a very unexpected and apparently, so far as plants are concerned, a unique discovery.

The centrosome and astrosphere of the male nucleus. The sperm-

nucleus soon after its entry into the oosphere, acquires a distinct centrosome and astrosphere. The centrosomes may have been present in the antheridia and fertilization-tubes in an unrecognizable condition, but the astrospheres are certainly new developments. The astral rays are always directed at first to the periphery of the oospore, as shown in Figs. 28 and 29, and they are associated with a mass of protoplasm having most of the characters of an ovocentrum. This mass of protoplasm might be regarded as homologous with the receptive spots found in the oogonia of the Peronosporeae. Apart from the fact that it appears in *Achlya* at the periphery of the oospore and not of the oogonium, there is good reason for believing that its affinity is really with the ovocentrum. One might go so far as to call it a spermocentrum to emphasize this point, but for the reason that the structure in question is not situated at the centre of the sperm. Fig. 27 shows the male centrosome and its astrosphere, with and without the associated protoplasm—at *a* and *b*. The nucleus is indicated separately at *c* for the sake of clearness. The astrosphere of the male nucleus was seen by me first in relatively poor preparations, when it was so badly fixed and stained that it was impossible to convince Mr. Pole-Evans—a very keen-eyed observer—of its presence. The figures are drawn from preparations in which the astrospheres were very conspicuous. These male astrospheres, in good preparations, are very striking objects. Mr. Pole-Evans made a drawing of one so that I might be able to compare our observations. The drawings contained exactly the same number of main rays. An inexperienced observer was also asked to examine a section, and he noticed the rays of the astrosphere at once. I have not been able to discover any record of a similar observation of astrospheres in association with male nuclei in plants.

Typical centrosomes and astrospheres appear to be much more rarely developed in plants than in animals. In Zimmermann's ('96) *résumé*, if we exclude, as we must, those cases resting on the evidence of Guignard, a very small number of genera are recorded as possessing centrosomes. Centrosomes together with their astrospheres have, however, been demonstrated very satisfactorily in *Sphacelaria* by Strasburger and Swingle ('97), in *Fucus* by Strasburger ('97) and Farmer and Williams ('96, '98), in *Dictyota* by Mottier ('98 and '00) and Williams ('04), in *Corallina* by Davis ('98)—the centrosome being represented in this case by a somewhat indefinite centrosphere—in *Surirella* by Karsten ('00), in *Peziza*, *Ascobolus*, and *Erysiphe* by Harper ('95, '97), in *Agaricus* by Wager ('94), in *Pellia* by Farmer and Reeves ('94), and in *Fossombronia* by Farmer ('95). A number of genera might be added to these on the basis of older and, yet in many cases, apparently thoroughly trustworthy observations. The phenomenon appears to be commonest amongst the Thallophytes, especially among Fungi, but to be rare in vascular plants, possibly altogether absent.

Strasburger ('01) has expressed himself very strongly as to the improbability of their presence in the higher plants. It may be regarded as doubtful whether the blepharoplasts described by Belajeff ('98 and '99), Shaw ('98), Webber, Ikeno, and Hirase in various Vascular Cryptogams and Gymnosperms are the homologues of the centrosomes found in Thallophytes and liverworts. In any case a critical summary of the present state of our knowledge as to the occurrence and distribution of centrosomes in plants appears to be a desideratum. In the genera mentioned above the male gametes have been critically examined in *Fucus* alone, and fortunately by such expert cytologists as Strasburger, Farmer, and Williams. Strasburger, in particular, paid special attention to the possibility of the existence of a male centrosome and astrosphere, being fully alive to the significance of its presence or absence in view of the current theories of fertilization. The observations of Farmer and Williams ('96, '98) are of special interest. They state ' the passage [of the antherozoid] through the cytoplasm [of the oosphere] is extremely rapid, as is proved by the rarity of specimens which we have obtained which show the antherozoid between the egg periphery and the centrally placed nucleus. In its path through the cytoplasm it rather resembles a chromatophore, from which, however, its reaction to stain readily serve to distinguish it. It travels towards its destination *with its blunter end forward*, and no trace of cilia could be discerned when it was once inside the egg.' An examination of the excellent figures of these three observers leaves little doubt that the sperm-nucleus in *Fucus* is destitute of a centrosome and astrosphere.

Male centrosomes and astrospheres are well known in animal eggs, and are especially well developed apparently in Echinoderms. It is interesting to note that that rotation of the axis of the sperm-nucleus and consequent change in position of the astrosphere which has been so often described, and is very beautifully illustrated for *Toxopneustes* by Wilson ('97), does not take place in *Achlya*. During the progress of the sperm-nucleus to the middle of the oosphere it appears to keep its astral rays directed constantly towards the periphery. This behaviour corresponds to that described as occurring in *Fucus* by Farmer and Williams, making the necessary allowance for the absence of the astrosphere in the Alga. The male astrosphere certainly influences the female, for that appears to be repelled and to be driven thus from its central position in the oospore (Figs. 28 and 29). When the sperm-nucleus reaches the central region of the oospore, the dense protoplasm accompanying it goes to swell the ovocentrum, and this regains its original central position (Fig. 28). The ovocentrum now encloses two nuclei, two centrosomes, and two astrospheres.

Disappearance of the astrospheres and ovocentra and the fusion of the gameto-nuclei. The detection of the male astrospheres proved difficult.

The study of their fate has been practically a failure. They, in fact, soon disappear, and the mode in which this takes place has still to be determined. The ovocentra gradually disappear too, being however still clearly visible when all traces of the astrospheres have vanished (Fig. 30). The male and female nuclei increase in size and capacity for staining during this period, are easily demonstrable, and are always found in close proximity to each other near the centre of the oospore. When the ovocentra have finally disappeared, the gameto-nuclei may be found either close together or at a considerable distance apart, and frequently at some distance from the centre of the oospore (Fig. 31). In view of these phenomena it seems useless to discuss Boveri's theory of the function of the centrosome in fertilization. At any rate, it is only necessary to state that the fusion of the gameto-nuclei is delayed for at least twenty-four hours, and that the division of the zygote-nucleus may not take place for months.

It is, however, worthy of note that the nuclei at this stage are easily fixed and stained. Binucleate oospores representing this stage would be, in fact, the most obvious structures in moderately good preparations. This must be the stage which Humphrey and Hartog saw and figured as representing the last of the nuclear fusions assumed by them to take place in the oogonium and oospheres. I examined critically three oogonia containing respectively fourteen, thirteen, and fifteen oospores, and all were binucleate. Thousands of sections of oospores must have been casually observed in this condition whilst searching for other stages, and they were always binucleate or uninucleate. If uninucleate, one could confidently predict that an adjacent section would be likewise uninucleate. The binucleate character of isolated oospores has often been proved in this way. It is only after repeated experiences of this kind that one becomes confident that uninucleate sections always go in pairs.

This stage in development is, of course, preceded by one in which fertilization is going on, and if attention be directed specially to it, and the oogonia are carefully examined, we find that fertilized and unfertilized oospheres occur together in the same oogonium. We see, side by side, genuinely uninucleate and binucleate eggs, not simply uninucleate and binucleate sections. Such a condition of affairs depends upon the obvious fact that the fertilization of the numerous oospheres is not affected simultaneously. Three oogonia in this condition were examined critically. No. 1 possessed eight uninucleate oospheres and two binucleate oospores; No. 2 possessed one uninucleate oosphere and six binucleate oospores; No. 3 possessed six uninucleate oospheres and two binucleate oospores.

During the maturation of the oospores the oospore-wall thickens and granules of reserve material collect in the protoplasm. It is only when these processes are well under way that the fusion of the gameto-nuclei takes place. A remarkable feature of this stage is that the chromosomes

seem to be still recognizable, being grouped apparently in fours (Fig. 32). This reminds one of the statements of Berlese ('97) as to the number of chromosomes counted by him in the oospores of the Peronosporeae under similar conditions—statements which certainly aroused considerable scepticism at the time they were published.

The further ripening of the oospores and their germination were not investigated in this species. The observations recorded suffice to prove once more the familiar succession in the number of the nuclei, the formula 1, 2, 1 being again demonstrated.

The number of chromosomes. As already indicated above, a study of the first mitosis in the oogonium led to the conclusion that the number of chromosomes in the nuclei was certainly more than four, and probably eight. The more difficult study of the second mitosis makes it pretty clear that the number of chromosomes is reduced therein to four. At metaphase in side views of the spindle we see clearly two or three chromosomes—numbers which would naturally occur if there were really four chromosomes in the nuclear-plate (Fig. 18 *k* and 20 *a*). It is not without significance, too, that four chromosomes can sometimes be seen in each of the conjugating gameto-nuclei (Fig. 32). Stress need not be laid on the actual numbers, as there are several sources of error in such determinations, but the reduction is definite enough. Such a division is to be regarded as a reducing division; a reduction in the number of chromosomes takes place in gameto-genesis in this plant as in most animals, and not in sporogenesis as in most plants. A detailed discussion of the physiological significance of such reductions may well be deferred until more information has been gathered as to their distribution amongst plants, and especially amongst the sexual and apogamous members of the Saprolegnieae. The view expressed by me in '99 as to the significance of these divisions during gameto-genesis must, for the present, suffice.

Anomalies. In the discussion of cytological problems a consideration of typical forms is of much more importance than that of anomalous ones. The conclusions reached by the modern cytological methods become quite untrustworthy in the presence of many anomalies, for the simple reason that anomalous and typical forms get grouped together to form an unreal series—a sequence is evolved which is purely imaginary. One must constantly bear in mind that the series of stages in development described in such a paper as this are not consecutive stages in the development of some one individual organ, but rather stages arranged consecutively with more or less success, according to the calibre of the observer, representing a series of such organs. With this difficulty always in view, great care has been taken in the selection and cultivation of the material employed in my researches on the Saprolegnieae. In certain preliminary studies, on material afterwards rejected, anomalies were only too abundant.

Two simple anomalies which came under observation in this species are, however, worthy of mention. In the case of one oospore—represented in Fig. 33—which contained a typical ovocentrum, an astrosphere and female nucleus, two sperm-nuclei had entered the oosphere through the fertilization-tube, and commenced to form astrospheres. The other oospores in the oogonium were normally fertilized. The fate of such sperm-nuclei is, of course, not ascertainable.

In one oogonium, containing about a dozen oospheres, three had the structure represented in Fig. 34. The impression given was that of two oospheres which had fused together, the line of fusion being indicated by the marginal indentations and the arrangement of the vacuoles in the plane connecting these. The nuclei, astrospheres, and ovocentra were well developed, and the mutual repulsion of these was very obvious. Such a case as this allows one to infer with some confidence that the size of the oosphere and the attracting (or repelling) forces associated with the nucleus and astrosphere stand in some definite quantitative relation to one another. The remaining oospheres in this oogonium were relatively small, but each had otherwise the normal structure. Although about thirty generations of cultures were examined in the fresh condition during the investigations made on this species, I never saw an oogonium with anomalous ripe oospores such as might be expected to result from the fertilization of such oospheres as these. It is upon such anomalous binucleate oospheres that Davis ('03) founds some of his most important conclusions. It would be useless to discuss these here. A theory founded upon exceptions and anomalies may be sound enough, but the exceptional facts recorded by Davis admit of a simple explanation, and certainly do not appear to have the phylogenetic significance which he attributes to them.

Summary of the evidence as to the occurrence of fertilization in the Saprolegnieae. In view of the facts recorded above, we may safely affirm that a normal fertilization takes place in *Achlya De Baryana*. For it has been shown that (1) the oosphere is uninucleate, the young oospore binucleate, and the old oospore uninucleate—the formula showing the number of nuclei in the reproductive organs is 1, 2, 1; (2) the protoplasm of the fertilization-tube becomes continuous with the ooplasm of the oosphere; (3) the entry into the oosphere of a sperm-nucleus can be traced; (4) the sperm-nucleus becomes associated with a special centrosome and astrosphere; and (5) the male and female nuclei fuse to form one zygote-nucleus. To *Achlya De Baryana* we may add, on the basis of my own earlier observations, fully concordant with the later ones so far as they go, four distinct species. The formulae as to the succession of the nuclei has been determined in these, and the cytological features are consistent throughout. The later results are, however, naturally more complete than

the earlier ones, as almost invariably occurs in all genuine investigations. The results in these four species are as follows:

1. *Achlya polyandra* has the formula 1, 2, 1, established in 1902.
2. *Achlya americana cambrica* „ 1, 2, 1, „ „ 1897.
3. *Saprolegnia dioica* „ 1, 2, 1? „ „ 1895.
4. *Saprolegnia mixta* „ 1, 1 or 2, 1 „ „ 1895.

Wager ('99) has confirmed the fundamental points of my earlier work. His formula would be 1, 2, *x*. He appears strongly inclined to the view that fertilization must take place, but expresses himself with commendable caution.

The negations of Hartog and Davis. Hartog apparently still adheres to his original view as to the wholesale fusion of nuclei in the oogonium, but since Davis has confirmed my results as to this point, and the above detailed observations show that no such fusions could possibly take place in these forms, there seems no adequate reason for subjecting his very pointed criticisms of my work to a detailed examination. The policy of appealing to fact, and of avoiding a war of words, finds ample justification.

Davis, however, is inclined to adopt Hartog's view—supported as it was a few years ago by the almost universal belief in the complete apogamy of the Saprolegnieae—that normal fertilization does not take place in this group. His contentions seem to me to be refuted by the facts described above, so that the necessity for a detailed examination of his criticisms likewise no longer exists. Moreover, the investigation of *Achlya De Baryana* having led to the discovery of the second mitosis, and of the centrosome and astrosphere associated with the sperm-nucleus in fertilization, it becomes necessary that Davis's species should be reinvestigated so as to test his view of the origin of the 'coenocentrum.' The question of the occurrence of a second mitosis in apandrous oogonia, and of the fate of the nuclei in these organs, forms a very difficult but interesting problem for future investigation—one, indeed, that should prove profitable in the highest degree.

Conclusion.

The problem of fertilization in the Saprolegnieae is, it seems to me, to a certain extent solved. Some species are typically sexual, others obviously apogamous. Between these extremes there are intermediate types. It is to be hoped that some of those able cytologists who have already done such good work on the Peronosporeae will turn their attention to the Saprolegnieae. Those who take up the study of such forms as *Achlya De Baryana* and *Achlya polyandra* will soon find themselves in a position to

bear witness to the existence of fertilization in this most interesting group of Fungi. I venture to predict that the Saprolegnieae will soon furnish many more examples of typically sexual species.

Summary of the Cytology in *Achlya De Baryana*.

1. The nuclei in the oogonia and antheridia all undergo a first mitosis, the number of chromosomes being apparently eight.

2. Some of the daughter-nuclei in both oogonia and antheridia undergo a second mitosis in which the number of chromosomes is apparently reduced to four.

3. Centrosomes and astrospheres are developed in the second mitosis in the oogonia, but have not yet been observed in the antheridia.

4. The supernumerary nuclei in the oogonium undergo degeneration and absorption by the ooplasm. There are no nuclear fusions. The process of degeneration is virtually completed when 'balling' begins.

5. The supernumerary nuclei in the antheridia and fertilization-tubes degenerate much more slowly than those in the oogonia.

6. The 'origins' and oospheres are uninucleate. Each nucleus is associated with a well-developed centrosome and astrosphere, and an ovocentrum.

7. In fertilization, an open communication is set up between the fertilization-tube and the oosphere, and a single male nucleus accompanied with a gonoplasm passes out of the fertilization-tube into the oosphere.

8. The sperm-nucleus acquires, in the oospore, a centrosome and astrosphere, and as it moves towards the oosphere-nucleus, the centrosome and astrosphere are directed outwards. There is no rotation of the axis of the sperm-nucleus as in animal eggs.

9. The fusion of the gameto-nuclei does not take place until centrosomes, astrospheres, and ovocentra have disappeared. The male centrosome does not seem to have any special significance in the act of fusion or of the subsequent mitosis. Boveri's theory of fertilization does not seem applicable to plants.

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EXPLANATION OF FIGURES IN PLATES XXXIV–XXXVI.

Illustrating Dr. Trow's paper on Fertilization in the Saprolegnieae.

All the figures were drawn with the help of the camera lucida. The finer details were of course filled in by freehand after the main outlines had been traced under the camera. All the nuclei present in a section were drawn, each generally in median optical section. The details of the cytoplasm are represented somewhat diagrammatically, as it is impossible to represent more than one optical section in the same drawing. All the sections figured were 5 μ thick. Various stains were used. The drawings were all made under a magnification of about 1,250 diameters. Figures 1 to 13, which represent sections of *Achlya polyandra*, have been reduced during the process of reproduction to about 800 diameters. The remaining figures, which represent sections of *Achlya De Baryana*, have, with the exception of 14 and 15, not been reduced. Some of the drawings of the more typical nuclei, as well as other details in the various sections, have been enlarged and inserted separately in the plates in the neighbourhood of the sections to which they belong, so that the structure might be more certainly demonstrated.

Achlya polyandra.

Fig. 1. A somewhat tangential section of an oogonium with the nuclei in metaphase and anaphase. *a* and *b* are apparently undergoing a second mitosis.

Fig. 2. A nearly median section of an oogonium with the nuclei in mitosis. One nucleus showing the polar view of the nuclear-plate.

Fig. 3. A median section of an oogonium, and a section of an antheridium with the nuclei in mitosis. One nucleus has a typical spindle and nuclear-plate.

Fig. 4. One section (the fourth of nine) through an oogonium which contained four origins, each uninucleate. Three origins shown, each with a functional nucleus (*f.n.*) and degenerate nuclei (*d.n.*).

Fig. 5. Section of an oogonium (the first of eleven examined) which contained seven uninucleate oospheres. A doubtful centrosome is shown at *a*.

Fig. 6. The third section of the same oogonium (as in Fig. 5).

Fig. 7. The fifth section of a series of five sections through an oogonium which contained five binucleate oospores. *a* had two nuclei in the fourth section and *b* had one. Two other oogonia on the same slide had eight and six binucleate oospores in series of seven and eight sections respectively.

Fig. 8. A young oospore. The two nuclei in contact by their anterior pointed ends, where is to be seen a pair of deeply stained granules.

Fig. 9. One of four half-matured oospores present in a small oogonium, all of which were uninucleate.

Fig. 10. One of four sections of an oogonium which contained three uninucleate oospores. The oospore wall well developed and as thick as the wall of the oogonium.

Figs. 11 and 12. Sections (two of five) of an oogonium which contained two oospores only. The fertilization-tube in eleven is anomalous, the normal tube being shown in twelve. The swollen fertilization-tube is likewise, so far as my experience goes, a unique anomaly.

Fig. 13. Section of an oosphere with fertilization-tube attached to it, the protoplasm in both being perfectly continuous.

Achlya De Baryana.

Fig. 14. Section of a young oogonium. Basal wall not yet formed.

Fig. 15. Median section of an oogonium and antheridium. All the nuclei in mitosis, excepting one or two which appear to be undergoing degeneration.

Fig. 16. Section of an oogonium with nuclei in mitosis. Nuclear figures somewhat irregular, suggesting a preparation for a second mitosis.

Fig. 17. Thin layer stage in development of the oogonium. The nuclei are either undergoing degeneration or are in the prophases of the second division. The two nuclei in the antheridium are in the metaphase stage of the first mitosis—one is seen in a profile, the other in a polar view. See text, pp. 553–4.

Fig. 18 $^{1+2+6+7+8+9+12+17}$ shows sections or portions of sections through one oogonium studied very thoroughly in a series of seventeen sections. The indices represent the numbers of the sections. Some of the nuclei are undergoing a second mitosis. The centrosomes and astrospheres make their first appearance at the poles of the spindles. Many nuclei are undergoing degeneration. See text, pp. 554–6.

Fig. 19. Section through an oogonium at the commencement of the formation of the 'origins.' Four origins are shown, each with a centrosome and astrosphere. A nucleus is present in each, too, but is only shown in *a*. There is a small supernumerary nucleus in *d*.

Fig. 20. Tangential section of an oogonium which contained four uninucleate 'origins.' At a lower focus the two origins of this section were continuous. The nuclei of the antheridium are in the metaphase of the second mitosis.

Fig. 21. Radial section of an oogonium with old origins. Mitoses completed in antheridia. Commencement of the formation of the ovocentrum. The nucleus has now no central chromatic mass. The centrosomes and astrospheres are very obvious structures.

Fig. 22. Small portion of an oogonium with the adjacent antheridium, drawn to show a typical oosphere. Seventeen similar ones were observed in a series of six sections, but fertilization-tubes were absent.

Fig. 23. Section of an oosphere at the moment of fertilization. A gonoplasm is present in the form of a thin strand which precedes the sperm-nucleus. The nucleus of the oosphere was in the adjacent section.

Fig. 24. Two serial sections of a small part of one oogonium showing the fertilization of one oosphere. The entry of the fertilization-tube and sperm-nucleus is shown in *a*. The median section of the oosphere is shown in *b*, the nucleus, however, not being inserted.

Fig. 25. Median section of a young oospore, showing female nucleus, centrosome, and astrosphere, the entry of the sperm-nucleus and the presence of a receptive spot.

Fig. 26. Portion of a section of an oogonium and antheridium showing the fertilization of two oospheres. In *a* the sperm-nucleus obscures the centrosome situated below or behind it. A portion of the ovocentrum is seen, but the female nucleus was in the adjacent section. In *b* the sperm-nucleus has apparently taken a tangential course through the oosphere.

Fig. 27. Tangential section of an oospore. At *a*, the male centrosome and astrosphere imbedded in the ooplasm; at *b*, the male centrosome and astrosphere without the ooplasm; at *c*, the associated male nucleus with the position of a portion of the astrosphere indicated.

Fig. 28. Fertilized oospheres, the gameto-nuclei being accompanied by centrosomes and astrospheres. Rays of male astrospheres directed outwards.

Fig. 29. Young oospores, in which the wall is clearly visible. The mode of attachment of the fertilization-tubes illustrated. The rays of the male astrospheres are directed outwards.

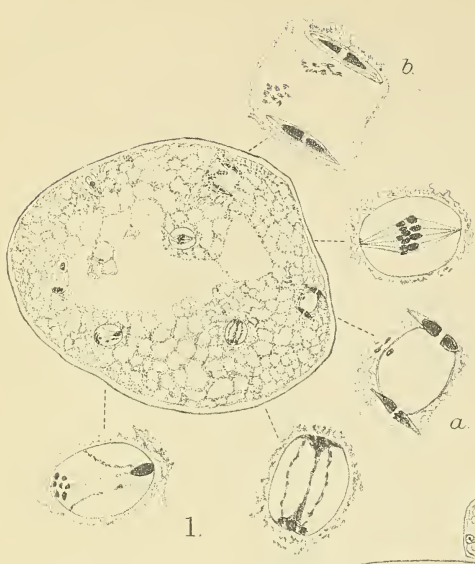
Fig. 30. Young oospore. The centrosomes and astrospheres have disappeared and the ovocentrum is relatively small, but still includes the two gameto-nuclei.

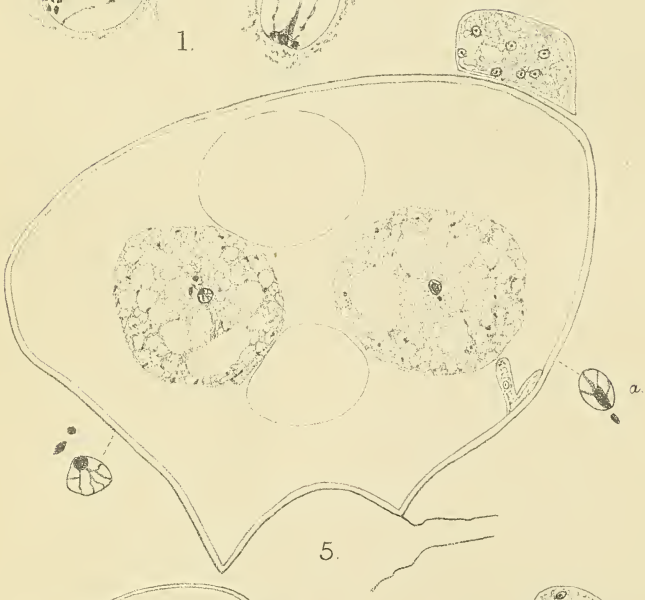
Fig. 31. Portion of a section of an oogonium with two binucleate oospores. The ovocentra have entirely disappeared, and the nuclei no longer occupy a central position. Deeply staining granules make their appearance in the protoplasm.

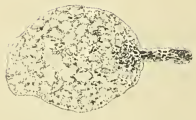
Fig. 32. Section through a portion of an oogonium showing three oospores with conjugating gameto-nuclei. The chromosomes of the nuclei appear to be still in evidence. The granules in the protoplasm are very conspicuous. In two of the oospores the nuclei are alone represented in full.

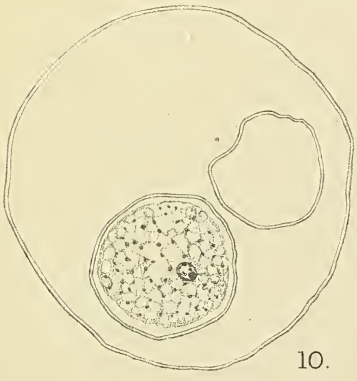
Fig. 33. Section through an oospore which contained two sperm-nuclei. The female nucleus was in the adjacent section.

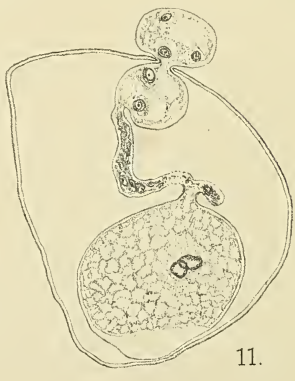
Fig. 34. An anomalous binucleate oosphere, one of three discovered in one oogonium.

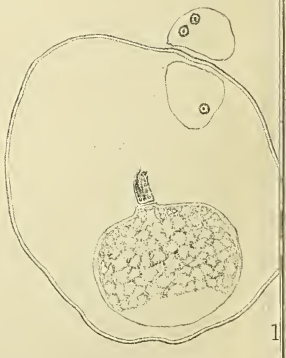












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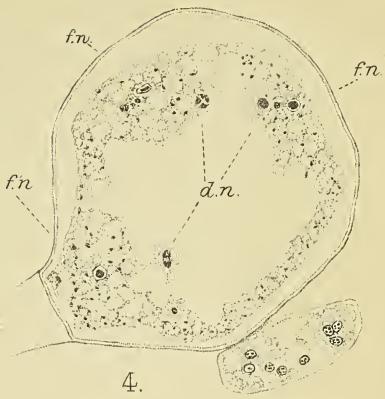

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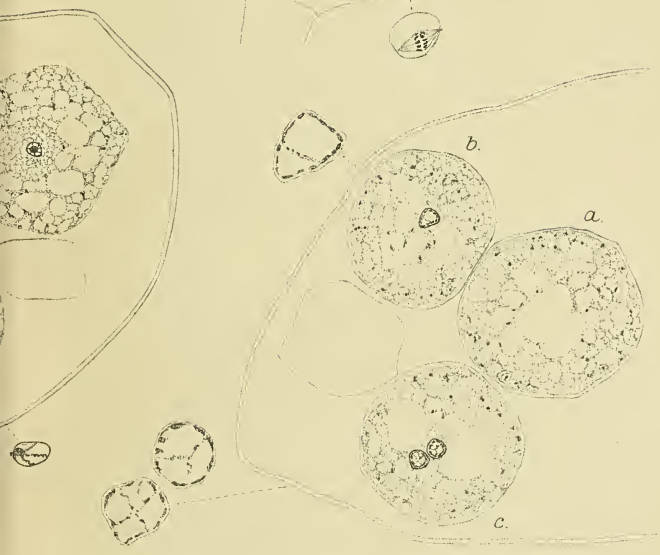

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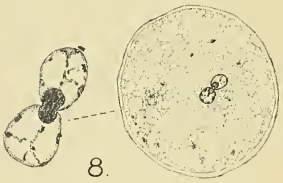

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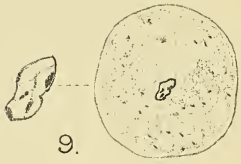

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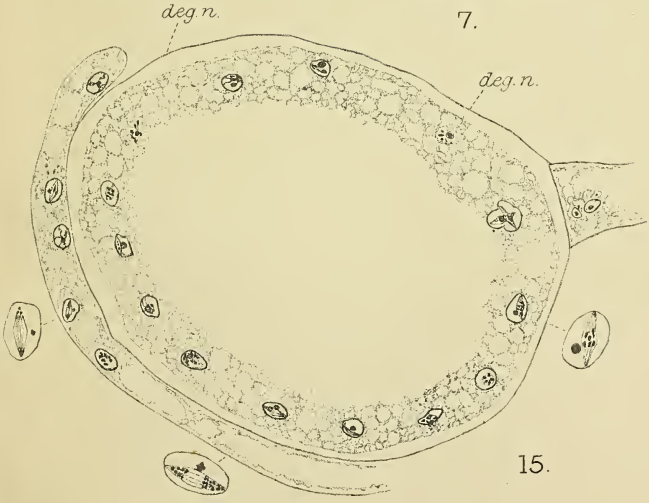

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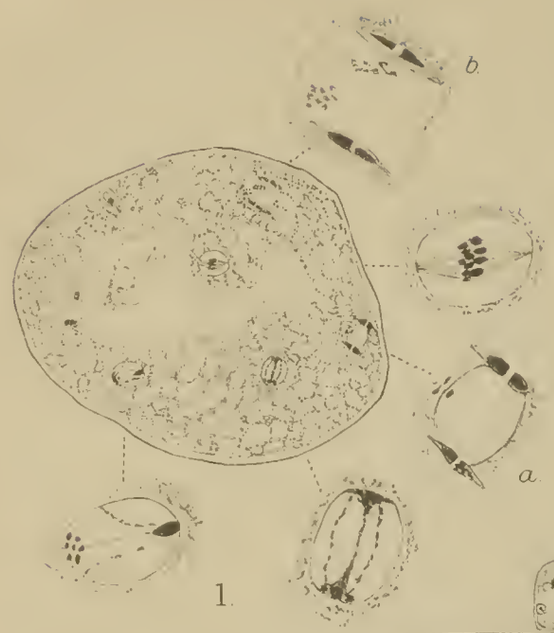

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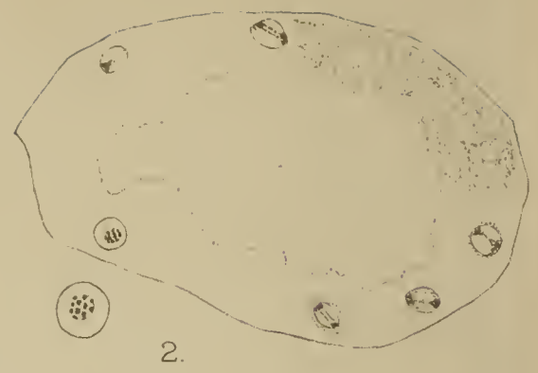

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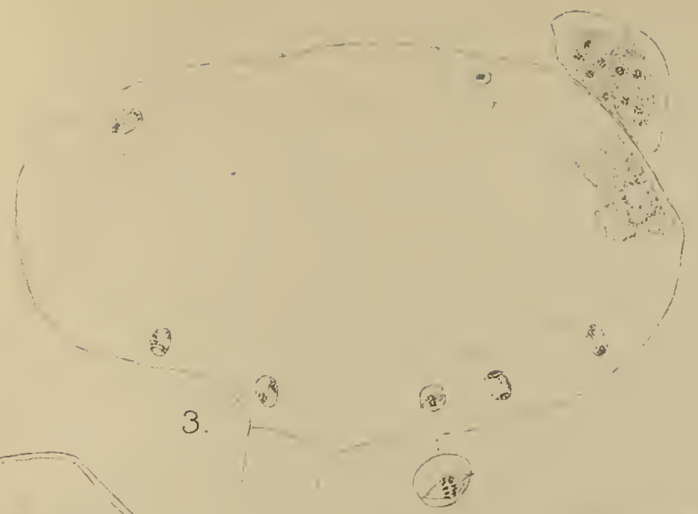

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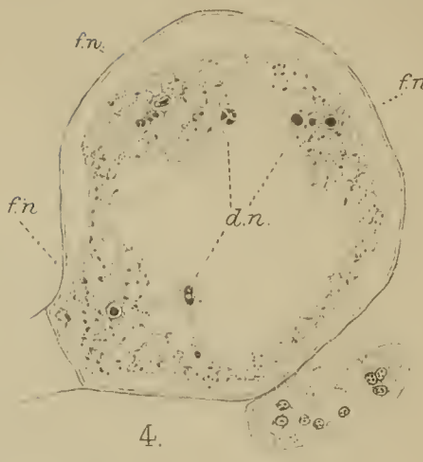

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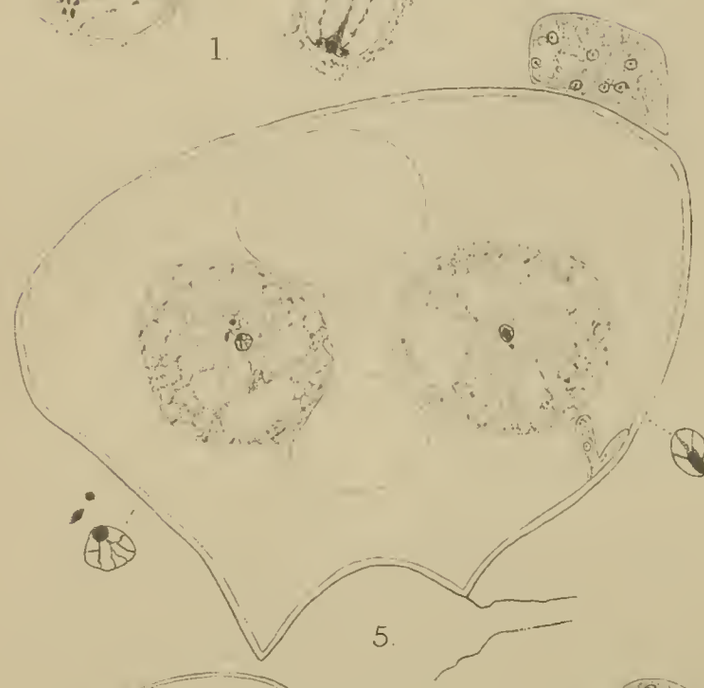

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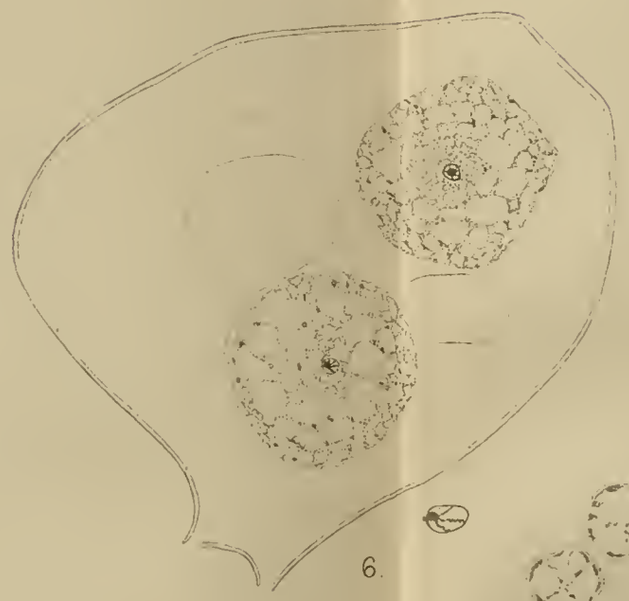

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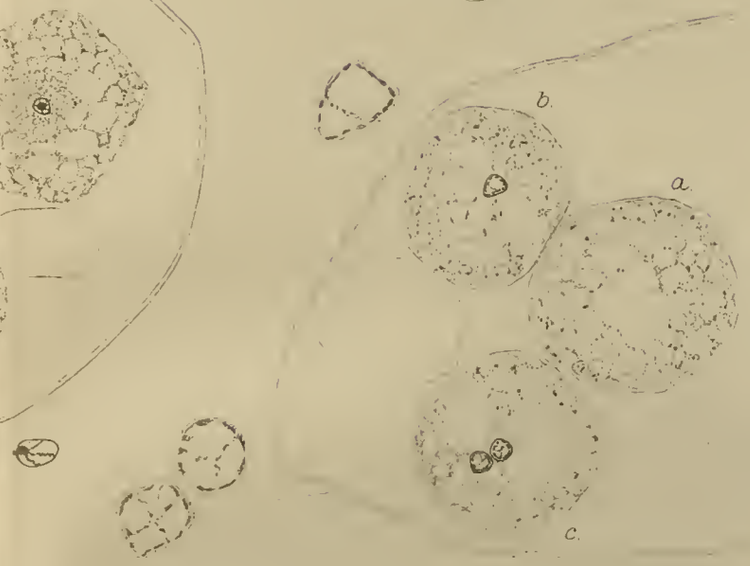

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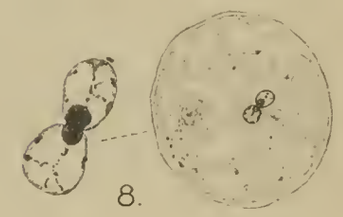

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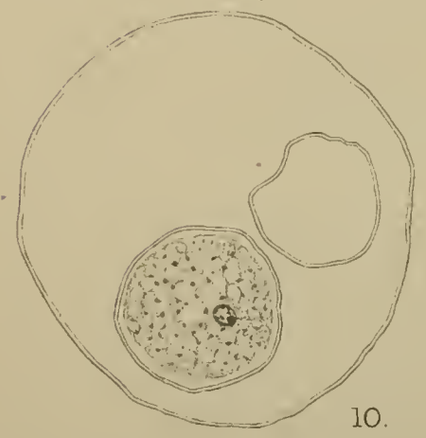

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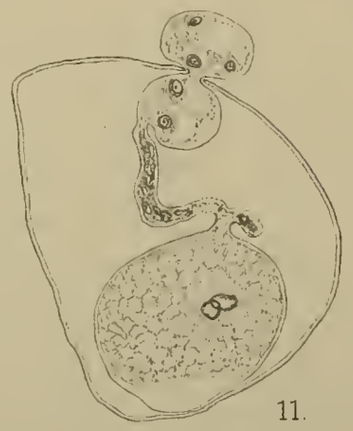

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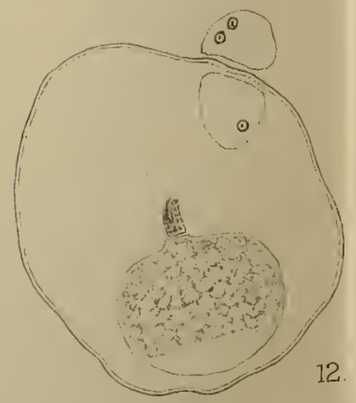

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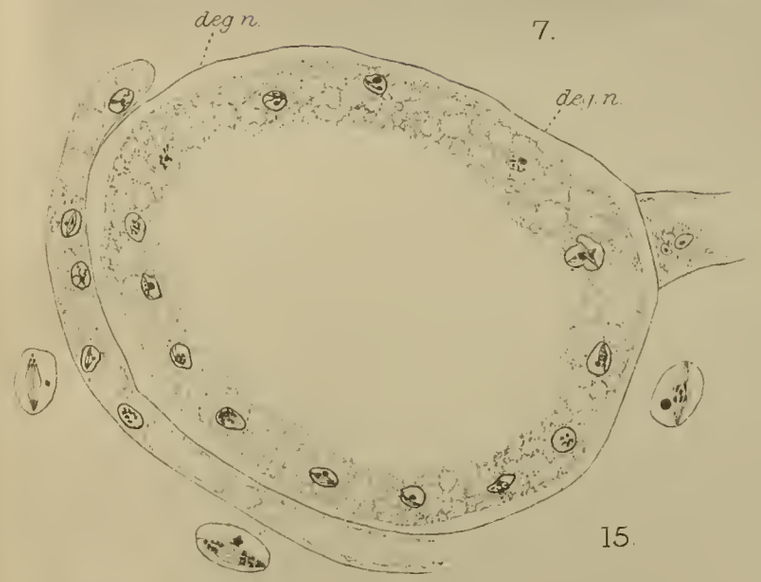

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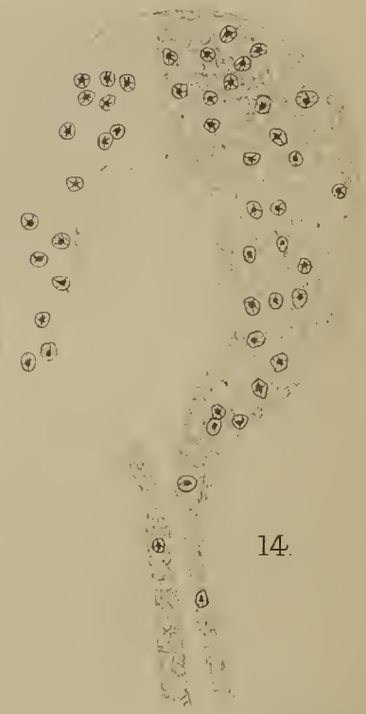
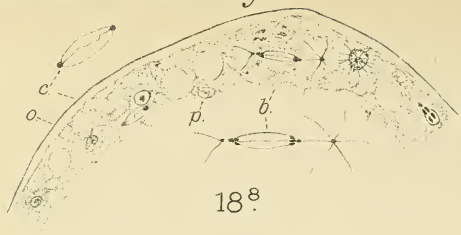


14.

A.H. Trow del.

Huth, lith. et imp.

TROW—SAPROLEGNIEÆ.



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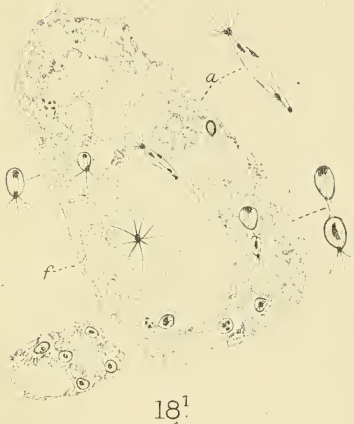

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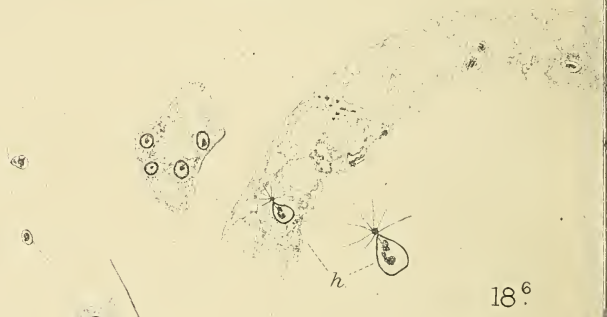

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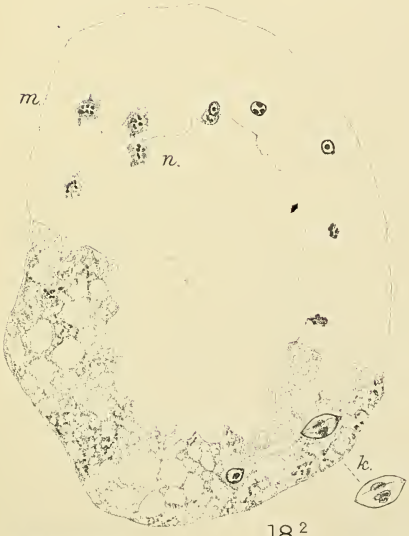

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18.9

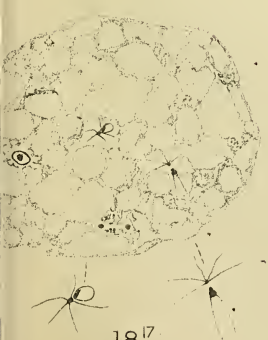
18.12

A.H.Trow del.

TROW—SAPROLEGNIEÆ.

17.

18.7



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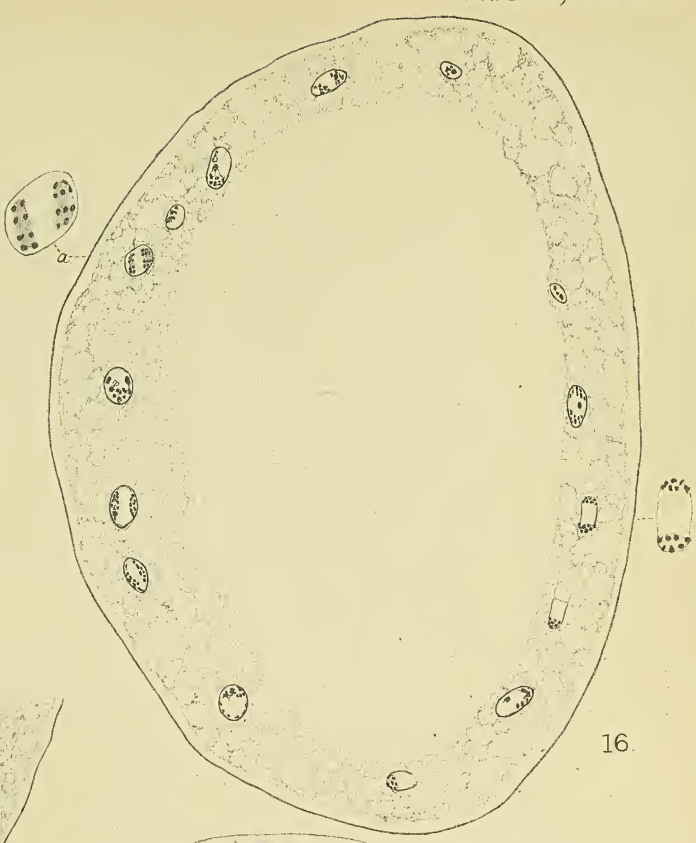

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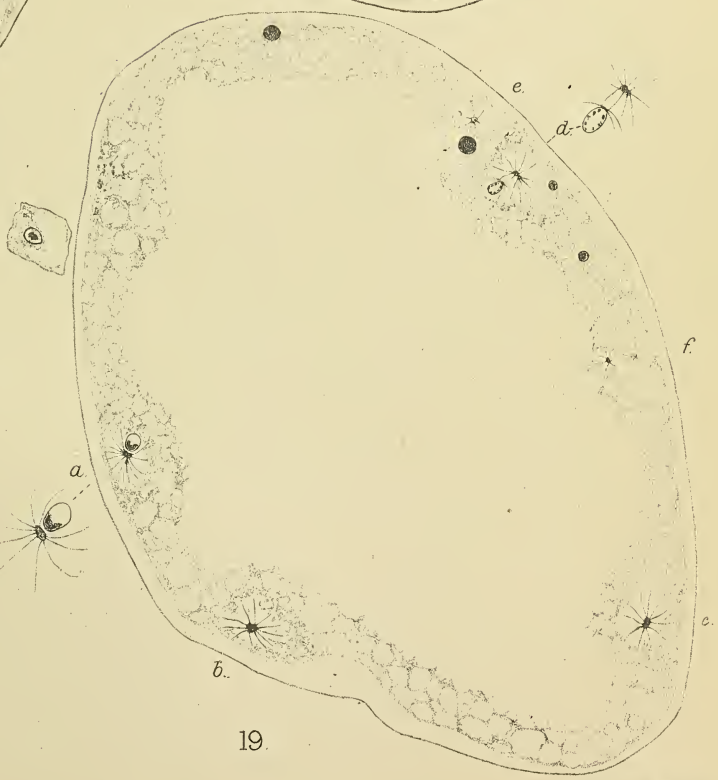

19.

Huth, lith et imp.

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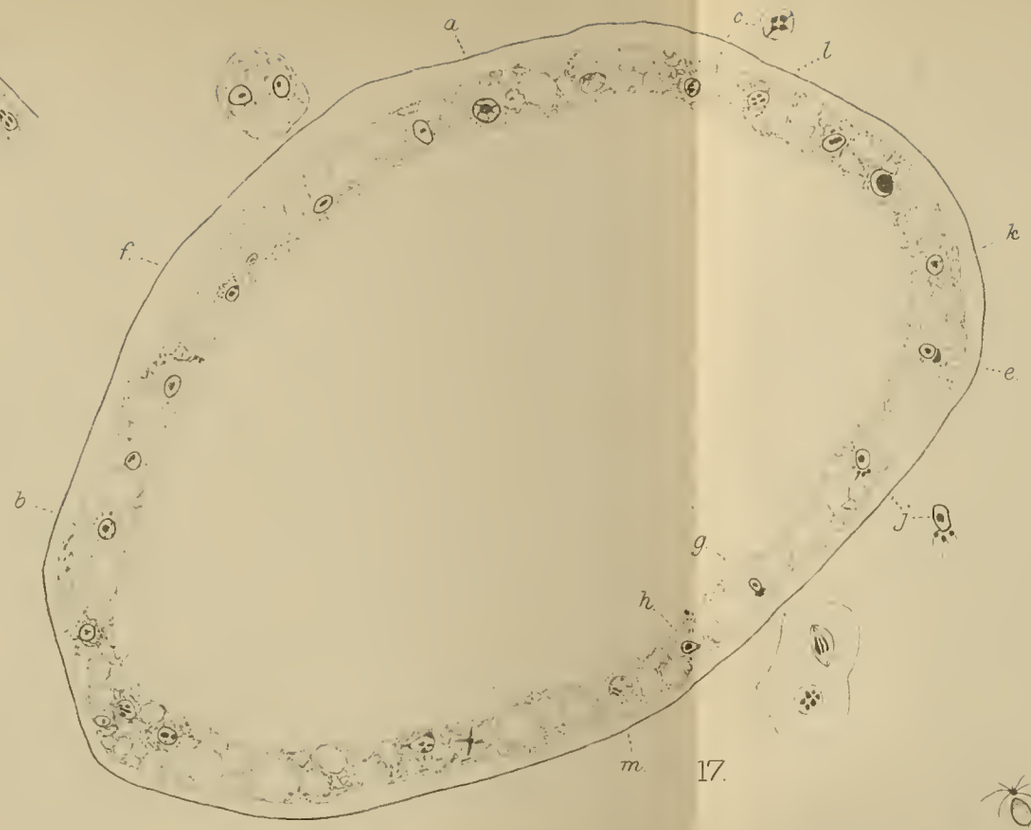

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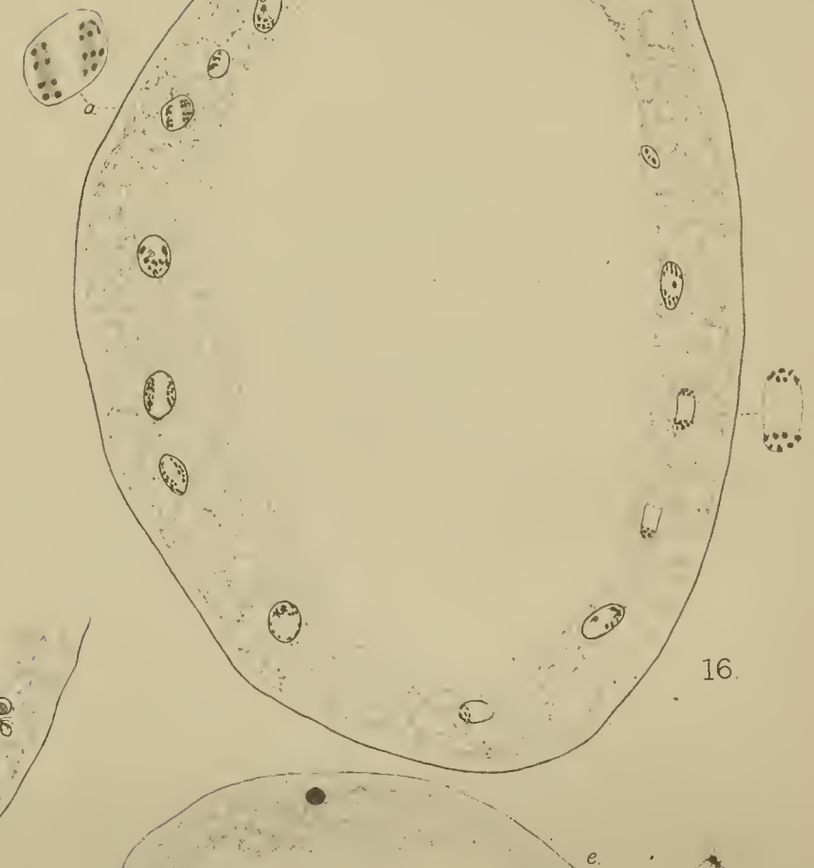

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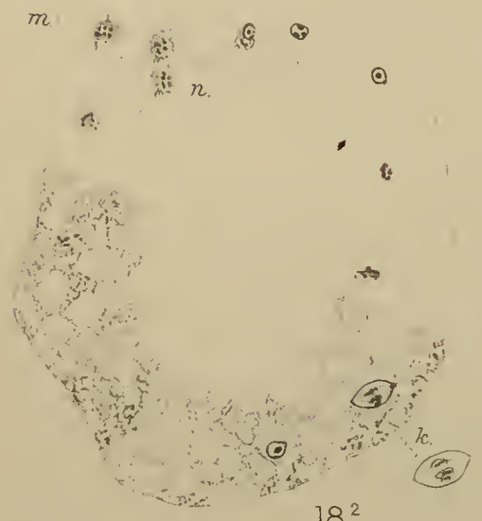

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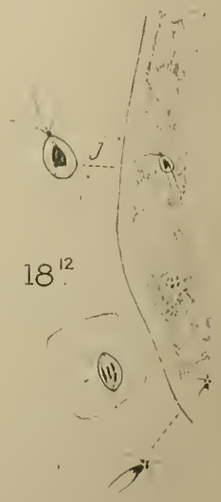

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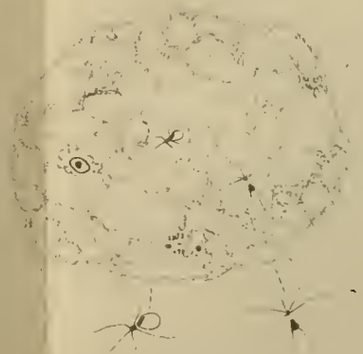

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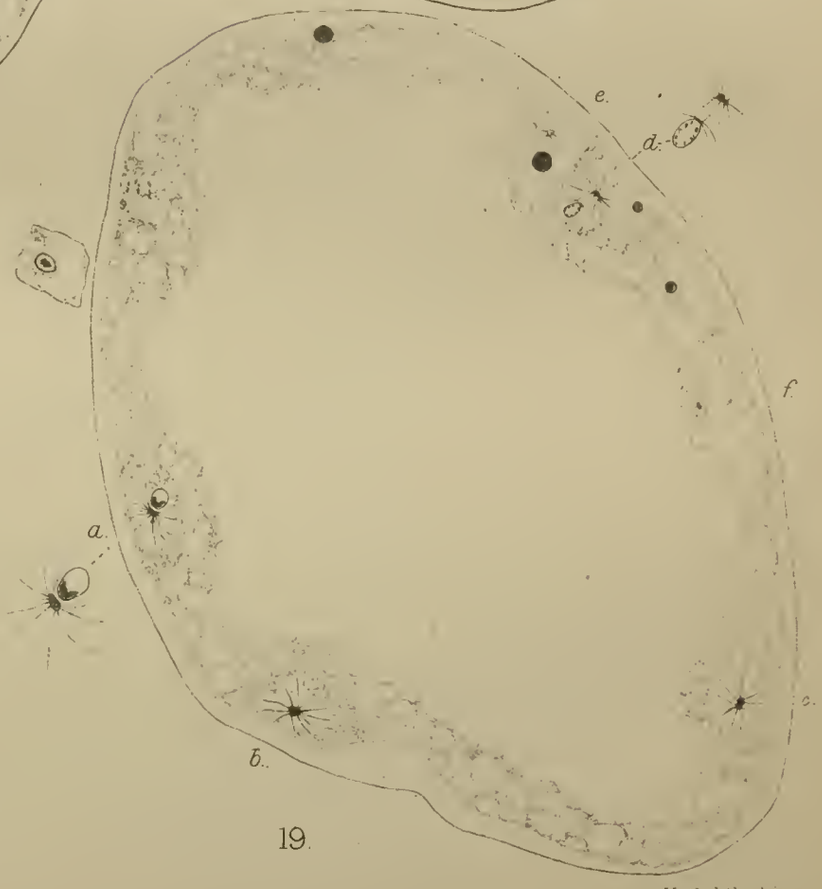
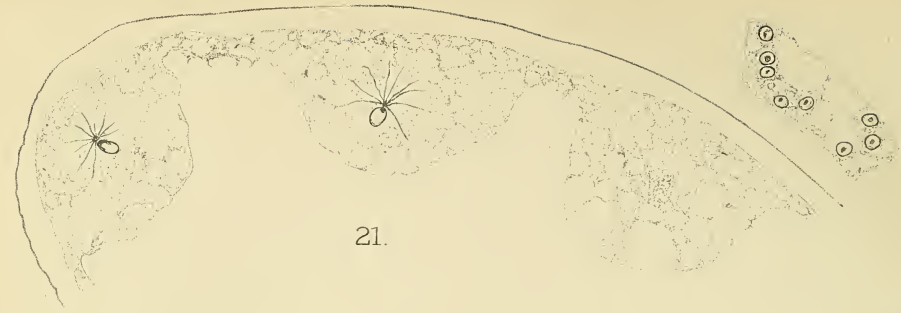


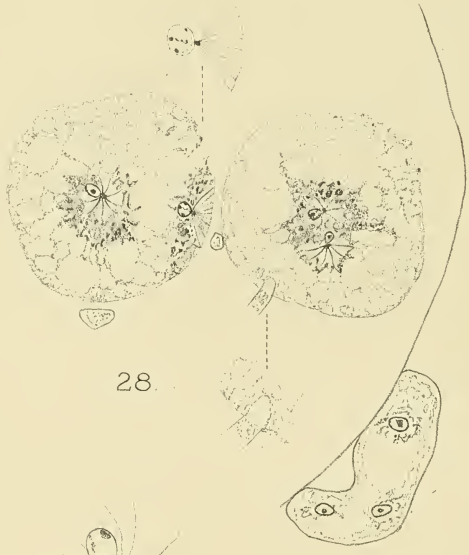
19.

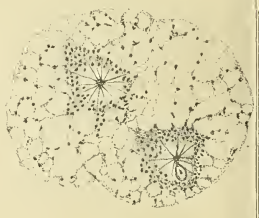
A. H. Trow del.

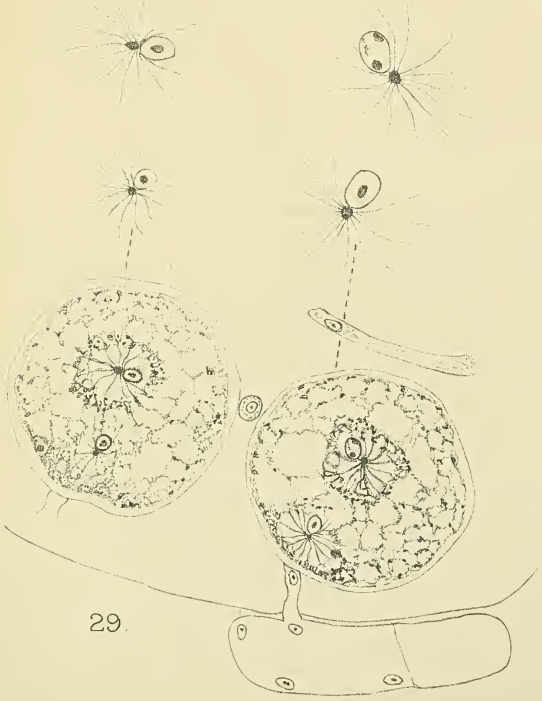
TROW—SAPROLEGNIEÆ.

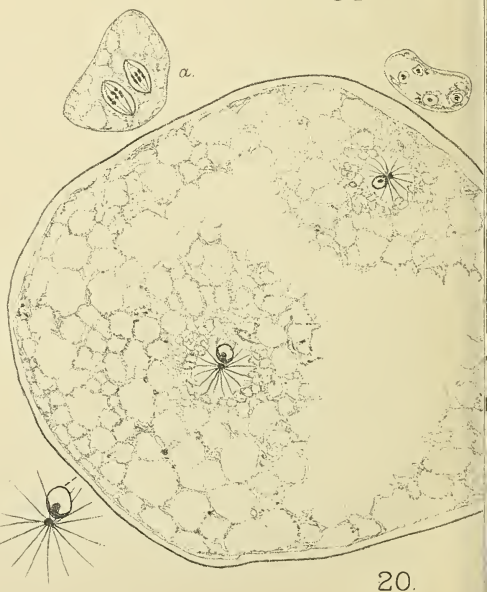
Huth, lith et imp





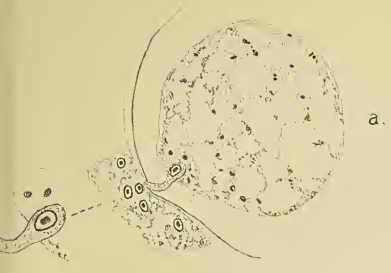






A. H. Trow del.

TROW – SAPROLEGNIEÆ.



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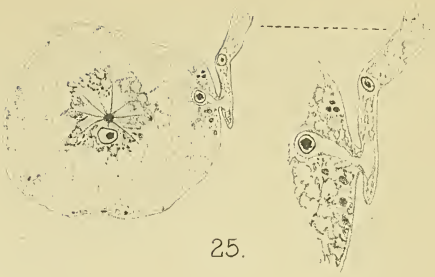

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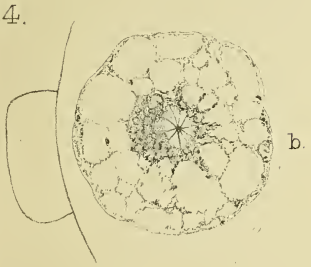

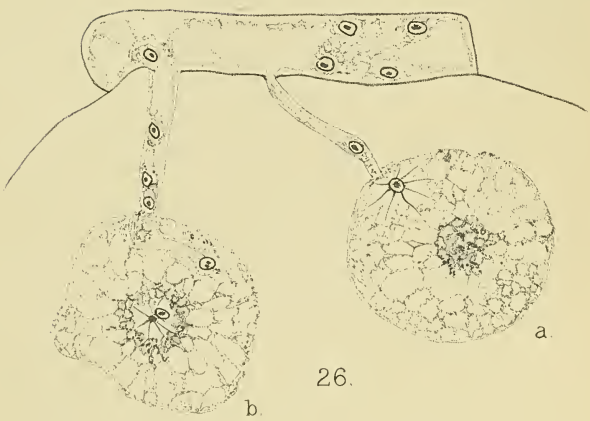

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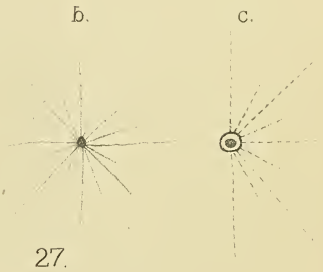

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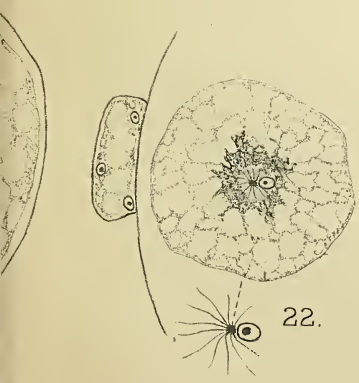

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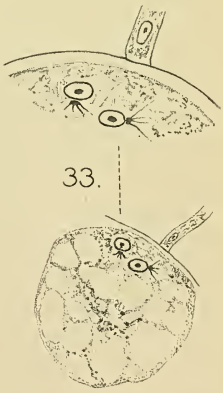

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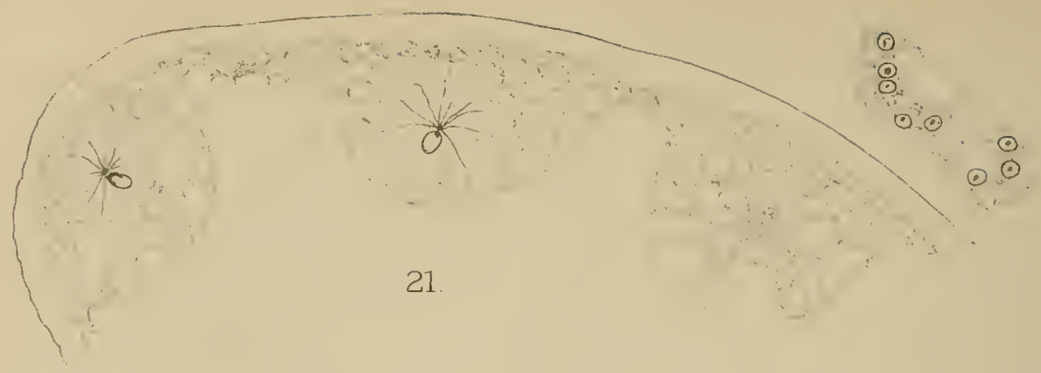

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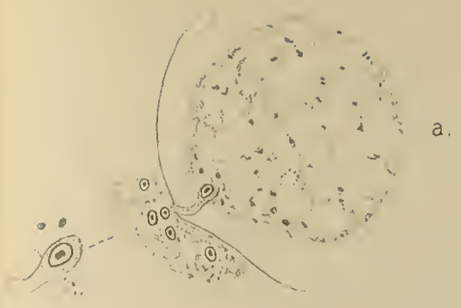

a.

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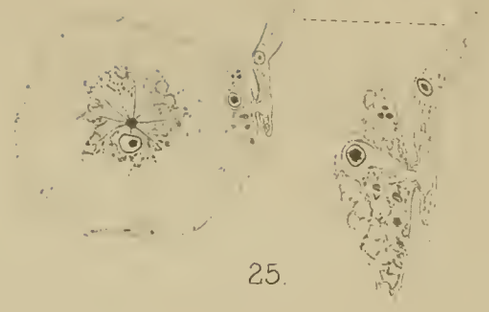

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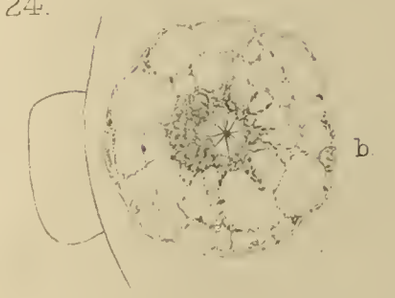
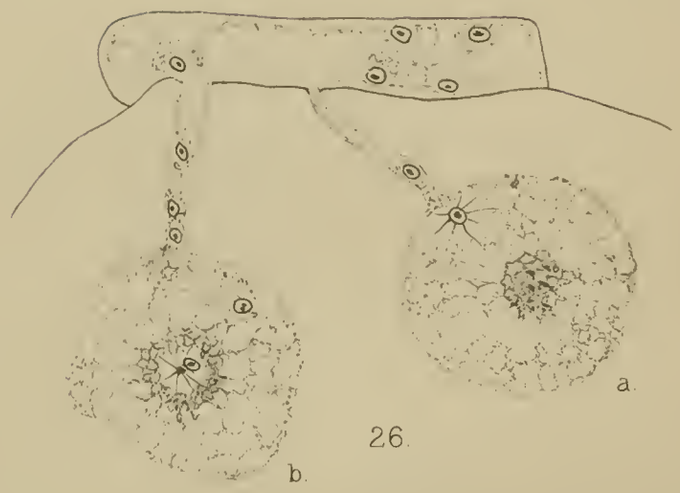


b



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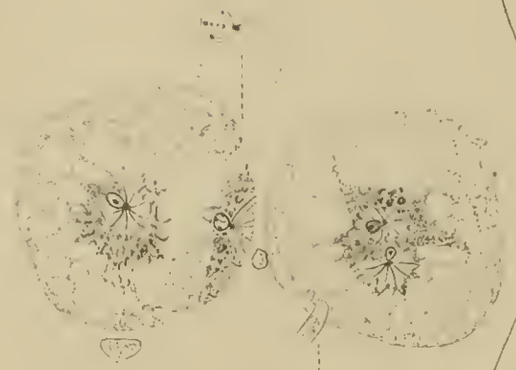
b.

26.

23.



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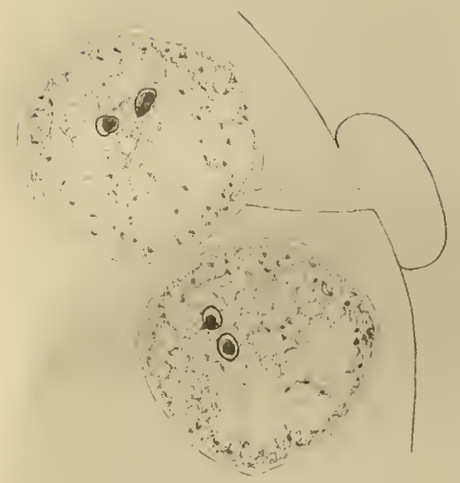

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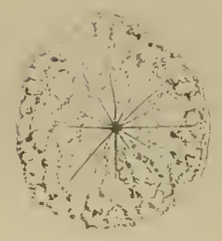
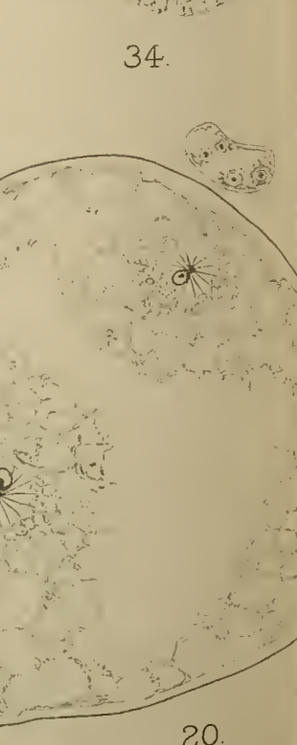


a.

b.

c.

27.



a.

20.

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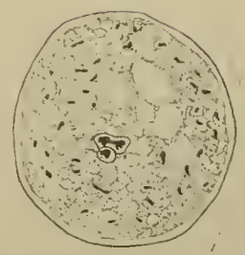

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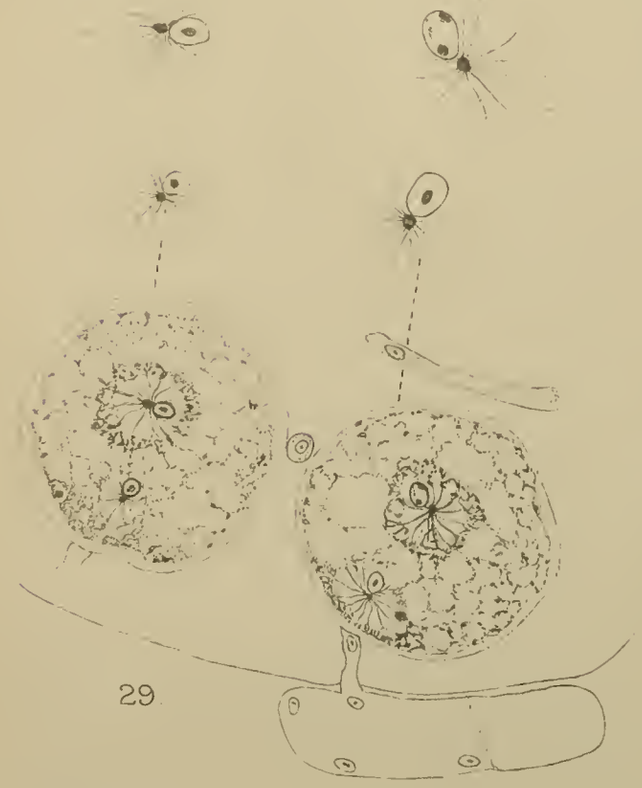


29.

A. H. Trow del.

TROW – SAPROLEGNIEÆ.

Huth, lith et imp

On a Prothallus provisionally referred to Psilotum.

BY

WILLIAM H. LANG, M.B., D.Sc.,

Lecturer in Botany at Queen Margaret College, University of Glasgow.

With Plate XXXVII.

THE only important group of the Pteridophyta in which the sexual generation is unknown is that of the Psilotaceae, comprising the two existing genera *Psilotum* and *Tmesipteris*. While travelling in localities in which *Psilotum* occurred, I spent considerable time in examining the neighbourhood of the plants met with in the hope of finding specimens of the prothallus. The sporophyte was found growing in various situations in a number of localities. In Ceylon it was once or twice seen growing in the soil below coco-nut trees near the coast, and very abundantly on the masses of roots which form the swollen base to the stems of these palms. In the mountain region it was seen growing as an epiphyte, its rhizome spreading through the humus accumulated in the fork of a tree, and occasionally growing on rocks. The observations made in Ceylon were without result; in most cases the start of any isolated portion of rhizome could be best accounted for by an origin from one of the gemmae borne in large numbers on the rhizome. In the localities I visited in the Malay Peninsula[1] *Psilotum* was not abundant, but in one of them, Maxwell's Hill in Perak, a number of plants were found growing on the stems of tree-ferns. The rhizome was embedded among the adventitious roots covering the stem of the tree-fern, while the aerial shoots projected more or less from the surface; they were not flattened, so that the species was presumably *P. triquetrum*. In close association with one of these plants the single prothallus, on the study of which the following description is based, was found. A preliminary account of its external form has already been published[2].

[1] The scientific expenses of my expedition to the Malayan Peninsula were met by a grant of the Royal Society.

[2] Proc. Roy. Soc., vol. lxviii, 1901, p. 405.

[Annals of Botany, Vol. XVIII. No. LXXII. October, 1904.]

The prothallus was almost certainly completely embedded among the adventitious roots, although, since some of the latter had been removed before it was noticed, this was not directly observed. Its general form will be evident from Figs. 1 and 2, which represent two views of the uninjured prothallus magnified seven times. Its natural size was about one quarter of an inch in length by three-sixteenths of an inch across at the upper end, which was the widest part. As the figures show, it was approximately cylindrical, agreeing in general form and symmetry with the prothalli of some species of *Lycopodium*. As in these, a lower vegetative region could be distinguished from an upper one, to which the sexual organs were confined. The vegetative region, which formed the larger portion of the prothallus, was of a brown colour and thickly clothed with rhizoids. Below, and somewhat to one side, it narrowed into a conical end, the relation of which to the general body of the prothallus at once suggests a comparison with the primary tubercle, which is more or less clearly distinguishable in most prothalli of *Lycopodium*. The upper portion of the prothallus consisted of a somewhat depressed central area and a thick overhanging margin, in which numerous antheridia were present. A comparison of Fig. 3, which represents a section through the whole prothallus, with Fig. 4, in which the overhanging margin is better seen, will make the relation of these parts clear without further description. It is sufficient to point out that, as in most types of *Lycopodium* prothalli, this prothallus is differentiated into a primary tubercle, a vegetative region, and a sexual region. There was clearly no apical growth, and, as will be shown below, the zone intervening between the vegetative and sexual regions must be regarded as the meristematic region. The fact that the sexual organs were confined to the margin of the sexual region indicates that the prothallus was a relatively old one. A comparison with prothalli of various ages of e. g. *Lycopodium clavatum*[1] makes the manner in which the existing relation of parts would follow from one in which the whole summit of the prothallus was covered with sexual organs perfectly clear.

There was no trace of the existence of any assimilating lobes such as occur among the sexual organs of *Lycopodium cernuum*. When fresh the upper portion of the prothallus had a faintly green tint, but examination on the spot did not reveal any chlorophyll corpuscles, nor could any be detected on more careful examination later. It is therefore probable that the prothallus was not exposed to light, and was incapable of independent assimilation.

The structure of the several regions of the prothallus must now be described in detail. The most convenient order in which to take them will be the sexual region, the vegetative region with the primary tubercle, and

[1] Bruchmann, Ueber die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien. Gotha, 1898. Cf. Taf. 3, Fig. 1, with the series of prothalli of various ages on Taf. 1.

the meristematic region. The symbiotic Fungus will be considered along with the vegetative region, in which it occurs.

The general relation of the sexual region to the rest of the prothallus has been described above. It only remains to point out that, as a comparison of the sections in Figs. 3 and 4 indicates, the thick margin was not equally developed all round. The former cut missed the overhanging edge on both sides, while the latter is taken through a place where it was well developed. The hypertrophied appearance of this edge was very striking; it was thrown into folds, and at places tears in its interior showed the strain which the development of the surface bearing the antheridia had caused. The antheridia originated in regular succession, the youngest being next the meristematic region, the position of which is marked with a cross in Fig. 4. From the few developmental stages observed it was clear that the antheridium originates in the same way as that of *Lycopodium*, the first division separating an outer cell, which forms the wall, from an inner one giving rise to the mass of spermatocytes. The outer wall of the mature antheridium (Figs. 5, 6) is one layer of cells thick, and is nearly level with the surface of the prothallus. Sometimes the antheridium was extended parallel to the surface as in those figured, while in other cases it was elongated at right angles to the surface. There were no hairs (paraphyses) growing from the surface among the sexual organs.

The vegetative region exhibited greater histological differentiation in relation to the presence within it of an endophytic Fungus, the definite distribution of which indicated its symbiotic nature. The central mass of tissue was entirely free from the Fungus, which was confined to a peripheral zone indicated by the shading on the cut surface of the prothallus in Fig. 3. The mycorhizal zone is continuous over the whole vegetative half of the prothallus (Figs. 3, 4, 8). On approaching the meristematic zone it narrows (Fig. 4), and in this situation the development of its constituent layers could be traced.

The relative position and appearance of these layers will be evident from the diagram in Fig. 8 and the slightly diagrammatic drawing in Fig. 7. The outermost layer (*a*) consisted of cells free from the Fungus, save for filaments passing across from the bases of the rhizoids and entering the cells of the deeper layers. The rhizoids are simple protrusions of cells of this layer, and most of them contained one or more fungal hyphae; whether these are to be regarded as passing inwards or outwards could not be determined, but the latter interpretation is more probable. Within the peripheral layer comes a zone of three or four layers of cells (*b*), the more external of which, like the superficial ones, were extended tangentially, while the inner cells have their longer axes at right angles to the surface, but do not form a distinct layer. These cells, each of which had a single healthy-looking nucleus in a central position, were filled with fine fungal hyphae,

which ran concentrically round the cell and almost completely obliterated the cell-cavity. So far as could be seen the hyphae were non-septate: they were very fine, and in well-stained specimens could be seen to contain numerous small nuclei. In a small proportion of the cells of this zone vesicular structures occurred in addition to the hyphae. They were relatively small and thin-walled, but otherwise resembled the vesicles, which are such a striking feature of the layer next to be described (*c*). This consisted of a single layer of long narrow cells standing at right angles to the surface. Fungal hyphae could in favourable preparations be demonstrated running between the cells in the thickened cell-wall, but the prominent feature of this layer was the presence of numerous oval vesicles, the position and general appearance of which are sufficiently shown in Fig. 7. The vesicles, which were intercellular, bulged out the septa in which they lay, and frequently obliterated the lumen of the cells on one or both sides. In suitable specimens it could be seen that they were borne on hyphae, but whether they were always terminal, as they appeared in all the cases observed, could not be determined. Close to the meristematic region they were thin-walled (Fig. 9, *a*), but in all the older regions the walls were thick and stained intensely with some dyes, e. g. safranin when used in combination with haematoxylin. The vesicles were filled with cytoplasm, no central vacuole being as a rule present; in most cases numerous small nuclei were evenly distributed through the cytoplasm (Fig. 9). In other cases the nuclei appeared to be aggregated in one or several groups, the rest of the cytoplasm being practically free from them. No signs of any further development was observed in the vesicles within the tissues of the prothallus. Internal to the layer of elongated cells is the parenchymatous tissue (*d*), which composes the central portion of the vegetative region of the prothallus. This tissue was entirely free from the endophyte, and its cells presented no characters which call for special note.

The arrangement of the various tissues in the conical base of the prothallus must be referred to in order to complete the description of the vegetative region. The diagram in Fig. 8, which is founded on a section passing through the middle of the projection, will make the matter clear. The region occupied by the Fungus is shaded, and the several layers are indicated by the same letters as in Fig. 7. It will be evident that in the upper portion of the conical projection the arrangement is exactly the same as has been described above. It is only at the extreme tip of the projection, the region which was first developed on germination, that a difference is found. There, as is the rule in the similar prothalli of *Lycopodium*, the endophytic Fungus inhabits the superficial cells as well as the layers beneath; the inner layers are also less regular here. Strictly speaking it is this tip only, and not the whole of the projection, that is comparable to

the primary tubercle. The state of things is much the same as in the prothallus of *Lycopodium clavatum*[1].

The zone intervening between the vegetative and sexual regions must be regarded as the meristem of the prothallus on account of the succession of antheridia in the tissues above and the gradual differentiation of the mycorhizal tissues below. But little or nothing in the character of the cells of this zone indicated their meristematic nature. Probably, as is the case also in the subterranean prothalli of *Lycopodium*, this may be placed in relation to the slowness of growth. Whether this is the true explanation or not, it was impossible to arrive at any conclusion as to the succession of divisions in the meristematic cells from the single specimen available for study.

Comparison with other prothalli, such as those of the Lycopodiaceae and Ophioglossaceae, justifies us in regarding the endophytic Fungus, the strictly limited distribution of which has been described above, as mycorhizal. The general differentiation of the tissues of the prothallus, the absence of chlorophyll, and the position in which the prothallus grew, all support the conclusion that it was a total saprophyte dependent in its nutrition upon the co-operation of the endophytic Fungus.

It now remains to inquire to what extent we are justified in ascribing this prothallus to *Psilotum*. Our knowledge of the characteristics of the gametophyte in the great groups of Vascular Cryptogams is sufficient to enable us at once to limit the inquiry as to the systematic position of this prothallus to the homosporous Lycopodiaceae and the Psilotaceae. The gametophyte is known in a considerable number of species of *Lycopodium* and in *Phylloglossum*, and I have previously discussed the several types occurring in the former genus[2]. The subsequent discovery of the prothallus of *Phylloglossum*[3], which appears to resemble most closely that of *Lycopodium cernuum*, though without the assimilatory lobes of the latter, confirms the view that this is the primitive type of prothallus in the group. In the paper cited I suggested several lines of adaptation to explain the various types of wholly saprophytic prothallus in the genus. A special type characteristic of the epiphytic forms (*L. Phlegmaria*, &c.) can be distinguished from the massive subterranean prothalli of a number of species normally growing in soil rich in humus. This latter type, as Bruchmann's[4] exhaustive work shows, presents various grades of specialization of the tissue containing the endophytic Fungus.

The prothallus under discussion, with its radial symmetry and its growth referable to a meristematic zone between the vegetative and sexual regions, is obviously constructed on the general plan traceable throughout the known prothalli of *Lycopodium*. In size and general appearance it

[1] Bruchmann, loc. cit.
[2] Annals of Botany, vol. xiii, 1899, p. 279.
[3] Thomas, Proc. Roy. Soc., vol. lxix, 1901, p. 285.
[4] Loc. cit.

approaches most closely to the wholly saprophytic subterranean type, and in the differentiation of its fungus-containing region is practically identical with *Lycopodium complanatum*[1]. It does not resemble closely any of the prothalli of tropical species hitherto described. Its form and structure would, however, be quite consistent with its belonging to some tropical species of *Lycopodium*, the life-history of which is at present unknown.

The only other possible position to assign to this prothallus is to regard it as belonging to *Psilotum*. Since direct evidence is lacking, we can only estimate the probability of this view indirectly. I shall therefore simply state in conclusion the reasons which incline me to provisionally assign this prothallus to *Psilotum* rather than to some species of *Lycopodium*. I recognize fully that no decisive weight can be attached to the arguments which follow, and that it must be left to future research to confirm or disprove my view.

In the first place, it appears to me that considerable weight may fairly be attached to the close association of the prothallus with a plant of *Psilotum*. It was found a few inches from this plant, embedded, as the rhizomes of the latter were, among the roots of the tree-fern. In my short stay in the locality I did not observe any species of *Lycopodium* growing in the same situation, though of course such may occur. My personal experience of searching for prothalli of *Lycopodium* would not lead me to expect those of any species to be so common as to be likely to turn up in situations not as a rule occupied by the sporophyte. At least I should regard the chances as being against a single prothallus found close to a plant of *Psilotum* being one of a species of *Lycopodium* sown from a distance. I incline to regard it as more probably related to the plant of *Psilotum* in its immediate neighbourhood, though I should hesitate to express an opinion as to whether it had sprung from a spore of this plant or whether the latter had originated from another prothallus sown at the same date. Our knowledge of the rate of growth alike of *Psilotum* and of saprophytic prothalli of this type is too imperfect to help us to come to a decision.

In the second place, the prothallus under consideration may, so far as generalizations are possible on the subject, be regarded as belonging to the wholly saprophytic subterranean type, such as that of *Lycopodium clavatum* or *L. complanatum*. It is remarkable to find such a prothallus in a situation to which a prothallus of the type of *L. Phlegmaria* would appear better adapted. But when the range of situation in which the plants of *Psilotum* are known to occur is taken into consideration this difficulty would admit of a satisfactory explanation. *Psilotum* is not an obligative epiphyte; it is known to grow in soil, and it would not be surprising if its gametophyte were of the subterranean type.

On these grounds I am disposed to regard it as probable that this

[1] Bruchmann, loc. cit., Taf. 5.

prothallus is really that of *Psilotum*. It will be of interest to see whether this assumption proves correct or not. In the meantime it would be obviously inadvisable to discuss the bearing of the type of prothallus on the question of the affinity of the Psilotaceae, further than to say that if this prothallus is proved to belong to *Psilotum*, it will lend support to the close association of the latter with the homosporous Lycopodiaceae.

EXPLANATION OF FIGURES IN PLATE XXXVII.

Illustrating Dr. Lang's paper on a Prothallus provisionally referred to *Psilotum*.

Fig. 1. The prothallus seen from the side. × 7.

Fig. 2. The prothallus seen from the side and from above. × 7.

Fig. 3. The prothallus halved in a plane parallel to but just avoiding the conical projection and viewed from the cut surface. × 7.

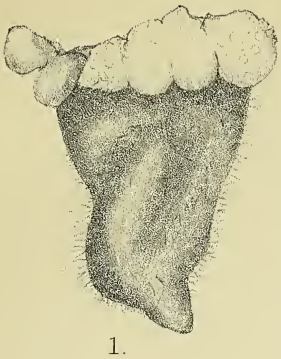
Fig. 4. Section through the junction of the vegetative and sexual regions, showing the protuberant edge of tissue bearing the antheridia; the cross indicates the position of the meristematic region. × 25.

Figs. 5, 6. Vertical sections through antheridia. × 375.

Fig. 7. Vertical section through the mycorhizal region, showing the arrangement of the layers of cells inhabited by the fungus. *a*. superficial layer, *b*. layers with intracellular hyphae, *c*. layer of elongated cells with intercellular hyphae and vesicles, *d*. parenchymatous tissue free from fungal hyphae. × 200.

Fig. 8. Diagrammatic section through the base of the prothallus and the primary tubercle, showing the distribution of the fungus. *a*. *b*. *c*. *d*., as in Fig. 7. × 25.

Fig. 9. Three of the multinucleate vesicles borne on the endophytic fungus. × 375.



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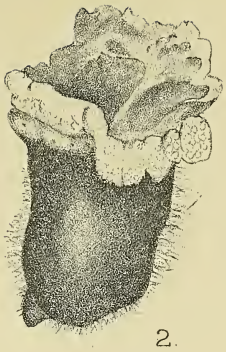

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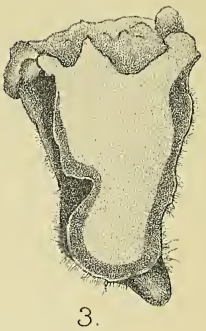

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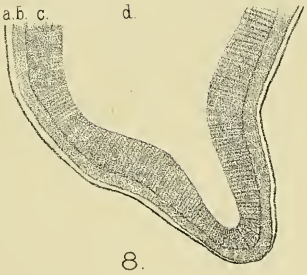

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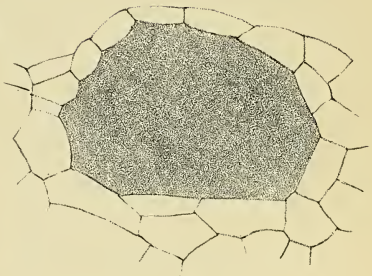

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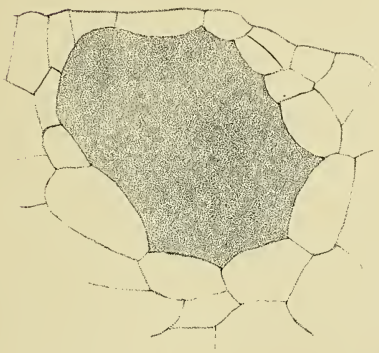

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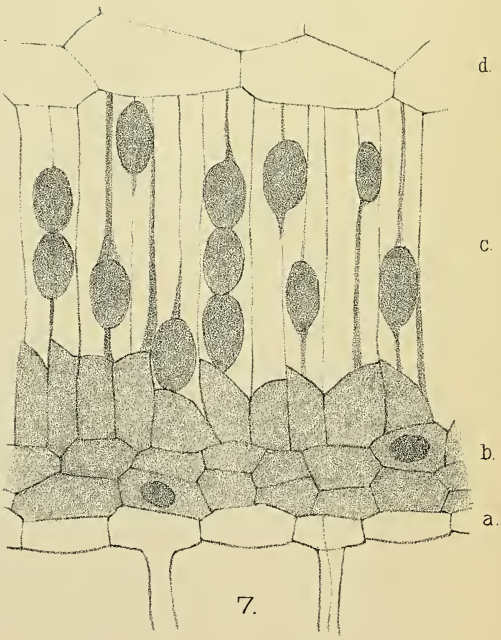

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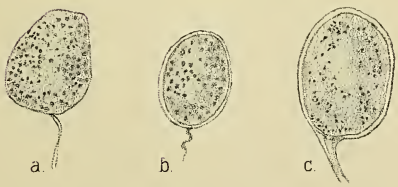

9

W.H.L. del. Huth, lith. et imp.

LANG — PSILOTUM.

Heterophylly in Proserpinaca palustris, L.

BY

GEORGE P. BURNS,

University of Michigan.

With Plate XXXVIII.

INDIVIDUALS of the same species growing under different conditions often show great variation in form and structure of their organs. These variations are, in some cases, very slight, but in others so marked that plants showing them have been taken for new species or at least varieties. Not only do we find great variety in form and structure of organs on separate individuals, but the same individual may form organs of very different structure at various stages of its development. These variations are in part caused by external conditions. Goebel (1) says: 'We know that external conditions may act as stimuli, but the influence of these depends upon the capacity of reaction of the individual plant.' The reactions of plants due to morphogenic stimuli have been studied for the most part on the leaves of amphibious plants. These vary in form and structure of the leaf. The leaf formed in and remaining in water is quite different from the leaf known as the 'land-type.' Many land plants also develop two kinds of leaves, and offer a very promising field for experiment, but so far very little has been done. Only in a few cases do we have any experimental knowledge of the stimuli causing the variations.

In working experimentally along this line two important points must be kept in mind. First, 'the capacity of reaction of the individual plant' toward external stimuli is not always the same. Goebel (2) cites numerous examples in which the same individual reacts differently in its juvenile and adult forms. Secondly, a given reaction is not necessarily the result of a single stimulus, but the same reaction may be produced by two or more stimuli. However desirable it may be to give a detailed analysis of all the possible factors which determine form, and to determine the influence of each component upon the protoplasm of the primordial cells, it seems that there is little possibility of accomplishing such an end.

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Two of the questions which arise in a study of variations in plant-organs are, in how far are they determined in form and structure by external stimuli? and, do these show a direct adaptation to the environment? This paper deals largely with the first question.

Most of the literature upon this subject deals primarily with the effect of external stimuli on the anatomical structure of the organs of amphibious plants. This side of the question is omitted in the present paper, although it is of great importance, because McCallum (3) promises a detailed account of the results of his experiments in the near future. For this reason the results of the work along this line will be briefly given.

Mer (4) refers the differences in structure to weakened illumination and poor nutrition. Constantin (5) finds that the aquatic form is due to poor vegetative conditions. Schenck (6) agrees on the whole with Mer.

On the other side of the question must be noted first the work of Goebel (7). In several cases he has shown that the form of the leaf is determined by external stimuli. This is true of *Sagittaria*, *Campanula rotundifolia*, and the Cacti. In these plants it depends upon the intensity of the light. In other cases, as *Ranunculus multifidus*, the form of leaf is dependent upon the water-environment to the extent, at least, that the leaves formed in water are more finely divided than those formed in air. However, in some cases, notably in the case of *Limnophylla heterophylla*, he could prove no direct relationship between the form of leaf and external stimuli. He thinks, however, that a direct causal relationship may have formerly existed even though it cannot now be proven (Org. p. 546).

McCallum (3) attempted to analyze more carefully the morphogenic factors working upon water-plants. From experimental work on *Proserpinaca palustris*, he concludes that the stimulus to the development of the water-form of leaf in this plant is not involved in the light-relations, in the nutritive conditions, temperature, the gaseous content of the water, nor contact-stimulus, but is due to the 'checking of transpiration and consequent increased amount of water in the protoplasm.' He continues, 'When the protoplasm of the primordial cells is in that condition of dilution which accompanies the absorption of a large amount of water, the nature of the growth and the orientation of the cell-division is such as to produce the water-form, while those physical and chemical conditions resulting from a partial withdrawal of water by evaporation (i.e. an increased density of protoplasm) result in that sort of cell-behaviour which produces the air-form of leaf.'

One other paper is that of Familler (8) on *Campanula rotundifolia*. He found that cuttings from stems of this plant, which were producing linear leaves, returned to the formation of round leaves. As we have seen, Goebel got the same reaction on this plant by decreasing the amount of light. Thus not only light, but a disturbance of vegetative activity—such

a disturbance is certainly made in the case of cuttings—may cause the same reaction in the plant named.

While engaged in an ecological study of certain parts of the Huron Valley, in the vicinity of Ann Arbor, my attention was repeatedly called to the great variations in the form of the leaves of the plants growing in the margins of the river, and especially to those of *Proserpinaca palustris*, *Ranunculus multifidus*, and *Ranunculus aquatilis*. The last two are familiar through the work of Goebel and others. *Proserpinaca* was first described by McCallum, who gives us a very satisfactory account of this plant, illustrated with some good photographs and drawings.

Proserpinaca palustris grows in great abundance in the vicinity of Ann Arbor, usually in water about 30 cm. deep. It may, however, grow in water one metre deep. At Dead Lake it grows in great abundance near the shore, and some plants are out of water during a large part of the summer. It is very easy of culture both on land and in water. The 'land-type' of leaf is lanceolate, from 3 to 5 cm. in length and 6 to 8 mm. broad. The margins are serrated (Pl. XXXVIII, Figs. 1 and 5). The 'water-type' of leaf is very different. It is finely divided, with a central rib and from three to five filamentous divisions on each side (Figs. 2 and 4). These divisions are almost round in cross-section.

A comparison of the development of the two types of leaves shows that they are exactly alike in form in the primordium, and that they continue to develop along the same lines for a comparatively long time, independent of external conditions. Thus, whatever factor or factors determine the type of leaf to be developed from a given primordium must come into play relatively late. Each type of leaf begins as a small protuberance on the side of the vegetation-point. Lobes soon appear on the margins in basipetal order. The leaf and each of the lobes ends in a peculiar gland-like structure which very soon reaches its full development. Thus far all leaves have the same history of development (Fig. 6). The final type of leaf formed depends upon the later growth of this primordium. If the growth is confined to the central portion, a broad leaf with serrated margins is the result (Fig. 5). On the other hand, if the growth is confined mostly to the ribs, a finely dissected leaf is produced (Fig. 4).

Development of the Seedling.

A large number of the seeds were gathered in October and divided into four lots of twenty seeds each. Those in lot 'a' were placed in a damp chamber between filter paper; those in lot 'b' were placed in soil under eight inches of water; those in lot 'c' were placed in two-inch pots; those in lot 'd' were planted in two-inch pots and then placed in a pail of water. The first three were left in the greenhouse under good conditions for

germination, the fourth was allowed to freeze and remain frozen during the winter.

In March of the following year seedlings began to develop in all four lots. Those in 'a' were transplanted to two-inch pots and kept in as saturated an atmosphere as it was possible to obtain. Lot 'd' was removed to the greenhouse. We have now seedlings growing in water, in air—under the same conditions as Geraniums—and in a saturated atmosphere. In every case, after the entire cotyledons, there appeared the 'water-type' of leaf. I cannot say how long this would continue, as my seedlings died when they had from eight to twelve leaves.

These experiments show that the young plant will produce a divided leaf regardless of external conditions as such.

Cuttings.

Reference has been made to the work of Familler on *Campanula rotundifolia*. Cuttings made from stems of this plant, which were producing linear leaves, reverted to the round form of leaf. To test this on *Proserpinaca* a large number of cuttings were made from that part of the plant growing in air and producing entire leaves. These cuttings were made on May 28, rooted in sand, and finally transplanted to two-inch pots. They were kept in the greenhouse in comparatively dry air. They all grew luxuriantly, producing entire leaves on the main stem, blossomed, and ripened fruit (Fig. 1). It would thus seem that this plant differed from *Campanula rotundifolia*. However, on August 12, these plants were divided into three groups. One was left in the same condition as before and served as a control, another set was placed in an aquarium under water, and cuttings were made from the third which were planted in earth in air. The control plants continued to produce entire leaves. Those in the aquarium did not all act alike. In some the vegetation-point of the main stem stopped producing leaves and side-branches developed, always with the 'water-type' of leaf. In other cases the plant on its main stem reverted to the production of the 'water-type' of leaf. But most interesting was the behaviour of the cuttings. These were left under exactly the same conditions of light, heat, &c., as the control plants, and yet they began immediately to produce the 'water-type' of leaf. Thus there is a stage in the development of *Proserpinaca* during which a reversion may be caused by propagating by cuttings.

A second set of cuttings was made from plants growing in water and producing the 'water-type' of leaf. These were started exactly as those in the first set. These cuttings were made in September. After transplanting to two-inch pots they were divided into three sets. One set was placed in an aquarium under water; another set was placed in 5 cm. of water, thus immersing the root-system only; the third set was placed

on the bench in ordinary conditions. All three sets were kept in the greenhouse. During the entire winter all of these plants produced the 'water-form' of leaf. None of them grew luxuriantly. The lobes on the leaves of the submerged plants were longer than those of the other two, but the type of leaf was the same. One fact observed was very interesting. The plants in the air did not grow erect, and those in the water seemed to grow aimlessly round, never projecting their vegetation-points above the surface, although the growth in length was more than sufficient.

Early in the spring the plants behaved quite differently. Those in the water projected above its surface and produced entire leaves; those not in water grew erect and they too produced the entire or 'land-type' of leaf (Fig. 2). In the late spring of the present year this change in behaviour took place about four weeks later than it did in the year previous, when we had an early spring. Had I been able to improve the vegetative conditions under which my plants were growing, I am confident the plants would have produced their entire leaves much earlier.

Cuttings made early in May, from stems producing the 'water-type' of leaf in water, begin to produce the 'land-type' of leaf when grown in air much earlier than the control plants left in water.

A number of cuttings were made from the water-form of *Ranunculus aquatilis*. These were rooted in sand and potted, the pots being out of water. They grew two years in the greenhouse, never growing erect, but forming a dense tuft on the earth in the flower-pot and rooting at every node. The water-form, on account of weakened illumination, stretches its internodes. None of the plants produced the three-cleft leaf 'opposite or in the immediate vicinity of the blossom.' The same finely divided 'water-leaf' was formed for two years in air, only differing from the leaf developed in water in the length of the lobes. None of my plants blossomed, but I found one plant growing in the air near the water's edge, which was producing flowers. This plant produced only the 'water-type' of leaf.

A comparison of the cross-sections of lobes from leaves developed in water and those developed in air shows marked differences in anatomical structure. The leaf of the latter is dorsiventral. The epidermal system is well developed both in regard to the thickness of the cuticula and the absence of chlorophyll-bodies in the epidermal cells. The mesophyll is differentiated into palisade and spongy parenchyma. The fibro-vascular bundle contains from six to ten well-developed vessels. Stomata are present. The leaf of the water-form is finely divided into cylindrical lobes whose tissues are little differentiated. The cuticula is very thin, and the epidermal cells contain fully as much chlorophyll as any cell in the mesophyll. The cells of the mesophyll are not differentiated. There is a marked reduction in the number of vessels found in the bundles. Only a few stomata are found, and these were not fully developed.

Returning to the cuttings (p. 582) which were made in May from *Proserpinaca* plants producing entire leaves, it will be remembered that they continued to produce entire leaves, blossomed, and in many cases produced fruit (Fig. 1). Early in September it was noticed that the main stem did not grow so rapidly and that several side-shoots developed at the surface of the pot. Here we meet a most interesting picture in leaf-formation. The main stem is orthotropic and produces the 'land-type' of leaf, while the side-stems are plagiotropic and produce the 'water-type' of leaf. Both vegetation-points are under the same external conditions, as far as they could be controlled, and yet what a marked difference in the leaf formed! Again a little later the main stem undergoes a change. It ceases growing erect and grows horizontally, and the vegetation-point, which since May has been producing the 'land-type' of leaf, begins to produce, in air, the 'water-type' of leaf (Fig. 5). This experiment finds its exact reproduction in nature and is of the greatest biological importance. As has been pointed out, *Proserpinaca*, during the summer, sends its branches out of water, producing entire leaves, flowers, and fruit. In the fall the main stem becomes plagiotropic, produces divided leaves, and finally sinks into the water. Here it passes the winter protected by the water, and in the late fall and early spring multiplies rapidly by the growth of axillary buds. It must be noted that this return to the water-form is not caused by the water-stimulus. I have fastened many plants so that the vegetation-point could not possibly be closer than 30 cm. to the water, and yet these plants returned to the formation of the 'water-type' of leaf.

In June of the present year the vegetation-point was removed from a number of plants producing entire leaves. This caused the lateral branches to develop. In many cases the leaves on these were more or less divided, although the plant grew in air under the same conditions as many other *Proserpinaca* plants, all of which were producing entire leaves (Fig. 3).

Thus we see that the presence of water is not necessary for the development of the 'water-leaf.'

On the other hand, *Proserpinaca palustris* may produce entire leaves in spite of a water-environment. In the spring I always found a number of plants producing the 'land-type' of leaf under 24 to 30 cm. of water. This I found only on rapidly growing branches. In June of the present year, in a large area at Dead Lake, I found that almost every plant was producing the 'land-type' of leaf, although dozens of them were growing under as much as 30 cm. of water (Fig. 4). This certainly points to other factors than water or checked transpiration as the cause of the division.

In Fig. 4 attention is called to the two leaves *a* and *b*. This tip was growing under 26 cm. of water. These two leaves show a transition from the one type to the other; they are 'water-leaves' at the tip and 'land-

leaves' at the base. A similar example is pictured by Goebel (9) in the case of *Ranunculus aquatilis*.

I will not give in detail a description of my experiments with such factors as light, temperature, nutrition, gaseous content of the water, &c., but only refer the reader to the work of McCallum, whose results are essentially the same as my own, especially agreeing with the results I obtained from experiments conducted in the winter.

I repeated the experiments cited by McCallum on p. 107 of his paper. In the fall of 1902 I set up the following: Cuttings were made from plants producing the divided leaf. They were divided into four sets; one set of plants was placed in a nutrient solution not quite strong enough to plasmolyze them; the second set was placed in a weak solution of potassium chloride; the third was placed in pots and cultivated as land plants; the fourth was placed in battery jars under about 18 cm. of water. In every case the leaf-form which developed was divided, although I allowed the solutions to become stronger by evaporation. In the early spring I repeated the experiments not only on *Proserpinaca*, but also on other plants, and my results were different. This time the plants in solution of potassium chloride and the nutrient solution showed a tendency to produce more entire leaves, at least on some stems. However, about the same time my control plants, growing in tap water, also began to show the same tendency, as did also the plants growing in air. As the control plants as well as those in the solutions showed the same tendency, it hardly seems possible that these salts either directly or indirectly could be the cause of the production of the entire leaf.

This phenomenon was explained by McCallum (p. 108) thus: 'It would seem here as if some of the plants after a time become accustomed to the stimulus and refuse to respond. Or it may be that as only the air-form is capable of fruiting, in the effort to produce flowers the plant has the ability of self-adjustment to its conditions and develops the air-form in spite of its environments.' . . . 'It is possible also that the protoplasm is able to adjust itself, perhaps by the expulsion of water, into that condition in which it exists when in air.'

Reference has been made to the biological importance of the change in the direction of growth of the stem which was observed in the fall. An effort was made to find the cause of this change. Why are all stems orthotropic in summer and plagiotropic in winter? Is it a different response on the part of the plant at different stages of its development; or, is it due to a change in external conditions? The facts observed in the field-work point to temperature as a controlling factor, but the work in the greenhouse excludes it as such. A solution of the question was finally reached in some experiments set up to study the effect of weakened illumination on leaf-formation. Two plants, both plagiotropic, one growing in air and

the other in water, were placed in the dark room. Within a few hours all the side-branches as well as the main stem were growing erect. The vegetation-point of stems growing in water soon appeared above the surface. This experiment was repeated with a large number of plants, and it was found that light was the determining factor. When light was removed the stems responded to the geotropic stimulus and turned up. When the plants were returned to the light the curves were straightened and the branches continued to grow horizontally.

The answer to our question seems clear. At one stage of its development *Proserpinaca* is positively heliotropic and at another it is diaheliotropic. That is, this plant changes its relation to light at different stages of its development. Furthermore, we find that the production of the 'water-type' of leaf is intimately connected with that stage when it is diaheliotropic; the 'land-type' of leaf with that stage when it is positively heliotropic.

Finally, we find that the primitive form of leaf is always the 'water-type'; that side-branches developing in the air from a plant whose main stem is producing entire leaves develop the 'water-type'; that all stems, regardless of all external conditions which I could control, produce the 'water-type' in the fall; that stems whose vegetation-points were removed in June threw out side-branches with the 'water-type' when other plants under the same external conditions were forming the 'land-type.' On the other hand, we find that at the time of flowering only entire leaves are formed; that in summer almost every plant, whether in water or in air, produces the 'land-type' of leaf; that the change from 'water-' to 'land-type' takes place earlier on strongly growing than on weak stems, and is dependent to a certain degree on external stimuli; that the plant in its positive heliotropic stage forms the 'land-type' of leaf, and in its diaheliotropic stage the 'water-type.'

These results do not point to one or more definite factors which will explain the form of the leaf in *Proserpinaca palustris*. It is evident the water-environment is not the cause of the division of the leaf. Nor does it depend upon light, temperature, gaseous content of the water, or contact-stimulus as such. The only conclusion that seems justified by my experiments seems to be that *Proserpinaca palustris* has two forms—an adult form and a juvenile form (10). Under good vegetative conditions it has a tendency to produce the adult form with the entire leaf, blossom, and fruit; under poor vegetative conditions it has a tendency to produce the juvenile form with the divided leaf. And furthermore, a reversion to the primitive form may be caused by unfavourably influencing the vegetative conditions.

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EXPLANATION OF FIGURES IN PLATE XXXVIII.

Illustrating Dr. Burns's paper on *Proserpinaca palustris*.

Fig. 1. Typical land plant, bearing fruit.

Fig. 2. This plant produced divided leaves in air during the winter, and is beginning to become orthotropic and to produce entire leaves. May 10, 1903.

Fig. 3. Explanation in text.

Fig. 4. Tip of stem found growing under 26 cm. of water. Leaves of 'a' and 'b' show transition forms. Collected in June, 1903.

Fig. 5. Photograph of plant started in the spring from a cutting. Taken November, 1902.

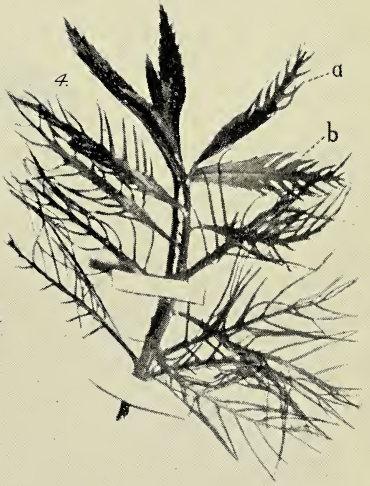
Fig. 6. Figure showing oldest stage in the development which is common to all 'types' of leaves. Magnified 65 times.


1.


5.


3.


2.


4.
a
b


6.

The Anatomy of Psilotum triquetrum.

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With Plate XXXIX.

THE genus *Psilotum* is represented by four species, *P. triquetrum*, *P. flaccidum*, *P. complanatum*, and *P. capillare*. Baker [1], however, gives only two distinct species, *P. triquetrum* and *P. complanatum*, regarding *P. capillare* as merely a variety of *P. triquetrum*, and, similarly, *P. flaccidum* of *P. complanatum*. Of these, *P. triquetrum* appears to be the most frequent in occurrence, being reported from practically all the warmer regions of the world [2]. The plant is found growing in the humus at the base of trees, amongst the roots of tree-ferns, and in the crevices of rocks. It is generally regarded as an epiphyte; but as young plants which had developed from bulbils, and also portions of the underground rhizome which had been broken off from the main plant, have been known to vegetate underground for a considerable length of time [3], the plant may also be regarded as being saprophytic in nature. A small, but well-developed, specimen grew for some time in the soil amongst the roots of *Norantea guianensis* in one of the tropical houses at the Cambridge Botanic Garden. It was unknown how the plant originally came there [4].

External Morphology.

The plant consists of a much-branched, green, aerial stem bearing the sporangia and small reduced leaves, and a brown underground rhizome, which is likewise much branched. There are no roots.

[1] Baker ('87), p. 30. Willis ('97), p. 314.

[2] Spring ('49), p. 269, *et seq.* Bertrand ('81), p. 257. [3] Solms-Laubach ('84), p. 141.

[4] Mr. Lynch tells me it is probably due to small pieces of the rhizome being broken off during potting, and one of these may have been unintentionally mixed with the soil in the pot of the Norantea.

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The Aerial Stem.

The aerial stem forms externally the most noticeable part of a *Psilotum* plant. At its base, where it issues from the soil, it is smooth, brown, and circular in outline, but higher up it gradually becomes green in colour, and the surface is conspicuously ribbed. Branching is apparently dichotomous, the first bifurcation occurring as a rule near the base of the stem, and is repeated many times. Here and there trichotomy may occur, not only in the lowest portion of the stem, as Solms-Laubach [1] states, but also at the second, third, or fourth level of forking. Prantl [2] states that the branching of *Psilotum* cannot be regarded as dichotomous, for one of the branches arises in the axil of a leaf, whilst the other continues the growth and the phyllotaxis of the main shoot. In the case of *P. flaccidum*, however, in which the leaf-insertion is much clearer than in *P. triquetrum*, and is represented by the fraction $\frac{1}{2}$, Solms-Laubach [3] has shown that the position of the leaves is not altogether regular in regard to the branching, whilst the phyllotaxis of the two branches points to dichotomy of the main shoot. As a rule, in *P. flaccidum* a leaf is found below each bifurcation. The first few leaves belonging to the two resulting branches continue the distichous arrangement as if no forking had occurred; thus, the first of these leaves is found on the outer side of the branch which is furthest away from the leaf below the bifurcation, whilst the second leaf is immediately above the latter and on the nearer branch. Higher up each branch assumes the usual distichous arrangement. It may, however, occasionally happen that the leaf usually found below the bifurcation is carried up, and is therefore situated on one of the two resulting branches. Judging from external appearances only, the branching would seem to be, as a rule, a regular dichotomy, although at times, especially towards the apex, branches of unequal size may be found. The question, however, will be referred to later in discussing the internal anatomy.

Leaves are present as small, scale-like structures. The phyllotaxis cannot be represented by a fraction which is constant throughout the plant. As Solms-Laubach [4] has pointed out, in the smaller, three-angled branches near the apex the leaf-insertion is easily ascertained, and is clearly represented by the fraction $\frac{1}{3}$; but in lower and stouter regions of the plant, owing to a certain extent to the twisting of the stem which often occurs, the leaf arrangement is much less clear and cannot be definitely or satisfactorily determined.

The sporangia occur, as a rule, on the later-formed branches, but they may be found on older branches near the base of the stem. The sporangial

[1] Solms-Laubach ('84), p. 163.
[2] Prantl ('72), p. 92.
[3] Solms-Laubach ('84), p. 165. Pl. XXIII, Figs. 10 and 11.
[4] Solms-Laubach, loc. cit., p. 164.

leaves are bi-lobed, a feature which makes it easy to locate even very young sporangia. The sporangia themselves are for the most part trilocular, but bilocular ones are occasionally found.

The Rhizome.

The much-branched rhizome of *Psilotum* bears no leaves or roots. In texture it is much softer than the aerial stem, and the surface is generally covered with fine, brown hairs, though these may be better developed in some regions than in others. The apices of the branches are white and naked, and the whole rhizome forms a somewhat confused mass of branches, which cannot be disentangled without breaking. Small circular, or oval, white spots occur at intervals on the branches ; these are destitute of hairs, and may be sunk or slightly raised above the surface. They represent dormant lateral buds which are capable of resuming growth under favourable conditions.

Here and there some of the rhizome-branches assume a vertical, instead of a horizontal, position, and grow upwards towards the surface of the soil ; these are future aerial branches. As they develop, the superficial hairs disappear and the surface becomes smooth and hard, and small scale-like leaves shortly make their appearance.

Before passing to the internal anatomy, reference must be made to Professor Bertrand's[1] detailed account of *Psilotum*. In regard to the external structure, Professor Bertrand has described the aerial stem under the heads of 'cladodes "souches,"' 'cladodes of the first, second, third, &c. order,' 'terminal and sporangiferous cladodes,' and 'simple, aerial branches,' the last being only occasionally found. Similarly, the underground portion has been subdivided into 'simple subterranean branches' and 'sympodia' of these, and 'subterranean cladodes' and 'sympodia of cladodes[2].' These structures have not the same morphological value. The 'simple branches' are to be considered as such, whilst the 'cladodes' and 'sympodia of cladodes' are to be regarded as consisting of two or more stems which are united throughout the greater part of their course. At the apex, however, their real nature may be detected, this being shown by the presence of two or more 'centres of growth' being always found, each of which may possess a distinct apical cell. The first elements of the xylem-strand to be differentiated below the apex consist of two or more separate groups of spiral tracheids, each group corresponding to an apical 'centre of growth.'

[1] Bertrand ('81), p. 252, *et seq.*

[2] It is of course open to question whether the term 'cladode' can rightly be used in regard to the rhizome ; cf. Goebel's definition of the term, viz. 'a branch consisting of one internode counterfeiting a leaf.' The branches of the rhizome of *Psilotum* are brown, and destitute of chlorophyll ; they fulfil the functions of roots, and have not the slightest resemblance to leaves.

In the case of the aerial stem the branching, as a rule, is fairly regular, and it might for some purposes be convenient to term the series of bifurcations as branches of the first, second, third, &c. order. Taking Professor Bertrand's sub-divisions as a whole, there is nevertheless some doubt whether such detailed and often confusing distinctions are satisfactory. In regard, however, to the rhizome of *Psilotum*, it must be stated that the above sub-divisions [1] have not been confirmed, although it may possibly be due to less well-developed material. The branching in the rhizome is as a rule very irregular, although here and there dichotomy may be found. In other cases, judging by the external appearance, ordinary lateral branches are found; these are generally smaller than the main branch from which they are given off, or again they may remain as dormant lateral buds, which may develop under suitable conditions.

Seeing that the apices of the branches are naked, they are in consequence easily injured. When this occurs, microscopical, as well as superficial, investigation shows that the apex itself is black and discoloured, whilst one of the lateral buds lying in its neighbourhood frequently exhibits distinct signs of renewed activity.

The question as to the presence of Professor Bertrand's centres of growth, as shown by the existence of the apical cells and the distinct protoxylem-groups, will be referred to later in dealing with the anatomy of the plant.

Anatomy.

The Aerial Stem.

Psilotum possesses a single central stele (Pl. XXXIX, Fig. 1). The stem is ribbed in the stouter parts of the plant; towards the apex it is triangular in outline. The epidermis is cuticularised, and stomata are present in fairly large numbers lying in the grooves of the stem between the ridges (Fig. 1, *st*). The guard-cells are slightly sunk below the level of the other epidermal cells, and the outer walls are cuticularised. The cortex is composed of three zones, an outer, which is parenchymatous and assimilating, a middle sclerenchymatous, and an inner parenchymatous surrounding the stele. The outer parenchymatous zone (Fig. 1, *pa*) is composed of peculiar cells, the structure of which is best seen in longitudinal sections (Fig. 3). The lateral walls bulge out at regular intervals, each cell having 2–4 such swellings. The swellings of adjacent cells touch each other, and in consequence a conspicuous row of intercellular spaces is formed. The cells themselves are nucleated and contain chlorophyll. The sclerenchymatous zone (Fig. 1, *sd*) consists of thick-walled fibrous elements with pointed ends, and the walls are perforated by small slit-like pits. Towards the centre this sclerenchymatous zone passes gradually into the inner

[1] Solms-Laubach ('84), p. 158.

parenchymatous region (Fig. 1, *p'a'*) immediately surrounding the stele. The cell-walls of this tissue are perforated by numerous oval or circular pits, which vary in size. Starch is generally found in the cells, and in the lower and older parts of the plant conspicuous nodules of varying shape and size (Fig. 1, *n*) are also present. These nodules are unaffected by acetic and nitric acids, and the fact that they are left as an insoluble residue after several days' maceration and boiling in Schulze's macerating fluid suggests the presence of silica.

The endodermis, which Bertrand describes as a badly defined layer, is however clearly recognizable, after suitable treatment or after staining, when the thickenings on the radial walls stand out very distinctly (Fig. 2).

The centre of the stele is occupied by a group of sclerenchymatous fibres (Fig. 1, *f*); the walls of these have numerous, small, simple pits. In the stouter parts of the plant the fibres form a conspicuous group; higher up the number gradually decreases until only two or three, and finally none at all, are present. The xylem surrounds the central group of fibres with a star-like outline, the projecting points marking the position of the protoxylem-groups, the number of which varies with the size and region of the stem. In stouter branches nine or ten may be found, in the small apical branches two or three only may be present. In the latter case the xylem may be present as a small central strand, or it may form two small masses lying close together, each containing one group of protoxylem. The xylem is made up of ordinary scalariform tracheids, the protoxylem of spiral elements.

Between the endodermis and the xylem a mass of tissue is found which is very difficult to differentiate. Seen in transverse section, there is no clearly defined pericycle, the tissue consisting of a mass of parenchymatous elements of varying size. Treatment with aniline chloride gives signs of lignification[1] in some of the cells, especially at the corners; and this is more noticeable in the tissue lying outside the projecting tips of protoxylem (Fig. 2). In longitudinal sections the main mass of the tissue is seen to be composed of elongated parenchymatous cells (Fig. 4, *pa*) with conspicuous nuclei. Staining with chlor-zinc-iodide shows that the walls of these cells are covered with numerous small oval or circular pits or pores. Besides the nucleated parenchyma, long tube-like elements (Fig. 4, *st*) occur singly, or occasionally one or two together, which may possibly be regarded as sieve-tubes. These tube-like elements are frequently crowded with granules which stain yellow with chlor-zinc-iodide. With Delafield's haematoxylin the walls stain a deeper blue than in the case of the ordinary parenchymatous cells, and scattered globular masses are found of varying size (Fig. 4, *m*) which stain very deeply. These are probably of the same nature as the

[1] Vaughan Jennings and Hall state that in *Tmesipteris* they failed to find lignification of the phloem, but the material at my disposal showed it far more noticeably and clearly than in *Psilotum*.

refractive spherules described by Poirault[1] as occurring in the sieve-tubes of many ferns. No nuclei could be found in these tubes, and no trace of callus could be demonstrated by treatment with coralline soda or with a watery solution of aniline blue. The phloem, or phloem tissue, is not stained blue by iodine as in the case of *Lycopodium*. Owing to the fact that no definite pores or sieve-plates have been observed in this tissue, it is of course open to question whether in *Psilotum* the term sieve-tube can be applied. It has been likewise impossible to locate any definite tissue corresponding to the protophloem, although Russow[2] in 1872 figured and described such elements as forming regular groups alternating with the protoxylem masses. Later, however, he modified his earlier view, stating that the protophloem (defining this as the first of the sieve tissue to be differentiated) consisted of numerous groups, each of which was composed of two or three elements lying at the periphery of the vascular strand.

Bifurcation of the stem is very simple (Fig. 1). The stele widens horizontally and new protoxylem-groups make their appearance (Fig. 1, *p'x'*). The central mass of fibres with the surrounding xylem splits into two approximately equal halves, and new tracheids arise so that each of the two resulting groups of fibres are surrounded as before by xylem, though for a short time they touch the phloem tissue directly. The two strands become further apart, the endodermis then breaks and closes up round each. This process is repeated at each level of bifurcation.

Unlike *Tmesipteris*, in which each leaf receives a small vascular strand from the stem, the leaves of *Psilotum* have no vascular bundle.

In regard to the region in which the sporangia occur, each sporangiophore receives a small vascular strand from the main stem. In stouter branches this strand is much the smaller, but towards the apex, where the stem branches are slighter, it is frequently equal in size to the main stele left in the stem.

The Leaf.

The leaf of *Psilotum* has a very simple structure (Fig. 5), the outermost parenchymatous zone and the epidermis of the stem alone being continuous with the tissues of the leaf. Externally the leaf is covered by a typical epidermis with cuticularised outer walls, but no stomata are present. The centre of the leaf is occupied by parenchymatous tissue; near the apex this consists of elongated, nucleated cells, which, lower down, gradually pass into the parenchyma of the stem with its characteristic intercellular spaces.

The Apex of the Aerial Stem.

The apex of an aerial branch of *Psilotum* ends in a conical prominence, at the summit of which a three-sided apical cell[3] is found. In the smaller

[1] Poirault ('93), p. 139. [2] Russow ('72), Taf. XI, Fig. 30; also ('75), pp. 20 and 40.
[3] Strasburger ascribes an apical cell only to the underground stem. Solms-Laubach has

branches the whole apex is surrounded by two or three foliage leaves, which serve as a protection, but it is easily recognized in longitudinal sections. In the stouter shoots, especially in those bearing the branches with fertile leaves and sporangia in all stages of development, the apex is much hidden. It is often difficult to distinguish from the leaves, each of which arises as an outgrowth of the apical tissues, and at an early stage may show considerable resemblance to the stem apex. The presence of an apical cell in the young leaf, however, cannot be stated as constant, though at times it appears to be present. Professor Bower [1] has also pointed out that it is often impossible to say at an early date whether a leaf will be a fertile one or not.

The apical cell of the stem (Figs. 6 and 7) has a large nucleus and granular protoplasmic contents. It has been termed by Professor Bertrand [2] a 'dermatogen' apical cell; but the subsequent divisions in the cells which are cut off from it do not appear to bear out this statement, other layers being derived from these divisions besides the dermatogen itself. It has not, however, been possible to trace the connexion between the cells cut off from the apical cell and the developing stele. Each cell which is cut off from the apex appears to divide by a tangential wall into an inner and outer cell (Fig. 7), and the inner again divides so that three result, an outer, middle, and an inner. A vertical wall also arises at an early stage, but it has not been possible to determine satisfactorily any of the subsequent divisions. The outermost of the three cells resulting from the tangential divisions forms one of the cells of the dermatogen layer, which passes over into the epidermis of the stem.

The stele in the stem branches ends in an apical meristem of undifferentiated elements with conspicuous nuclei. Between this meristem and the apex itself is a mass of tissue, the product of the subsequent divisions of the segments cut off from the apical cell; the tissue is composed of irregular cells with comparatively large nuclei.

In regard to the dichotomy of the aerial shoot it is difficult to make any definite statement. As Solms-Laubach [3] has pointed out, the behaviour of the apical cell, if present, is an important factor in such a question. In dichotomy the single apical cell may on the one hand divide medianly into two, or again it may disappear altogether before bifurcation occurs, two new apical cells being differentiated in the two resulting branches. The nature of the apparent dichotomy would be open to doubt if it was shown that the single original apical cell continued to exist as the apical cell of one of the branches, a new one being cut off laterally for the second branch. It has been impossible to say definitely what occurs in *Psilotum*. The

confirmed this view, and is inclined to agree with Strasburger in regard to the absence of a distinct apical cell in the aerial stem, although he finds one to be present in the fruiting branches.

[1] Bower ('94), pp. 42 and 49; also Juranyi ('71), p. 177.

[2] Bertrand ('81), p. 441, *et seq.*

[3] Solms-Laubach ('84), p. 170.

apical cell has never been observed dividing into two. On the other hand, radial sections of bifurcating branches have never shown an apical cell to be present at the summit of either branch, although we have seen that it is generally found in older ones. From this we might then conclude that a new apical cell arises later in each resulting branch in place of the original single one which was present below the level of forking.

The Subterranean Stem.

In accordance with his definitions of the external structure, Professor Bertrand has described in some detail the anatomy of the rhizome of *Psilotum*. The structure of a simple branch and of the various 'cladodes' is given, and more or less hard and fast rules laid down as to the appearance presented by the stele in each sub-division. As already stated in this paper, it has been impossible to distinguish satisfactorily these various underground structures from their external appearance; and investigation of the anatomy, moreover, has not led in any way to their confirmation. The structure of the stele depends, for the most part, on the size and development of the branch in question, and not on the position it occupies in regard to the main mass of the rhizome. According to Bertrand, the typical structure of the stele in a simple branch consists of a diarch band of xylem, with spiral protoxylem-elements at the two extremities of the band. The present investigations have not confirmed this as being in any way constant or typical. The xylem-strand varies considerably in size and shape (Figs. 8, 9 and 10); in smaller branches two or three tracheids alone may be found, in stouter and more mature regions of the rhizome an irregular mass (Fig. 8) or a band-shaped strand [1], or again a central group of xylem more or less circular in outline may be found. The tracheids are scalariform, with no parenchyma interposed, and the spiral protoxylem elements of Bertrand appear to be generally absent. Both in transverse sections and in series of longitudinal sections cut on a microtome no trace of spiral or annular elements are often found, the first elements to be differentiated at the apex being as a rule of the ordinary scalariform type [2]. These results therefore agree with the earlier researches of Russow [3], who came to a similar conclusion in regard to the absence of typical spiral or annular elements in the protoxylem.

A similar absence of typical protoxylem also occurs in the rhizomes of *Loxsoma*, and of some species of *Schizaea* and *Anemia* [4].

Figs. 10 and 11 represent the xylem of a branch of a rhizome below

[1] See also Boodle ('04), p. 500, Fig. 46.

[2] In some cases transverse sections have given one or two tracheids of smaller size than the rest, which might be taken for protoxylem. Further investigation of sections belonging to the same series have almost invariably shown that these elements merely represent the narrow pointed ends of scalariform tracheids of ordinary size.

[3] Russow ('75), p. 21.

[4] Gwynne Vaughan ('01), p. 79; Boodle ('01), pp. 375, 381.

the level of bifurcation. In neither case is the bifurcation quite equal. Fig. 10 shows a fully developed and fairly stout branch, whilst the section given in Fig. 11 was taken a little below the apex of a rhizome branch. The main stem has only three tracheids lignified ; whilst the smaller branch, which in this case ended in a dormant lateral bud, contains but two lignified elements separated from each other by parenchyma.

Surrounding the xylem is the phloem tissue, which is similar in general respects to that found in the aerial stem, but is less well developed. The so-called sieve-tubes and nucleated parenchymatous cells are present, but the pitting on the walls is less noticeable, and there is practically no lignification of the sieve-tubes. The endodermis has its usual features, although not so easily distinguishable as in the aerial stem, and the thickenings often extend to the tangential walls (Fig. 8 *a*).

The main mass of the cortex is composed of large thin-walled cells containing starch. The two or three layers, however, lying next to the endodermis, have their walls coloured a dark brown ; the coloration being due, according to Bertrand, to 'gélification' and 'humifaction' of the cell-wall. Investigation of this brown substance, however, shows that, whilst unchanged by the action of acids, it is slightly soluble in eau-de-javelle, but dissolves in potash, after which the cell-walls give the usual blue coloration on treatment with chlor-zinc-iodide. It is probably of the same nature as that described by other writers[1] as occurring in Ferns, and which has been termed 'phlobaphene.'

Many of the cells of this outer thin-walled cortex are crowded with filaments of the Fungus which occurs so noticeably in the rhizome of *Psilotum* and *Tmesipteris*. The filaments in some cases form a thick mass in the centre of the cell, in which it is impossible to distinguish individual threads ; in other cells, in which the mycelium is actively living, the coils of threads are loose, and by staining with alum and Haidenhain's haematoxylin it is possible with an oil immersion lens to make out distinct nuclei. Globular or pear-shaped swellings occur often very abundantly on the mycelial threads ; as many as thirteen were counted on one occasion in one cell alone. The walls of these structures, which according to Bernatsky[2] are aborted sporangia or 'sporangoïds,' also show nuclei with Haidenhain's haematoxylin. Bernatsky also states that he has obtained successful cultures of this Fungus, which he ranks as *Hypomyces*.

The cells of the outermost cortical layer grow out into absorptive hairs. Each hair is composed of two cells, a basal cell and the main mass of the hair itself. The walls are coloured brown.

[1] Poirault ('93), p. 127. Boodle ('01), p. 361. Yapp ('02), p. 194. [2] Bernatsky ('99).

The Apex of the Subterranean Stem.

The apex of an underground branch of *Psilotum* is terminated, as in the aerial branches, by a three-sided apical cell. The walls, too, arising in the segments cut off from this apex appear to be similar to those in the aerial part of the plant; and again no connexion has been traced between the apical meristem, in which the stelar tissue ends, and the apical cell itself.

The first xylem tissue to be differentiated and lignified appears to be often a single, ordinary, scalariform tracheid (Fig. 12); but in other cases two tracheids may appear at approximately the same time, lying either side by side, or separated from each other by one or more parenchymatous cells.

The presence of one of Professor Bertrand's 'cladodes' is said to be marked by more than one 'centre of growth' being found at the apex, each of which has a distinct apical cell. The apical cells in question may lie in the same or different planes. It is certainly true that towards the apex of a branch the lateral buds may often be present in large numbers, and these may either grow out directly into ordinary branches, or again may remain dormant, becoming more widely separated from each other by the subsequent increase in length of the branch. Transverse and longitudinal sections cut on a microtome do not, however, seem to show at any time that more than one apical cell is present at the actual apex itself, although a somewhat lateral cell may at times be found belonging to a dormant bud. It is often impossible to detect an apical cell or cells in a branch which is about to bifurcate, although after forking these may make their appearance in the two resulting branches. This may point, as in the case of the aerial stem, to the fact that the cells in question are developed secondarily from the apical meristem of their respective branches. In no case has an apical cell been observed which is dividing medianly into two, this fact agreeing with the investigations of Solms-Laubach[1].

The Intermediate Region.

This region includes the base of any aerial stem, from the level at which the external appearance gradually changes from the green ribbed surface to the smooth brown exterior, down to the point of insertion of this vertical stem on the rhizome.

At the extreme base the stele of the stem possesses more or less the structure found in an ordinary rhizome branch, viz. a central strand of xylem of varying size and shape, with no clearly defined protoxylem, and surrounded by phloem-tissue. The walls of the two or three cortical

[1] Solms-Laubach ('84), p. 171.

layers next to the endodermis have deeply coloured brown walls. Passing gradually up the stem, it is seen that the superficial hairs have disappeared, and the whole thin-walled cortex is gradually replaced by the brown-walled tissue. The xylem increases in amount, parenchymatous cells may be present scattered irregularly amongst the tracheids, but the protoxylem is often hard to determine, although further up the stem two and then three or more groups make their appearance. The secondary tracheids, which were first observed and have recently been described by Boodle[1], appear in this region. Higher up, the stele passes through an interesting stage (Figs. 13 and 14). The amount of xylem increases in extent, the three or four protoxylem-groups can be readily distinguished, and the parenchyma, instead of being scattered amongst the tracheids, lies in the centre as a more or less distinct pith (*pa*). In Fig. 13 it is seen that one tracheid (*t*) alone is left in the centre, whilst the xylem forms a ring broken only at one point (*g*). Further up again, the xylem forms for a short time a complete, unbroken ring round the central pith. This tissue is composed of nucleated elements, with thin areas on their walls, but no sieve-tubes have been observed, nor has any internal endodermis been detected. A few sections higher up, the first of the central fibres makes its appearance (Fig. 14, *f*) in the pith, the number then gradually increases, until the typical central mass is formed, surrounded by the xylem with its radiating points of protoxylem. The cortex gradually changes from the uniform mass of brown-walled cells to the usual arrangement of parenchymatous and sclerenchymatous zones found in the aerial stem.

The Reproductive Organs.

The nature of the sporangial apparatus in the Psilotaceae has given rise in the past to much controversy. The researches and results of Professor Bower[2] seem, however, to be conclusive in ranking the sporangium itself, not as a terminal outgrowth of a reduced axis which bears two leaves, but as an outgrowth from the sporangial leaf, which is bi-lobed possibly for a protective purpose. The whole sporangiophore is then a single foliar member. Investigations on the origin of the sporangium during the work on this paper have led to similar conclusions.

With the exception of the vegetative reproduction by means of bulbils, described by Solms-Laubach[3], and the prothallus found and described by Lang[4] as possibly belonging to *Psilotum*, nothing is known of the development of either *Psilotum* or *Tmesipteris*. Attempts have

[1] Boodle ('04), p. 499.

[2] Bower ('94), loc. cit.

[3] Solms-Laubach ('84), loc. cit. 139.

[4] Lang ('01), p. 405. See also Dr. Lang's paper in the present number of the Annals of Botany, 'On a Prothallus provisionally referred to *Psilotum*.'

been made to germinate spores of *Psilotum*, but without success. The spores have been sown in ordinary soil, in sterilised soil and sand, with varying conditions of heat, light, and moisture, at the base and in the crevices of tree and fern trunks in the tropical houses at Cambridge. Spores also have been sown in hanging-drop cultures, in water, and in different solutions of salts. Cultivations of the Fungus have also been made, and either the soil in which the spores were sown or the spores themselves were infected with portions of Fungus, but with no success. At the time of writing more attempts are being made under different conditions, though it seems improbable that any favourable results will follow.

Theoretical Conclusions.

Psilotum is generally regarded as representing an ancient type, which has retained to a certain extent some of its primitive features, whilst showing at the same time modifications due to its manner of life. Mr. Boodle [1] considers that the secondary tracheids in the stem probably represent a more normal secondary thickening, the reduction being in correlation with that of the leaves. Prof. Lignier [2], however, regards the Psiloteae as a very ancient type, this being shown by the primitive nature of the leaves.

In relation to the foliar structure the question as to the origin of the pith in *Psilotum* is of interest. If the small scale-like leaves with no vascular supply are to be regarded as primitive, then there is no doubt as to the stelar nature of the pith, for unlike other Pteridophyta there are no leaf traces to be considered, and the branching is not altogether constant at this level and cannot be taken into account. There are, therefore, no obvious points at which any continuity can be traced and any intrusion of the cortical into the stelar tissues can be observed. The protostele in the lower part of the stem is therefore succeeded by a medullated condition, and no further stage is found except the substitution, for a purely mechanical reason, of sclerenchymatous fibres for the parenchyma in the aerial stem. As each aerial branch from the rhizome turns to grow vertically upwards above the soil, cortical as well as stelar tissues undergo considerable change. More mechanical support is needed by the plant, and this for a short time is undertaken by the whole brown-walled cortex. Later the stele increases somewhat in size, though the xylem remains approximately the same in amount, and parenchyma replaces the conducting tissue of the stele for a short distance. Sclerenchyma, however, soon takes the place of this thin-walled pith, and in correlation with the necessary increase in the assimilating tissue of the stem the cortical sclerenchyma decreases.

[1] Boodle ('04), p. 505.

[2] Lignier ('03), p. 106.

It seems, however, to be more likely that the leaves of *Psilotum* represent reduced, and not primitive, structures, for in *Tmesipteris* the leaves and leaf traces are well developed. In this case a suggestion, which has been given by Tansley and Chick[1] in their paper on *Schizaea Malaccana*, may be also made for *Psilotum*. The stele remains the same in size, but the demand for water conduction is less, and hence tracheids, which originally occupied the centre of the stem, are no longer developed and their place is taken by ordinary parenchyma. The great reduction of the leaves in *Psilotum* is a point in favour of this suggestion.

The position of the Psilotaceae amongst the Pteridophyta is not very clear. The family was formerly placed as a sub-group of the Lycopodineae, but of late this affinity has been regarded as being somewhat remote, whilst a stronger relationship has been shown to exist with the fossil group of the Sphenophyllales. Anatomically, however, the Psilotaceae show certain Lycopodinean features. Professor Bower in 1893[2] drew attention to the resemblance between the central stele of the Psilotaceae and that found in the axis of *Lepidostrobus Brownii*. He enumerates the four chief points of resemblance, the crenulated margin of the xylem, the definite layer of endodermis as in *Psilotum*, the slight bulk of phloem tissue and absence of distinctive characters in it, and lastly, the presence of a parenchymatous pith in *Lepidostrobus* which resembles that 'well known to occur in *Tmesipteris*, though its place is taken in *Psilotum* by thick-walled sclerenchyma.' The presence, therefore, of a central thin-walled pith in the stem of *Psilotum* helps to strengthen the possible relationship.

There is again a strong resemblance between the aerial and intermediate regions of the stem of *Psilotum* and the fossil stems of *Lepidodendron mundum*[3]. Some sections of the latter in the Manchester Museum show a distinct medulla of varying size, the xylem forming a definite ring which may be broken at some levels. Other sections, again, show this parenchymatous pith replaced by thick-walled tissue as in *Psilotum*.

The 'secretory' zone[4] of some Lepidodendroid stems is absent in *Psilotum*, but the somewhat unsatisfactory nature of the phloem may possibly be another link, though a slight one, between the two forms.

The affinity of the Psilotaceae with the Sphenophyllales is based not only on anatomical grounds, but also on the nature of the sporangial apparatus[5]. From the anatomical point of view, the characteristic triangular mass of primary wood, which is so noticeable in the stem of *Sphenophyllum*, recalls the structure of the smaller branches of an aerial stem of *Psilotum*, and the discovery of secondary tracheids serves to strengthen this

[1] Tansley and Chick ('03), p. 499.
[2] Bower ('93), p. 337.
[3] Williamson ('89), p. 197, Pl. 6, Figs. 7–14.
[4] Seward ('00), p. 155; ('02), p. 38. Weiss ('01), p. 3.
[5] It has already been stated in this paper (p. 599) that in the Psilotaceae the synangium and its axis are to be regarded as the product of a single fertile leaf.

resemblance[1]. In his account of the structure of *Cheirostrobus* in 1897[2], and again later in 1900, Dr. Scott has compared the synangium and its axis in the Psilotaceae to the ventral sporangiophore of the Sphenophyllales, regarding the vascular supply of the leaf and synangium in *Tmesipteris* as comparable to that found in the bract and sporangiophore of a *Sphenophyllum*. A still closer affinity between the Psilotaceae and the Sphenophyllales was suggested by Thomas[3] in 1902, and based largely upon certain variations commonly met with in the fertile structures of *Tmesipteris*. Dichotomy may occur in some of the sporophylls, accompanied by an increase in the number of synangia; and this condition, which is also found, but less frequently, in *Psilotum*, is regarded by Thomas as an ancient feature of the Psilotaceae. Again, the synangium in *Tmesipteris* may be raised on a stalk or pedicel, and this is compared to the sporangiophore of *Bowmanites Römeri* with its two sporangia. Whether we accept such variations as representing normal conditions or otherwise, there nevertheless seems to be little doubt that a strong resemblance exists between the Psilotaceae and the Sphenophyllales in regard to the sporangial structures.

Professor Bower[4] has adopted Thomas's view as to the close relationship of the two groups, and would include the Psilotaceae and the Sphenophylleae together in the Sphenophyllales. The two series are regarded as being related, but as having at the same time two distinct lines of evolution. Whilst admitting with Professor Bower the great importance of the spore-producing members in plants, nevertheless other considerations must also be taken into account. The structure and development of the young sporophyte, the anatomy of the mature plant, and again, the nature of the gametophyte, are points which must be considered, even though they may be ranked as secondary in importance. Dr. Scott[5] has suggested that the Psilotaceae may be allied, though very remotely, to the Lycopodieae, having perhaps branched off from the main line of Lycopod descent very far back, at a point where some of the characters common to the Sphenophyllales were still retained; a knowledge therefore of the gametophyte and of the young sporophyte might throw fresh light on this possible relationship. As matters stand the affinity of the Psilotaceae with the Sphenophyllales is clearly the most marked, but until a further knowledge of the development of the living genera of *Psilotum* and *Tmesipteris* is known it would seem a little premature to assign to them a definite position in a group which hitherto has only included fossil forms.

In regard to the rhizome of *Psilotum*, a considerable similarity in structure has been found to exist with the fossil root or rhizome recently described and figured by Professor Weiss[6]. The structure of the two

[1] Boodle ('04), p. 506.
[2] Scott ('97), p. 27; ('00), p. 499.
[3] Thomas ('02), p. 342.
[4] Bower ('03), p. 229.
[5] Scott ('00), p. 499.
[6] Weiss ('04), p. 255.

groups of xylem with the somewhat irregular protoxylem, and again the whole appearance of the cortex with its absorptive hairs and cells with clearly defined fungal filaments and probable reproductive bodies, recall very forcibly some sections of *Psilotum*. As Professor Weiss has pointed out, it is impossible to decide the systematic position of this fossil root or rhizome until more is known of the whole plant to which it belongs; too much stress, therefore, must not be laid on these resemblances to *Psilotum*, however strongly marked and interesting they may be.

Summary.

1. *Psilotum* possesses a much-branched aerial and subterranean stem, and greatly reduced leaves with no vascular supply. There are no roots.

2. The plant is monostelic throughout. A protostele is found at the base of the aerial stem, and this is often succeeded by a medullated stage. In the aerial branches a central core of sclerenchymatous fibres is found.

3. The protoxylem is often absent in the underground branches. The phloem throughout is poorly developed, though elements resembling sieve-tubes are present. Lignification of the phloem tissue may occur in the aerial stem.

4. Owing to its saprophytic manner of life, *Psilotum* probably represents a much reduced form, which may have retained some primitive characters. The relationship to any of the living Lycopodiaceae is somewhat distant, but the structure of the aerial stem shows resemblance to the fossil Lycopod *Lepidodendron mundum*, as well as to the axis of the cone of *Lepidostrobus Brownii*. On anatomical grounds, as well as on the nature of the sporangial structures, the Psilotaceae appear to be somewhat closely allied to the fossil group of the Sphenophyllales.

In conclusion, I must add that this paper was begun at Cambridge at the suggestion of Mr. Seward, but was continued and completed at the Owens College, Manchester. To Mr. Seward, to Professor Weiss, and to Dr. Scott I owe my warm thanks for suggestions and advice given me throughout. My thanks are also due to Mr. Lynch for the material and information with which he has so often supplied me.

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EXPLANATION OF PLATE XXXIX.

Illustrating Miss Ford's paper on *Psilotum triquetrum*.

Fig. 1. Transverse section of an aerial shoot of *Psilotum* below a bifurcation. *st* = stomata, *pa* = outer assimilating layer of parenchyma, *scl* = sclerenchymatous zone, *p' a'* = inner parenchymatous zone, *n* = siliceous nodules, *px* = protoxylem, *x* = xylem, *ph* = phloem, *f* = central fibres, and *p' x'* = secondary protoxylem. × 34.

Fig. 2. Transverse section of portion of aerial stem, showing lignification of phloem tissue. *px* = protoxylem, *st* = phloem tissue, *en* = endodermis. × 250.

Fig. 3. Assimilating cells from the outer cortex in longitudinal view. × 150.

Fig. 4. Longitudinal section of phloem tissue from an aerial shoot. *st* = sieve-tubes, *pa* = parenchyma, *m* = globular deeply staining masses of substance. × 300.

Fig. 5. Longitudinal section of a leaf of *Psilotum*. × 40.

Fig. 6. Transverse section of the apex of an aerial shoot, showing the apical cell. × 300.

Fig. 7. Longitudinal section of the same. × 300.

Fig. 8. Part of a transverse section of an underground stem of *Psilotum*. *en* = endodermis, *ph* = phloem tissue.

Fig. 9. Transverse section of a branch nearer the apex. × 195.

Fig. 10. Transverse section of a branch below bifurcation. × 180.

Fig. 11. Ditto, but near the apex and showing tracheids passing off to a lateral bud *l*. × 173.

Fig. 12. Transverse section below the apex of an underground branch, showing a single lignified tracheid. × 215.

Fig. 13. Transverse section of the intermediate portion of the aerial stem. *ph* = phloem tissue, x^2 = secondary xylem, *pa* = central parenchyma, *t* = single central tracheid. At *g* the break in the xylem ring is seen. × 90.

Fig. 14. Ditto, higher up. *en* = endodermis, *f* = central fibres. × 42.

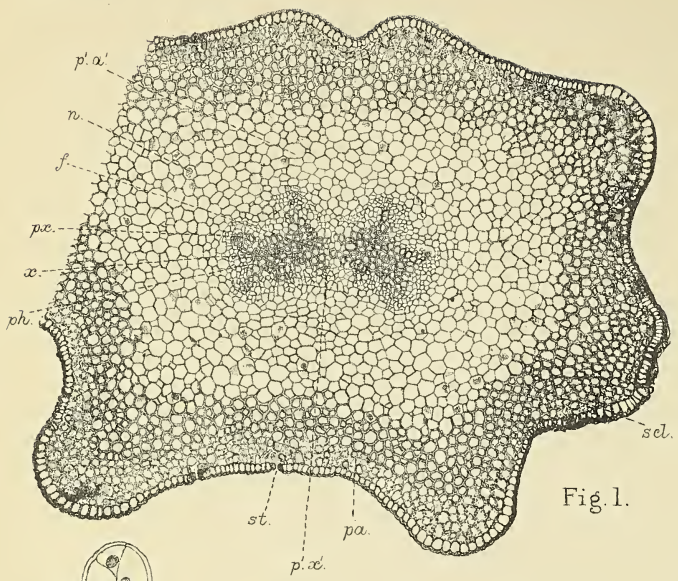


Fig. 1.

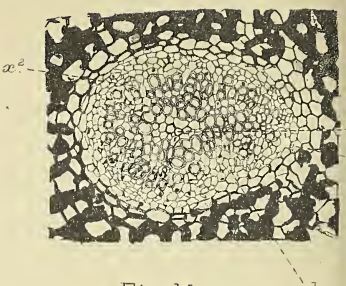
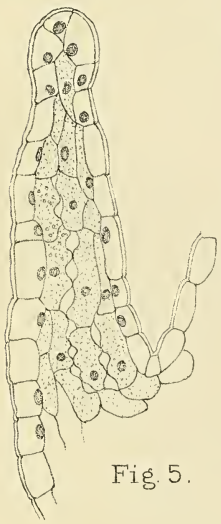


Fig. 14.



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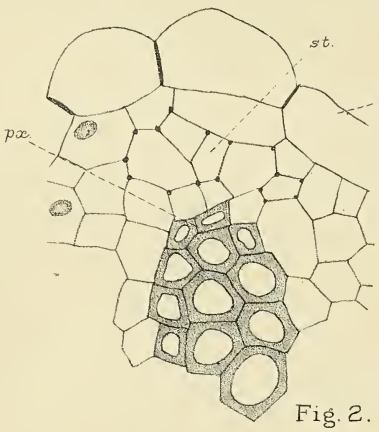

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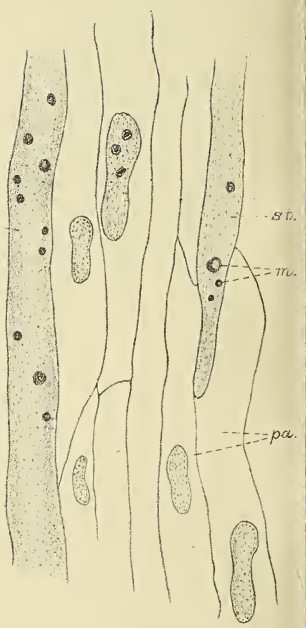

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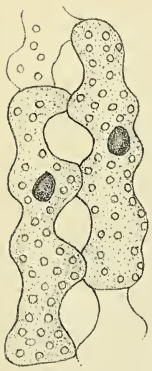

Fig. 3.

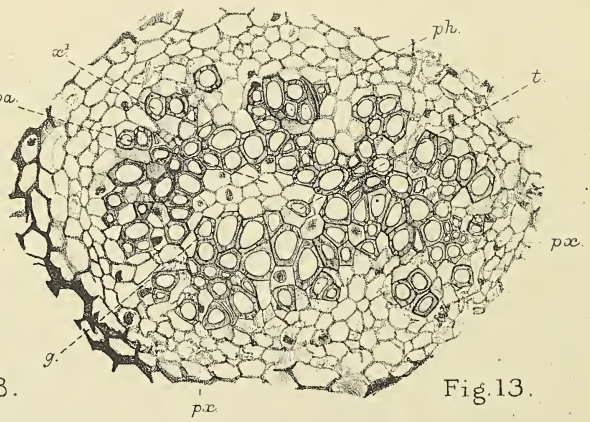

Fig. 13.

Fig. 6.

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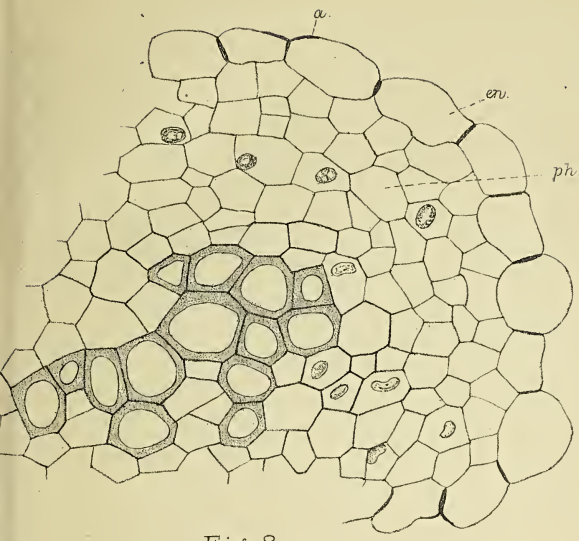

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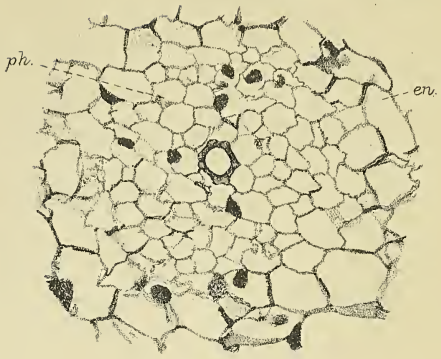

Fig. 12.

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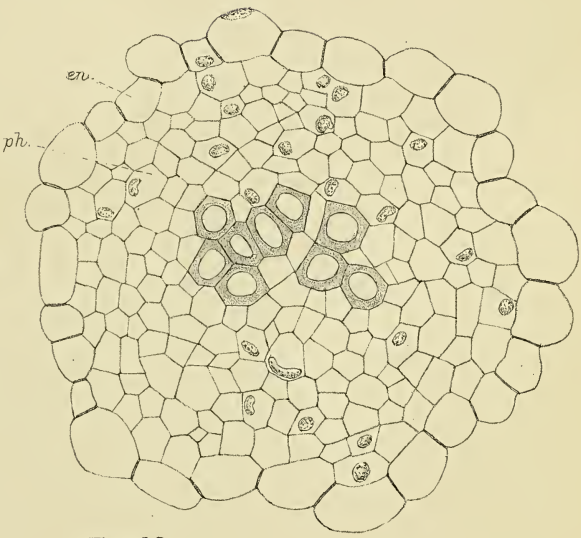

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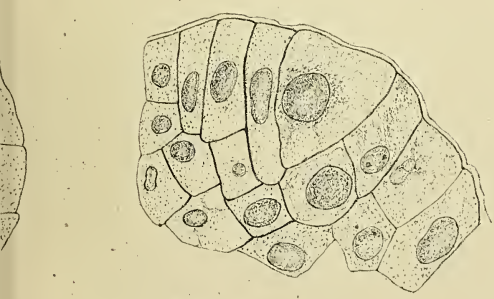

Fig. 7.

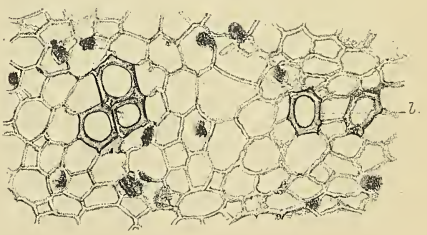

Fig. 11.

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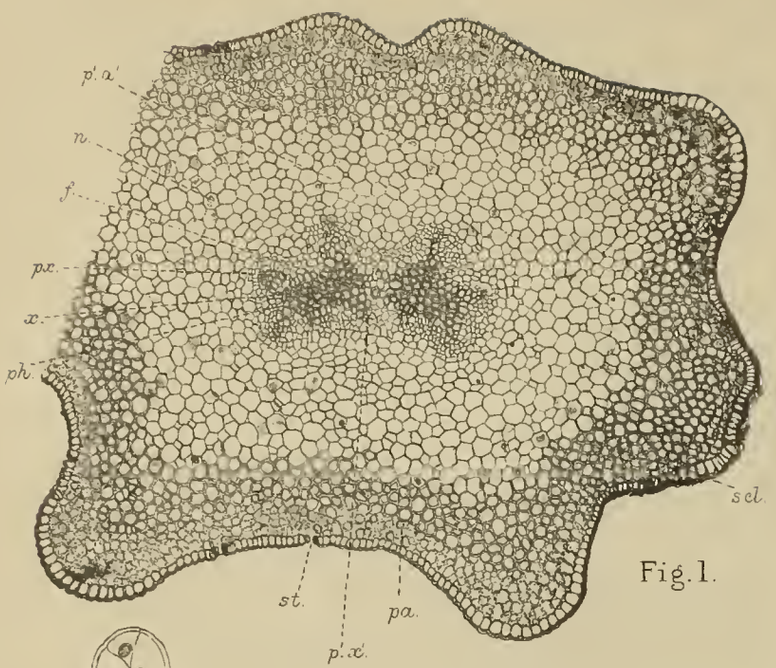


Fig. 1.

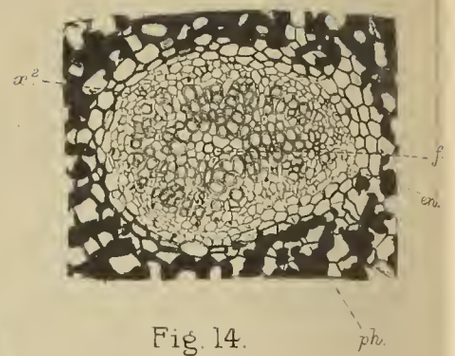
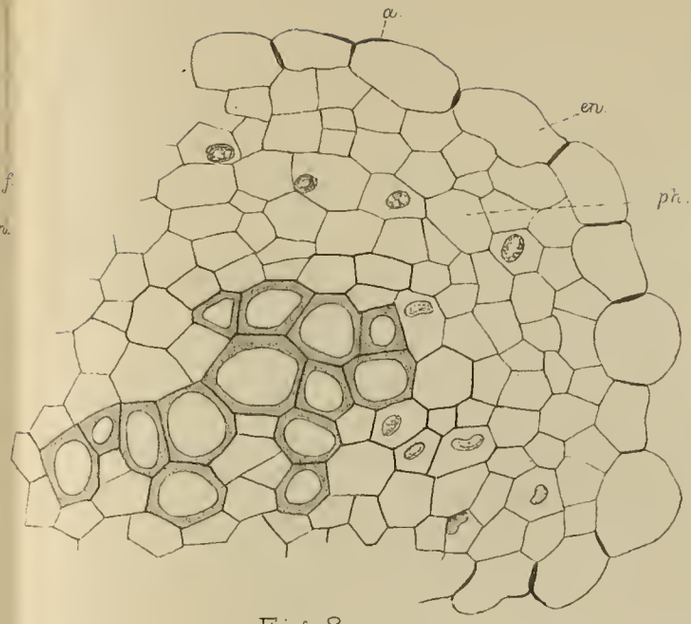


Fig. 14.



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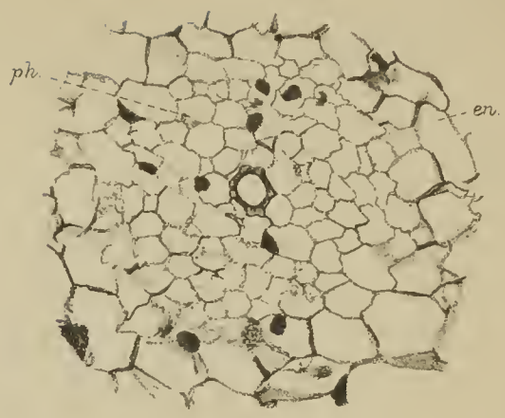

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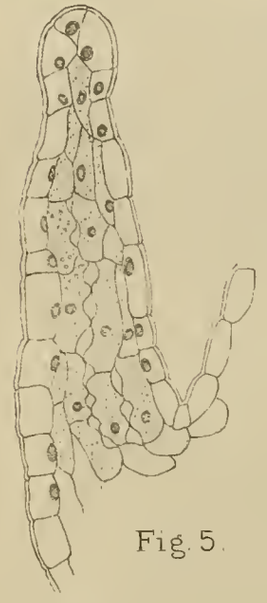

Fig. 5.

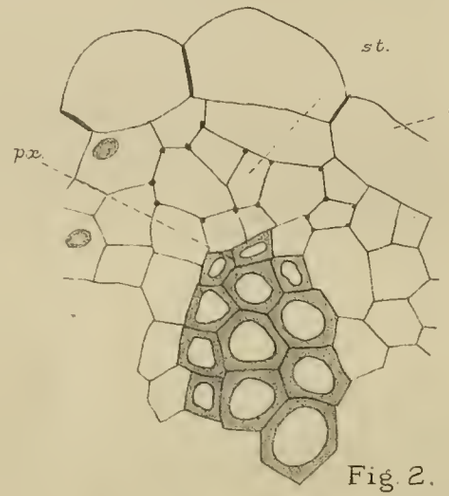

Fig. 2.

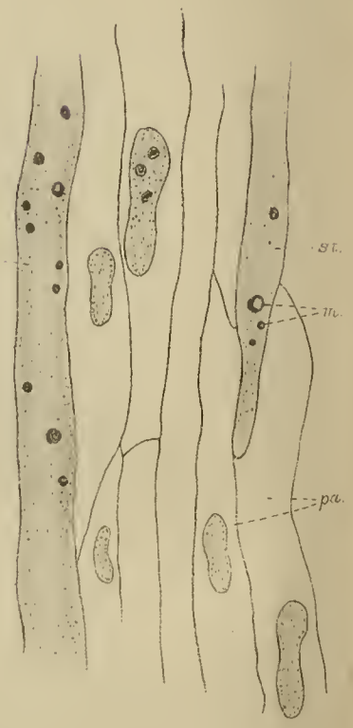

Fig. 4.

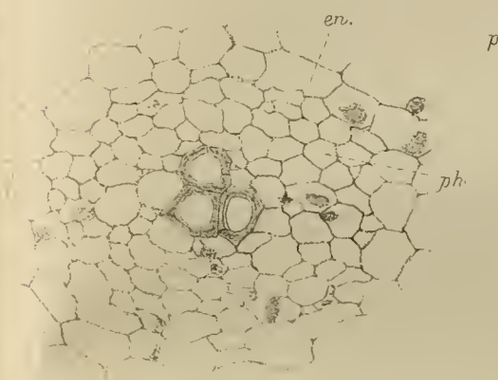

Fig. 9.

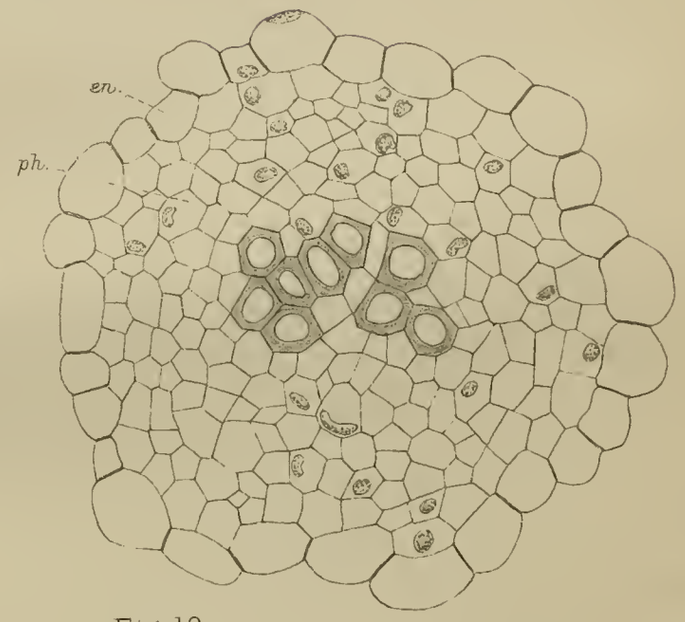

Fig. 10.

Fig. 3.

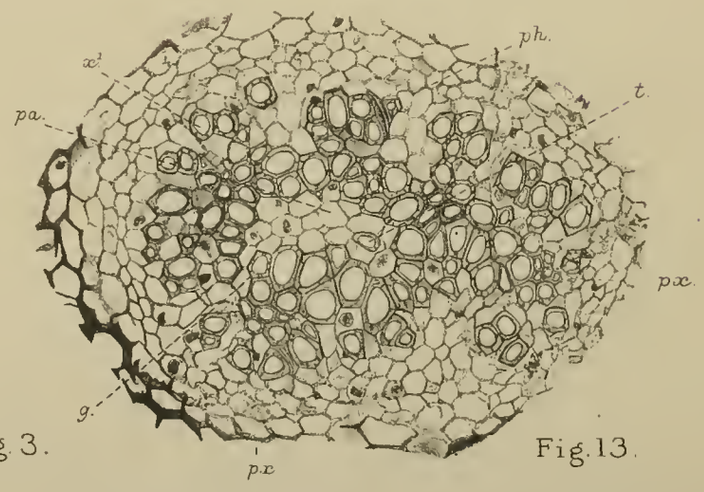

Fig. 13.

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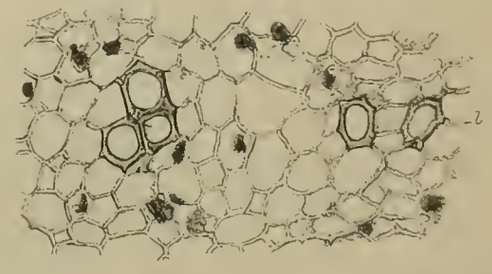

Fig. 11.

S.O.F. del.

Huth, lith. et imp.

FORD.—ANATOMY OF PSILOTUM.

Cytological Studies on Nemalion [1].

BY

J. J. WOLFE.

With Plates XL and XLI and a Figure in the Text.

WITHIN the past half-century only four papers of broad scope bearing on the Florideae have appeared. The work of Bornet and Thuret ('67), and that of Janczewski ('76), both models of their kind, presented clearly and exhaustively the external morphology of the sexual organs and the cystocarp. Antedating, however, critical cytological methods they left much to be desired. Schmitz ('83) attacked the subject in somewhat greater detail, but was misled by the cytoplasmic fusions so characteristic of the group. Attaching a deeper significance to this than the facts warranted, he believed that those fusions actually involved the nuclei, and that here we have a sort of 'double fertilization.' This was, of course, confusing, as it left the group presenting anomalous conditions. Oltmanns ('98), reinvestigating the subject, reached the conclusion that only the cytoplasm is involved, and that the ooblastema-filaments of *Dudresnaya*, for example, simply graft a cell derived from the fertilized egg upon the auxiliary cell. The original nucleus of the auxiliary cell disintegrates, leaving the nucleus derived from the zygote, which by repeated division gives rise to the favella of spores. This condition of affairs is entirely in harmony with current views as to the significance of the sexual act. Sachs ('74) had already extended the theory of 'Alternation of Generations' to the Ascomycetes, the higher Chlorophyceae, and the Florideae. The results of Oltmanns go far toward establishing the correctness of this view, which holds that in these forms what may conveniently be termed the interval between the spore and the fertilized egg is homologous with the sporophyte in higher plants. Before this can be regarded as established, however, a comparative investigation of the chromosome-relations in the nuclei of this interval and of the vegetative cells remains to be made.

It is not clear why, in view of the great number of cytological studies that have appeared in recent years, the Florideae have received so little

[1] Contributions from the Cryptogamic Laboratory of Harvard University.—LIX.

[Annals of Botany, Vol. XVIII. No. LXXII. October, 1904.]

attention. The minuteness of the cell and cell structures, together with the complexities attendant upon the act of fertilization, may be in a measure responsible for this. Perhaps also the group has been less attractive owing to the fact that it appears to be modern, highly specialized and terminal, hence not in itself a factor in the evolution of the plant series. Nevertheless, it has seemed to the writer that it would be interesting to know how far the nuclear phenomena in this side line parallel those in the direct line of evolution

With a view to filling this gap in our knowledge a large supply of *Nemalion multifidum*, Ag., was collected at Woods Holl, Massachusetts, at different times during the summer of 1901. The structure of the frond and the simple sexual relations make this a favourable type for such a study. Consisting of a central axis of intertwining filaments from which branches radiate perpendicularly, and, in addition, having apical growth, a longitudinal section presents vegetative cells in division at the growing point, and all stages from the youngest procarp backward to the mature cystocarp. Other demands upon the writer's time have delayed the completion of this work. It was begun in the Botanical Laboratories of the University of Chicago and continued at the Marine Biological Laboratory at Woods Holl, under the direction of Dr. B. M. Davis, and brought to completion under the supervision of Professor Roland Thaxter at Harvard University. The writer wishes here to express his thanks both to Professor Thaxter and to Dr. Davis for constant suggestion and criticism.

Aside from phenomena related to chromosome-reduction, which was the primary purpose of this investigation, during its progress a considerable body of information has been gathered bearing on other matters of cytological interest. For convenience these results will be discussed under the following heads:—

A. Methods.

B. The Cell.

- (*a*) *Structure of the Chromatophore.*
- (*b*) *Division of the Chromatophore.*

C. Maturation and Sexual Reproduction.

- (*a*) *Oogenesis.*
- (*b*) *Spermatogenesis.*
- (*c*) *Fertilization and Development of the Cystocarp.*

D. Mitosis (*centrosome, spindle, &c.*).

- (*a*) *The Nucleolus.*
- (*b*) *Reduction.*

Methods.

The chrom-acetic mixtures gave entire satisfaction as killing fluids. Material fixed when collected was suitable for most purposes, but a greater abundance of dividing nuclei was seen in that kept in running water in the laboratory and killed at various times during the night. Material killed in 2 % formaldehyde in sea-water and gradually transferred to pure glycerine kept its colour perfectly, and, except for a slight shrinkage and the loss of the small amount of chlorophyll, presented much the appearance of the living plant.

In connexion with the study of fertilization a method was employed which made it possible to examine a great number of procarps at all stages of development with a much smaller expenditure of time and labour than would have been the case if sections had been used. Young tips were crushed in water under a cover-glass and on a slide that had previously been treated with fixative; the cover was then removed and the water on the slide allowed to evaporate. The gelatinous nature of the wall prevents the contents of the cell from being affected by this treatment even when the albumen has hardened sufficiently to hold the filaments firmly in place. Preparations made in this way were then double stained in safranin and gentian-violet by the usual method and mounted in balsam. This was usually accomplished without any shrinkage whatever; and, as the decolorization could be controlled with the aid of the microscope, a finer differentiation was secured than is possible by staining in bulk.

For the study of nuclear detail iron-alum-haematoxylin after the method of Heidenhain gave excellent definition, and, in addition, was easy to control. Erythrosin, or eosin, was used where a plasma stain was desired. Flemming's triple stain was repeatedly and persistently tried, but in no case were results secured in any way approaching those obtained with the Heidenhain.

Sections 3 μ in thickness were used in most cases, but in order to establish certain points it was occasionally found to be necessary to cut them as thin as 1 μ.

The Cell.

The cell-wall is relatively thick, and under high powers is evidently lamellate in structure. It has been figured by Wille ('94) as a double wall, and certainly such an appearance may be seen in certain cases (Pl. XL, Fig. 24). It is by no means easy, however, to determine whether this appearance is due to the actual presence of a double wall, or whether it may not more probably be a misleading appearance resulting from its often very distinctly lamellate structure. The usual tests produce a characteristic though delicate cellulose reaction, the weakness of which is evidently due to the partially gelatified condition of the walls.

The contents of adjacent cells are united by means of the characteristic protoplasmic connexions usually conspicuous in these plants, and in each a chromatophore, surrounding a body which has been called a pyrenoid, and a well-defined nucleus are present.

The nucleus occupies a characteristic position in the basal portion of the cell just below the chromatophore. Its structure is a matter of considerable interest, inasmuch as not the slightest indication of a reticulum has ever been seen, the chromatin being collected in a single, large, densely staining, centrally placed 'nucleolus.' The nuclear wall is somewhat remote from this body, extremely thin and usually difficult to differentiate, and, moreover, connected with the nucleolus by means of delicate radiating fibrillae.

The cytoplasm forms a finely granular layer closely applied to the inner surface of the wall, and extending throughout the cell, envelops the nucleus together with the intricately constructed chromatophore, between the processes of which are left vacuoles of irregular contour (Fig. 25).

Structure of the Chromatophore.

By far the most striking member of the cell in both living and prepared specimens is a highly complex structure, the chromatophore. In the living condition the chromatophore is seen to consist of a centrally placed ellipsoidal body more deeply pigmented than the numerous strands which radiate from it in all directions. These strands, which are of the same material as the central body and continuous with it, as they approach the cell-wall flatten out gradually on all sides, forming a continuous membrane which lies just within the peripheral layer of cytoplasm, and is clathrate through the presence of numerous openings, as seen in Fig. 24. These unpigmented areas thus represent breaks in the continuity of this peripheral portion of the chromatophore, the substance of which is not sharply delimited from the surrounding cytoplasm, and the transition from the one to the other is thus somewhat gradual, the distinction in nature being less abrupt than is represented in the figure. Nothing was observed which appears to indicate that there was a difference either in composition or structure between the various portions of this complex organ other than that of relative density, the material being denser in the central body and gradually becoming less so toward the periphery.

Material crushed on the slide and stained in safranin and gentian-violet shows that this central body is not a homogeneous solid. Sections reveal more clearly a wall layer of the same material as the rest of the chromatophore, surrounding what appears to be a mere vacuolar cavity. This vacuole represents the so-called pyrenoid, which earlier writers have described as characteristic of the vegetative cells of this plant. In the effort to differentiate the substance of this 'pyrenoid' a great number

of stains and other reagents were thoroughly tested, but in no instance was it found possible to demonstrate the presence of any organized material in this central region. Since this cavity is only about 3 μ in diameter its absolute section is secured only in the thinnest sections, and not in such as were chiefly used in this investigation. In these preparations the central body of the chromatophore is seen as a hollow ellipsoid, from the upper portion of which a slice has been removed in another section; therefore, even at the median focus, the concave wall always appears, and being out of focus, presents a granular appearance as is shown in most of the figures. The fact, however, that such confusing appearances are not seen in the absolute median section, like that represented in Fig. 25, which is drawn from a section 1 μ in thickness, demonstrates, to the satisfaction of the writer at least, the absence of any body of such organization as is assumed to be characteristic of the pyrenoid by all recent investigators. It may be objected that the conditions just mentioned might be due to the fact that the contents had dropped out of such a thin section; the essential agreement, however, seen in thicker sections and even in material which has not been sectioned, it would seem, make this supposition, to say the least, in the highest degree improbable.

It should be mentioned in this connexion that Schmitz ('82) in speaking of the pyrenoid in the Bangiaceae and Nemaliae remarks (p. 52, l. c.): 'Bei diesen Algen nämlich quellen die Pyrenoide bei Einwirkung von süssem Wasser, Spiritus, verdünnter Essigsäure u. s. w. auf und vertheilen sich schliesslich vollständig in dem umgebenden Lösungsmittel.' The possession of such remarkable powers of solubility by a distinct organ of the cell would certainly seem to be anomalous; and, although in the somewhat extensive literature of the subject there is so little agreement as to the nature of the pyrenoid, it is perhaps fair to assume that we are here dealing with a wholly different structure. To indicate this diversity of opinion: Meyer ('83), for example, representing perhaps an extreme view, holds that the pyrenoid is of a crystalloidal nature and has no function other than that of serving as reserve material; Boubier ('99), on the other hand, finds it to be a crystalloid, but with a characteristic reaction to stains and surrounded by a membrane of distinct and definite structure; and further, Timberlake ('01) has given a most detailed and admirable description of the structure and function of this body in *Hydrodictyon*, regarding it as proteid in nature and manifesting its activity by cutting off segments which are directly converted into starch. Whatever may be the true nature of the bodies investigated by these writers, it is very evident that in *Nemalion* there is no structure present which could be said even remotely to approach the conditions described.

The chromatophore alone thus appears to be responsible for the constructive metabolism of the plant. No starch, however, could be

demonstrated, although with several other Florideae entirely satisfactory tests were obtained with iodine. The inner surface of the chromatophore surrounding the vacuole presents a granular appearance (see Figs. 24 and 25), but the nature of these granules was not further investigated.

Assuming, then, that the views above expressed are correct, the chromatophore may be described as consisting of a hollow ellipsoid with vacuolar contents, the thick wall of which, granular on its inner surface, becomes gradually less dense in its peripheral region, whence numerous diverticula radiate to the cell-wall, within which they form a continuous, thin, clathrate membrane.

Division of the Chromatophore.

The accounts that have been given of this process in other plants suggest a considerable degree of variability in the process; Davis, for example ('99), has figured a simple elongation and constriction as accompanying the division of the single chromatophore in the spore-mother-cell of *Anthoceros*. Chmilowsky ('97), investigating the mode of multiplication in several green Algae, finds that contemporaneously with the division of that body the chromatophore splits from centre to circumference.

The process in *Nemalion* is somewhat different, and recalls in appearance at least the method of cell-formation by gemmation so characteristic of this plant. In this connexion it should be noted that cell-formation precedes nuclear division by a considerable period. When the daughter-cell has very nearly reached its adult size, the thick wall of the central body of the chromatophore bulges out toward the young cell as if pushed from within (Figs. 26, 27, and 29). The bulge increases in size until the two together present the appearance of one elongated chromatophore (Fig. 30). Soon after this a slight constriction may be observed (Fig. 31), which rapidly increases until the two walls of the central body are in contact, when the two daughter chromatophores begin to separate from each other (Fig. 32).

At about the time when this separation occurs, the cytoplasm loses its granular character, and becomes absolutely hyaline in the region that marks the position of the wall which is to divide the two cells (Figs. 32 and 33). The appearances presented in these figures, and in Fig. 34, suggest that the cytoplasm in this region is transformed into the substance of the future wall.

As can be seen from the figures, there is no exact correspondence in time between the events of nuclear and chromatophore divisions, although in general the nucleus divides contemporaneously with the chromatophore. The conditions seen in Fig. 28, which represents a terminal cell of a filament of the young cystocarp, suggest that the process may be very

transient and entirely independent of nuclear activity in rapidly growing cells.

Although this is the normal process of chromatophore division, in exceptional cases it seems to take place in a different manner; a new chromatophore being organized from one of the coarser strands of the other. This condition, which was described by Schmitz ('82), and has been seen by the writer, may perhaps account for the not infrequent presence of two chromatophores in old cells.

This body is constantly present in all phases of the life history of the plant, and in all cases arises from a pre-existing body of the same nature. The fact that in sperm-cells it disappears in a manner that will be hereafter described, and that, for a short time after the *second* division of the zygote, it has been impossible to demonstrate it, owing no doubt to the dense surrounding cytoplasm, does not militate against the accuracy of this statement.

Maturation and Sexual Reproduction.

Oogenesis.

The carpogonium, as is well known, is borne at the end of a short branch, developed in connexion with sterile branchlets at right angles to the longitudinal axis of the frond. This carpogenic branch is composed usually of three cells; since, however, the number varies from two, in the simplest noted, to as many as five, it cannot be considered as in any way significant. It can be readily recognized even when, as in its youngest stage, it consists of but a single cell. Its peculiar shape (Fig. 1), as well as its capacity for absorbing dyes, serves to differentiate it at once in stained preparations. In life its almost colourless condition renders it equally conspicuous. The members of this branch arise by the divisions of the primary carpogonial cell, which acts as an apical cell by cutting off successive basal segments.

The cells of the branch, excepting in shape and colour, are organized practically just as are the ordinary thallus-cells (Fig. 2). Janczewski ('76) describes the cells of which it is composed as being without nuclei and chromatophores; a manifestly erroneous statement, since there is present a well-defined nucleus differing in no way from the usual type. The chromatophore is not conspicuous in the living cell, because of the small amount of pigment which it contains, but staining brings it out readily. In some cases, at least, a distinctly appreciable amount of colour is present even in the living condition. The cells are short cylindrical blocks contrasting rather sharply in form with the usually ellipsoid cells, which are characteristic of vegetative filaments (Fig. 24). The lowest cell of the series partakes to some extent of the characters of both, and thus lessens the abruptness of the transition between the two types.

As the carpogenic branch becomes differentiated its terminal cell

begins to develop into a procarp by sending out the trichogyne, which consists of a small finger-like protoplasmic process surrounded by a distinct wall; while the chromatophore and the primary egg-nucleus, both normally organized, assume their characteristic positions in the basal portion as indicated in Fig. 3. A little later the tip of the trichogyne becomes much swollen, and in this swollen portion an unmistakable nucleus is always present, which, although the mitotic figure has not been seen, must be assumed to have resulted from the division of the primary nucleus of the procarp. After this division the egg-nucleus, as it may now be called, lies in that portion of the procarp immediately above the chromatophore. Very probably it migrates to this position as a preparation for this mitosis, but such procedure is unusual, and it is but fair to call attention to the fact that it is not a phenomenon usually accompanying mitosis in this plant. While actual division of the primary nucleus was not observed, these conditions presented in Fig. 4, which were seen in several cases, can hardly be explained on any other supposition. Figs. 5 and 6, in which the egg-nucleus has returned to its characteristic position, make clear the fact that this occurrence is not to be explained upon the assumption of an artifact. At all events a well-marked nucleus is now present in the trichogyne. The wall of the trichogyne, which has hitherto been relatively thick, now becomes extremely thin and delicate throughout the terminal receptive portion, and fusion with the spermatium is thus accomplished without difficulty (Figs. 4–11).

Soon after this nucleus has entered the trichogyne it begins to break up into a variable number of nuclear fragments which retain their staining power for a considerable period (Figs. 8 and 9), and become gradually smaller and less markedly differentiated as the trichogyne approaches maturity (Fig. 10). At the period just preceding fertilization inconspicuous aggregations of slightly staining material may often be distinguished, which probably represent the last stages in this process of nuclear dissolution. These facts are of importance, since were such differentiated nuclear-like masses present, it would prove difficult or even impossible to distinguish them from the sperm-nuclei after the entrance of the latter. While this might be regarded as negative evidence, the writer feels reasonably certain of the facts, as a very careful examination was made of a large number of well-stained trichogynes before reaching this conclusion.

So far as the writer is aware, the earliest detailed account of a nucleated trichogyne is that of Davis ('96) in his paper on *Batrachospermum*. It is true, of course, that Schmitz ('83), together with some of the earlier observers, found certain differentiated granules present in the unfertilized trichogyne. Schmitz, however (p. 12, l. c.), dismisses them with the statement that they react towards stains like chromatin. Further, Schmitz noted the presence of chromatic bodies in the trichogyne after fertilization,

which he regarded as 'Richtungskörper.' These have, however, been correctly explained by Schmidle ('99) as supernumerary male nuclei discharged into the trichogyne. Oltmanns ('98) also noted the granules, previously observed by Schmitz, in the trichogyne of *Gloeosiphonia* (p. 110, l. c.), but concludes that they have absolutely no relation to nuclei. Schmidle, reinvestigating *Batrachospermum*, failed to find the nucleus described by Davis, and Osterhaut, in his paper on the same genus ('00), remarks (p. 113, l. c.): 'Ich habe in meinen Präparaten nicht die geringste Andeutung eines Kernes im Trichogyne gesehen vor Eintritt des Spermatiumkernes.' In view of these conflicting statements, the evidence in the present case was examined with extreme precaution; but the conditions were so evident that the writer has been forced to conclude with Davis that the trichogyne (in *Nemalion* at least), instead of being a mere hairlike outgrowth from a cell, is at first a cell in the strictest sense of the word, which only later is specially modified in connexion with the reproductive processes.

For purposes of comparison, it may be instructive to note that a corresponding condition of the female sexual apparatus is described in the case of the Laboulbeniaceae by Thaxter ('96). In these plants, which closely resemble the Florideae in their sexual processes, the procarp consists of a carpogenic cell and trichogyne connected by an intermediate cell, and the trichogyne presents every degree of complexity from a unicellular vesicular prominence to a highly developed branching system of nucleated cells, the terminal cells in these structures constituting the receptive portion, and being often specially modified by spiral twisting, or otherwise, as, for example, in the genus *Compsomyces*.

It is also of interest to note, as a further indication of the probable differentiation of the trichogyne as an independent cell, that in two cases at least conditions were observed which strongly suggest that a chromatophore derived from that body in the procarp also passes into the young trichogyne. This, of course, is indeed the more probable from the fact that the chromatophore is clearly present in the trichogyne of the nearly related *Batrachospermum*. However, the protoplasm of the procarp stains so heavily at this time that a satisfactory demonstration of this point was not secured.

Spermatogenesis.

The general morphology of the antheridial branch has already been figured in the works of Bornet and Thuret ('76, '78, and '80), Guignard ('89), and others. In *Nemalion* this branch arises from what appears to be an ordinary vegetative cell, and like the carpogenic branch is remarkable for the variability in the number of cells of which it is composed. Similarly, too, it can be distinguished when very young by the absence of pigment and the short cylindrical form of its cells (Figs. 35 and 36).

Each cell of the antheridial branchlet usually gives rise to four antheridia which bud out radially, although even here the number is not constant. Fig. 37 represents a median section of such a branch in which only those antheridia developed laterally are shown.

The divisions of the nucleus of the mother-cell from which these antheridia arise correspond to those of other vegetative cells in all respects, and the chromosomes, as usual, are about eight in number. The minuteness of the figure necessarily renders accurate counting a difficult matter, but so many were seen in both longitudinal and polar view, and the cytoplasm is so clear at this time, that there is little reason to doubt that this estimate is approximately correct. The spindle is in no way peculiar, except that it is smaller and details are relatively even more difficult to differentiate than in the case of other cells of the plant. The spindle appears to be intranuclear, as is certainly the case in other mitoses. A centrosome was occasionally distinct at the poles of the spindle at metaphase (Fig. 39), but could not be made out in the majority of the cases examined (Fig. 38).

The chromatophore divides after the nucleus (Fig. 42), and for a short time can be recognized as a faintly staining body, with a not very definite outline in the antheridium (Fig. 43). Soon, however, the contents of the cell undergo a remarkable reorganization, after which it is no longer possible to recognize any differentiated bodies in the protoplasm, except the nucleus itself (Fig. 47). In this reorganization the chromatophore disappears and a mass of densely staining matter is seen at the distal end of the antheridium (Fig. 44), a portion of which seems certainly to be derived from the disorganization of the chromatophore. For a time the nucleus is seen lying below this mass, but very soon is indistinctly visible in its midst (Fig. 45). This matter begins to stain less deeply, and well-marked, deeply staining, fairly uniform granules, twenty to thirty in number, appear on the nuclear wall. These granules suggest those bodies figured as chromatophore-derivatives by earlier writers (Figs. 44–46). Although the phenomenon bears a striking resemblance to the conditions seen in the early prophase of ordinary mitoses (see Figs. 54 and 55), it is evident that it cannot be thus interpreted as a prophase character, from the fact that the nucleus resumes again its normal organization preparatory to the succeeding mitoses (Fig. 16, the spermatium at the left). As to the origin of these granules, it seems more reasonable to suppose that they represent food material, a part of which at least is derived from the chromatophore, and is now passing into the nucleolus. It should here be stated as further evidence in support of this interpretation that preparations of the cell at this stage (Fig. 47 in median section, and Fig. 48 in surface view), as well as in the stages immediately preceding, show that the nucleolus retains its normal size and appearance (Figs.

37–44), while after the disappearance of the granules in question its size becomes greatly increased. The condition just described, in which the nucleus is surrounded by granules (Figs. 46–48), is seen at the time when the contents of the antheridium are discharged into the water as a naked or thin-walled mass of protoplasm; and although the terms antherozoid, pollinoid, and spermatium, have been variously applied to it, the subsequent history seems to indicate that it may more properly be termed an antheridium. Since hundreds of instances of such antheridia showing these conditions are presented in almost any well-stained preparation, it would seem that the arrangement persists for a considerable period. At the time, however, when the spermatium has reached the trichogyne these granules have usually disappeared (Fig. 16) and the nucleus has resumed its normal appearance, although, as already mentioned, the nucleolus has undergone a conspicuous increase in size. That the so-called spermatium cannot be regarded as a sexual element, but, as just noted, is to be homologized with the antheridium, is shown by the fact that a rapid division into two sperm-cells now takes place (Figs. 49, 50), which, so far as it has been possible to determine, is an invariable preliminary to actual fecundation, and takes place shortly after conjugation with the trichogyne. The figure in this mitosis is of the type already described, and, so far as could be certainly determined, presents no unusual features. In several cases the number of chromosomes could be fairly well estimated, and is certainly very close to the number eight, which is seen in the preceding mitosis. From the above facts it would appear that, although the term spermatium might be conveniently retained in conformity with previous usage, it should be distinctly understood that it is not the homologue of a sexual element.

It should be mentioned that a binucleate spermatium has been described by Schmidle ('99) in his paper on *Batrachospermum*, but he leaves us somewhat in doubt as to when and how this division occurs, which is of importance, as Osterhout ('00) found this body in the same genus to be uninucleate and suggests that a binucleate appearance is probably due to disorganization. Further, Davis ('96) noted the rare occurrence of a binucleate appearance in the spermatium of this plant, assuming, however, that they were fragmentation-products. In presenting the mitotic figures from which these two nuclei arise, and which are of very common occurrence, the writer believes that he has established the fact that this binucleate condition is a normal phenomenon.

Fertilization and Development of the Cystocarp.

The trichogyne, as has been stated above, contains at this time no organized nucleus. Both the male cells, as we have already seen, seemed normally to be discharged into the trichogyne. The processes of ferti-

lization, as they have been observed by the writer, correspond to the account given by Wille ('94) with the following exception. This author states (p. 59, l. c.): 'Wenn der Spermakern sich der Verengung des Carpogoniums nähert, wandert der Eikern diesem entgegen.' In the numerous preparations of this stage in the writer's material, the male nucleus is present in the upper part of the carpogonium, while the female still occupies its usual position (Fig. 12).

Soon after, however, the female passes into the upper part of the cell, and there fusion takes place (Figs. 13–15). The male nucleus is often somewhat smaller than the female, although more frequently it is very nearly of the same size. Differences in staining power, such as were noted by Wille, were not observed. The staining of the protoplasm, according to the method employed to demonstrate these stages, somewhat veiled the processes, rendering an accurate examination of the finer details in fusion of the chromatic masses unsatisfactory. Actual fusion is evidently a very rapid process. In the scores of cases examined, no stages intermediate between these presented in Figs. 13 and 16 were observed.

Just about the time of fusion, the protoplasm of the trichogyne separates from that of the carpogonium (Figs. 13–16). No cellulose plug appears to be formed, as stated by the earlier observers, but a distinct wall is thrown around the zygote within the original wall of the carpogenic cell, in much the same manner as has been described for the fertilized egg-cell of the Bryophytes. This wall, however, does not sever the original protoplasmic connexion with the cell immediately below (Figs. 17 and 20), which Oltmanns ('98) has characterized as the hypogynous cell; and soon after it is laid down, the fusion-nucleus passes from its position in the upper portion of the carpogonium to its normal one below the chromatophore. These characteristic positions of the nuclei are of importance, since they eliminate all possibility of mistaking late stages of zygote-division for fusion.

As seen in Fig. 16, many spermatia may conjugate with the same trichogyne. Sperm-nuclei in all stages of dissolution can be noted at different points in this organ. Nuclei thus discharged appear to disintegrate almost immediately. The protoplasm of the trichogyne contracts away from the delicate wall soon after fertilization, and the structure as a whole withers very rapidly. Small bodies resembling male nuclei (Fig. 14) were noted in several cases, indicating, perhaps, that more than one male nucleus may enter the carpogonium. This is, of course, not impossible, since the protoplasmic passage between the egg and the trichogyne is not broken until fusion is practically complete. Further information, however, concerning the origin and fate of these bodies was not obtained.

When the fusion-nucleus returns to the basal portion of the carpogonium, division begins at once, and, as stated by both Wille and Janczewski, a basal cell is first cut off by a transverse wall. This cell usually undergoes no further division, but a single case was observed in which it divided once longitudinally; suggesting that although it normally functions as a sterile 'stalk-cell,' it may be considered as potentially similar to the primary sporogenous cell above it, from which the sporogenous filaments, or gonimoblasts, are developed.

Wille has already noted that at the time of the division of the zygote into these two superposed cells, the chromatophore also divides (Figs. 13 and 19). The history of this body, and of the chromatic elements as well, could not be satisfactorily followed through the two or three immediately succeeding divisions, owing to the intense staining of the surrounding cytoplasm. At the period, however, when the young cysto-

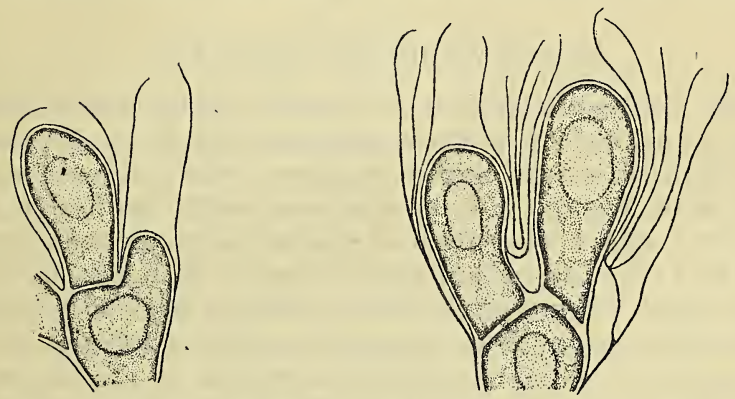

Fig. 51. Mother-cells of carpospores proliferating from a subterminal cell. The walls of their predecessors persistent. (Optical section. × 1500, reduced in reproduction.)

carp consists of six or eight cells the divisions of nucleus and chromatophore can be followed with certainty. These divisions present double the number of chromosomes characteristic of the thallus-cells. This phase of the subject will be more thoroughly presented in a subsequent portion of this paper. The details of the structure of the chromatophore can be seen at this period of development and its presence proved in every cell of the cystocarp, from such stages as are illustrated by Fig. 22, to the mature condition (Fig. 23). Its demonstration in the stages intervening between the two and the six-celled condition will no doubt be possible with the development of special technique.

The gonimoblasts when fully developed are more or less copiously and repeatedly branched, and the cells in the peripheral region of the resultant structure ultimately give rise distally to numerous subterminal and terminal mother-cells, the contents of which, severing their protoplasmic

connexions, escape as carpospores through a terminal break in the mother-cell wall, which is left behind as an empty adherent sheath, and within which, by further successive proliferations, several more mother-cells of carpospores may be produced, from which a corresponding number of spores are successively discharged (Text-fig. 51).

The function of the stalk-cells of the carpogenic branch can be stated in brief to be that of furnishing organized material to the rapidly growing cystocarp. Whether or not the heavily staining masses appearing in these cells (Figs. 21 and 22) represent food material, as is suggested by Wille and as further indicated by their disappearance as the cystocarp develops, is a matter of some uncertainty. At least a portion of this material, in the writer's opinion, results from the disorganization of the chromatophores which disappear during the early development of the cystocarp (Figs. 21 and 22).

Mitosis (Centrosome, Spindle, &c.).

In the mitoses of this plant two heavily staining masses invariably appear at the poles of the spindle at metaphase, with the possible exception of the antheridial mitoses already mentioned, which, it would seem, are certainly to be identified with the structures usually described as centrosomes. In *Nemalion* this body is of relatively large size and always surrounded by a delicately outlined hyaline area (Pl. XLI, Figs. 63, 67, &c.), although within this region no fibrous radiations could be satisfactorily observed. The occurrence of instances in which it appeared to divide (Figs. 63 and 70), together with such conditions as are illustrated in Fig. 53, make it probable that the centrosome is always present even in the resting condition, and corresponds to the bodies thus designated in many plant and animal-cells. While its size alone would make it a favourable subject for research, the fact that it has not been shown to be accompanied by an aster, or any other very characteristic structure which might serve to differentiate it clearly from the granules of food and other material which react to stain in much the same manner, has led the writer to refrain from attempting at this time to examine it in more detail.

The spindle is organized within the original nuclear cavity, and possibly from a residual non-chromatic nucleolar substance which may be seen as a lightly staining mass, apparently fibrous in character, occupying the central region of the nucleolus after the chromatic substance has all passed to the nuclear wall (Figs. 56 and 60). No indication whatever of an extra-nuclear origin for the achromatic structure was ever observed. The suspending fibrillae already mentioned (Fig. 54) may of course co-operate with this nucleolar matter. The spindle may be

best observed at metaphase, at which stage the shape of this structure in mitoses occurring in the early development of the gonimoblasts presents a strong contrast to that assumed by it in the vegetative phase, since in the latter it is relatively long and narrow, with the fibres meeting at the poles at a fairly acute angle, whereas, on the other hand, in the cells of the gonimoblasts its length remains about the same, while its width has become nearly doubled, and consequently the angle at the poles is now markedly obtuse (cf. Fig. 75 with 65, for example). It should be mentioned, perhaps, that the formation of the cell-wall which has been described above as occurring after mitosis is quite independent of these fibres. The central portion of the spindle could be observed for a short time after the separation of the daughter-chromosomes (Fig. 66), while its cones remain intact, somewhat shortened perhaps, for a considerable time (Fig. 69).

Nucleolus.

The nucleus, as has been already briefly pointed out, consists of a relatively large cavity bounded by a delicate wall and filled with nuclear sap, in the centre of which appears a single, large, heavily staining 'nucleolus,' attached to the wall by a considerable number of radiating fibrillae (see figs.). The dimensions of the nucleus, and nucleolus as well, vary to some extent, as may be seen from the figures, its increase in size being apparently connected with preparations for division. The nucleolus is not homogeneous in structure, and consists of a peripheral region of denser material surrounding a central substance which shows a much less marked capacity for absorbing stains. This condition of affairs is represented in Fig. 51, which illustrates a favourable section passing through the body in question. As mitosis approaches, this peripheral substance aggregates in heaps, leaving thinner areas between (Fig. 52); and in some cases these aggregations are of such uniform character as to suggest that they may be the units from which the chromosomes are to be formed, their number being decidedly in excess of, and probably about double that of the chromosomes (Fig. 57). At this period the nucleolus in many cases assumes a lenticular shape, its short axis probably coincident with the long axis of the future spindle. The correctness of this assumption is indicated by the conditions present in later stages (Figs. 59, 61, and 62), in which the chromosomes are gathered chiefly in the equatorial region of the dividing nucleus.

At this period the radiating fibrillae stain somewhat more conspicuously, the nucleolus becomes irregular in outline and often slightly drawn out along them, while distinct, deeply staining masses appear at the points where they intersect the nuclear wall (Figs. 54–56). Inasmuch as previously no stainable material whatever could be detected in this

region, there is, it would seem, little reason to doubt that a substance originally stored in the nucleolus is passing in the manner indicated to this position, and further, that this substance is, from its subsequent history, certainly to be identified with chromatin.

These chromatic masses are now directly organized into chromosomes without, so far as could be certainly determined, the intervention of a spireme stage (Figs. 58–60), and very soon are apparently drawn from a general equatorial position to the nuclear plate (Figs. 61–65). After splitting has occurred, the daughter-chromosomes remain separate for a considerable time (Figs. 66 and 69), eventually, however, fusing into two or three masses (Figs. 70 and 71), which further fuse into the single centrally placed nucleolus (Fig. 72), finally assuming the appearances already described as characteristic of the resting nucleus.

In the development of the gonimoblasts, but not elsewhere, conditions were observed which cannot, it would seem, be explained upon any other assumption than that a portion of the nucleolar substance is expelled from the nucleus in the prophases of division (Figs. 56 and 68). It could not be determined whether or not such extruded masses represent a substance other than chromatin located in the nucleolus, and the homologue of the so-called plasmosomes, or true nucleoli. Inasmuch, however, as its reaction to stain, as well as its mode of origin, appears to be entirely similar to that of the chromatin, the presumption is in favour of identifying it with that substance.

In a recent paper on the nucleolar conditions of *Phaseolus* ('04), Wager has shown that the nucleolus in this plant is simply a storehouse for the greater portion of the chromatin, which, as division approaches, passes out of that body into the spireme-thread through fibrous connexions. Although in *Nemalion* no reticulum is present and no spireme is formed, the chromatin, which, as we have seen, is in this instance wholly confined to the nucleolus, passes out from it along fibrillae in much the same manner. The nucleolus of *Spirogyra*, according to numerous investigators, is also concerned to a greater or less extent in the formation of the chromosomes; Moll ('93), for instance, regarding all the chromatin as derived from that body, while Van Wisseling ('98) finds that only portions of two chromosomes owe their origin to the nucleolus. Golenkin ('00), in his paper on *Sphaeroplea*, describes conditions according to which the nucleolus breaks up directly into morphological chromosomes. This author goes further in stating that nuclei organized upon this plan are characteristic of many green Algae and Musci. In view of the fact that Golenkin did not section his material, it would seem entirely possible that the fibrillae above described might easily escape such an examination, and altogether likely that a method better adapted to reveal such detail would show conditions which would bring these forms more nearly into harmony with the accounts

of these phenomena as described in *Nemalion*, *Phaseolus*, and many other plants.

Such nucleoli have been variously characterized as false nucleoli, chromatin nucleoli, or karyosomes, in contradistinction from the true nucleoli, or plasmosomes, which appear to contain no chromatin, and are supposed by some to be cast out of the nucleus at mitosis. Since, however, they disappear immediately before the formation of the achromatic spindle, they are held by other observers to be concerned in its formation. In general, the former have been supposed to be confined to lower plants, while the so-called plasmosomes were believed to be characteristic of vascular plants. Evidence is accumulating, however, which appears to indicate that even in the Phanerogams the body hitherto distinguished as the plasmosome is also concerned in the formation of the chromosomes, and suggests that further research in this direction is likely to show that similar conditions are, at least, of very general occurrence throughout the plant kingdom. The data at present available would thus seem to indicate that the differences existing between the various structures which in the nuclei of plants have been termed nucleoli are not such as would justify their separation under two distinct categories; and that, although forms may exist which contain little or no chromatin, every gradation may be observed through a gradual increase of the chromatin content, to such conditions as are illustrated by *Nemalion* or *Sphaeroplea*, in which the nucleolus represents the entire chromatin content; and therefore the writer would agree with Wager in the conclusion (p. 50, l. c.) that 'the nucleolus is intimately bound up with the formation of the chromosomes, and Strasburger's contention that it is only concerned in spindle or kinoplasmic formatin does not hold good, although it is not impossible that a portion of it—the plastin or pyrenin of Zacharias and Schwarz—may be used up in this way.'

Reduction.

As has already been mentioned in the introduction, the primary object for which the present investigation was undertaken was to determine whether in the plant under consideration a differentiation into gametophyte and sporophyte could be proved, and, if so, to what extent the limits of these generations could be fixed by such cytological evidence as might be obtained. As will be seen by the following account, we may assume the existence of such an alternation, and hence with propriety characterize the period comprising the earlier divisions of the gonimoblasts as the sporophyte, and as the gametophyte that beginning with spore-formation and ending with the differentiation of the sexual elements.

As previously stated in the description of the behaviour of the nucleolus, the number of chromatic masses appearing upon the nuclear wall in

prophase is far in excess of the chromosome-number characteristic of that generation, sporophyte, or gametophyte, respectively, in which the division occurs. Since the diameter of the nucleus does not exceed 3 μ, it is evident that an exact enumeration is a very difficult matter, and, in fact, in the earlier divisions of the gonimoblasts estimates of the number of these masses varied from twenty to thirty, and appeared to be correspondingly less in the gametophyte. Inasmuch as the number of chromosomes eventually appearing is less than the number of granules by about half, it is not impossible that these granules may be chromatic units which may fuse two and two at the time of their organization into morphological chromosomes, although the details of this process could not be determined.

The chromosomes are rounded bodies which stain intensely, and are thus, notwithstanding their small size, fairly distinct. From a number of estimates very carefully made and repeatedly confirmed, the writer feels entirely safe in stating that in the divisions of vegetative nuclei they are about eight in number; certainly not less than seven, nor more than nine (Figs. 29 and 75).

In the cystocarp the details of mitosis could not be followed for the first two or three divisions of the zygote, owing to the fact, previously mentioned, that the cytoplasm at this time stains so intensely. In all divisions subsequent to this period, however, mitotic figures were observed in a variety of stages and in great abundance. In the earlier divisions in which the mitotic figures could be clearly differentiated, the chromosomes, owing to their crowded position upon the nuclear plate, form at times an almost continuous band (Fig. 65), and in general their number could not be ascertained with the same degree of certainty as in the vegetative phase. Owing to the fact that these preliminary aggregations of chromatin, illustrated in Fig. 56 in the prophase stages, might possibly be mistaken for polar views of the spindle at metaphase, polar views were taken as corroborative only, and such approximations alone relied upon as could be obtained from longitudinal and oblique views in which, since both centrosomes were present and the spindle clearly differentiated, it could be definitely determined that the bodies in question were morphologically chromosomes. A great number of estimates were made, based upon such stages, and certainly the number is greatly in excess of that characteristic of the vegetative phase, approximations varying from twelve to sixteen, with the weight of probability in favour of the latter number (Figs. 61–65). The decided increase in breadth of the spindle, which has previously been noted as characteristic of the earlier mitoses in the cystocarp, further indicates the presence of a greater number of chromosomes in these divisions. In this connexion, perhaps, it should be stated that the phenomenon of 'splitting' presents characteristic features and cannot be

easily mistaken for the conditions above described, since the daughter-chromosomes are at this period, the early anaphase, approximately thirty-two in number, and are, in addition, arranged uniformly in two receding circles (Fig. 66).

Since, as we have seen, the number of chromosomes in the earlier dividing nuclei of the cystocarp are approximately double that seen in the vegetative cells, a reduction must be assumed to occur at some point in this life history. By analogy with the conditions seen in higher plants, it might be expected that this reduction would be associated with the formation of spores; and such, in fact, has been found to be the case. At a more advanced stage in the development of the cystocarp (Figs. 67 and 68), mitotic figures were seen in terminal cells, which present a marked contrast to those previously occurring in these divisions (Figs. 61–65), but agreeing absolutely in number of chromosomes, shape of spindle, and all other details, so far as could be seen, with the figures observed in vegetative cells (Figs. 29 and 75). Moreover, it seems certain that the new cell resulting from this reduction division is destined to become a spore. The further history of the sister-nucleus which remains within the cell in which this reduction division occurs was, however, not followed, owing to the great difficulties of orientation involved. The habit of proliferation, already briefly described, apparently admits of a rather wide variability in the number of spores that may be formed from such a cell, inasmuch as it gives rise terminally and laterally to spores as well as to short branches bearing spores. The number is further multiplied by proliferation, within the mother-cell, more than once repeated after the carpospores have been successively discharged (Text-fig. 51). Although it has been impossible to follow out this matter in detail and with certainty, owing to the complicated conditions just mentioned, it is entirely probable that the reduced number of chromosomes once established in a given cell persists throughout its further divisions, and is continued in all its products. Since, however, the reduction division in this plant is not associated with the formation of a tetrad of spores, a parallelism with higher plants does not exist beyond the phenomenon of actual reduction.

The process by which the chromatin is reorganized into the reduced number of chromosomes in the prophases of this reduction division could not be followed, owing, as may be readily seen, to the fact that in the reconstruction of the daughter-nuclei resulting from the preceding division, these structures necessarily lose all morphological identity, since it is from their fusion that the nucleoli are formed. Inasmuch as an extrusion of chromatic substance has in some cases been described as being associated with the reduction division, it might perhaps be well to mention in this connexion the fact that the chromatic extrusion previously referred to in this paper, while seen to occur in connexion with reduction (Fig. 68),

does not appear to be confined to such divisions alone, being seen in connexion with what appeared to be a much larger number of chromosomes.

The events above described constitute, in the opinion of the writer, conclusive evidence in support of a view long maintained, according to which the cystocarp of the red Algae is held to be the homologue of the sporophyte in higher plants. Resulting from a sexual act, intervening between that sexual act and the formation of spores, bearing a spore which gives rise to an individual unlike that upon which it is borne, and finally presenting double the number of chromosomes seen in the vegetative phase, it possesses the essential characters of such a sporophyte.

Summary.

Since it has seemed to the writer that the present condition of our information concerning the phenomena of reduction in Thallophytes is far too meagre and conflicting to render any further discussion of the subject at the present time of value, he has chosen to content himself with the presentation of such matters of fact as appear to have been developed in the course of this investigation. It may, however, be pointed out that in the Florideae, a family usually regarded as an independent and terminal phylum, or at least in *Nemalion*, which is not considered as an illustration of their highest development, a condition of alternation is present which, in so far as the cytological phenomena accompanying it are concerned, is very closely comparable with that which is familiar in the archegoniate series—the double number of chromosomes appearing after the union of the gametes and continuing in the sporophyte to the point at which spore-mother-cells are formed. Regarding, then, the present paper as a contribution which may be of value when a sufficient body of information concerning such cytological conditions in the lower plants renders their co-ordination possible, the general results obtained may be summarized as follows:—

(1) The chromatophore of *Nemalion* is in the form of a hollow ellipsoid from which processes radiate to the periphery of the cell, and there flatten out to form a clathrate membrane.

(2) The region surrounded by the ellipsoid portion of the chromatophore, and generally regarded as a pyrenoid, consists entirely of vacuolar material, and hence cannot be considered as constituting any such definite organ of the cell.

(3) The chromatophore is present in all cells of the plant, with the exception of the mature antheridium and the two sperm-cells to which it gives rise; and in all cases originates from a pre-existing body of the same nature.

(4) The sex-organs cannot be regarded as unicellular structures: since

(5) In earlier stages the trichogyne possesses a well-organized nucleus, which fragments as that organ matures; and, the egg-cell thus becoming an intercalary cell, the trichogyne must be regarded as a cell which has been specialized in connexion with the reproductive processes, and further,

(6) The contents of the spermatium normally divide into two fertilizing elements which are discharged into the trichogyne: these events constitute this so-called spermatium an antheridium.

(7) Fertilization consists essentially in the union of a male and a female gamete, and from the further divisions of the resultant fusion-nucleus the cystocarp arises after the separation of the stalk-cell.

(8) In the mature cystocarp the ultimate cells give rise terminally, as well as by subterminal proliferation, to a variable number of carpospores, which is further augmented by repeated proliferation within the successively formed mother-cell walls.

(9) In the nucleus the entire chromatin content is stored in the nucleolus, and in the prophases of division passes to the nuclear wall along delicate fibrillae.

(10) The spindle is intra-nuclear, and centrosomes are distinctly visible at the poles at metaphase.

(11) The conclusion that *Nemalion* presents the essentials of an antithetic alternation of generations, and that the cystocarp is, therefore, the homologue of the sporophyte in higher plants, is indicated by the cytological evidence—since

(12) Approximately sixteen chromosomes are present in the divisions of the cells of the cystocarp up to the period of spore-formation, and approximately eight in those of the thallus; the reduction division being immediately associated with the production of the carpospores.

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EXPLANATION OF FIGURES IN PLATES XL and XLI.

Illustrating Dr. Wolfe's Cytological Studies on *Nemalion*.

All figures are drawn at the level of the stage with the aid of a camera lucida, and using the apochromatic 2.0 mm. objective of Zeiss with compensating ocular 18, giving an approximate magnification of 2250 diameters. Plate XL is reduced about four-sevenths in reproduction, the other is reproduced without reduction. Figs. 2–19, 49 and 50 are drawn from specimens crushed and stained on the slide in safranin and gentian-violet; 24, from material preserved in formatin, and mounted in glycerine; 25 from a section 1 μ, 22, 35, and 44–48 from sections 3 μ in thickness, and stained in safranin and gentian-violet; all others are from 3 μ preparations stained in haematoxylin after Heidenhain.

Oogenesis.

Fig. 1. Young carpogenic branch, consisting of the primary carpogonial cell borne on a vegetative cell. A nucleus and chromatophore are present in each.

Fig. 2. Older. The procarp is supported upon three modified cells. The branch arises from a normal vegetative cell. The procarp is bulging out to form the trichogyne.

Fig. 3. The trichogyne is now a narrow protoplasmic process surrounded by a heavy wall. The primary egg-nucleus is in its characteristic position below the chromatophore.

Fig. 4. The egg-nucleus is now above the chromatophore—an unusual position. The primary egg-nucleus has probably divided, giving rise to the egg-nucleus, and to that of the trichogyne. The wall of the trichogyne is now, and also in later stages, very thin at the tip.

Fig. 5. The egg-nucleus has resumed its normal position below the chromatophore and in the basal portion of the cell.

Fig. 6. The trichogyne is elongating, and the nucleus is still intact.

Fig. 7. Fragmentation of the nucleus of the trichogyne is beginning to take place.

Figs. 8–10. Later stages in the process of fragmentation. The fragments gradually stain less deeply. A thin wall surrounds the entire receptive portion of the trichogyne.

Fig. 11. The carpogonium is now ready for fertilization, and shows what are probably the last traces of a disorganizing nucleus.

Fertilization and Development of the Cystocarp.

Fig. 12. An early stage in fertilization. Residual protoplasm and fragments, probably, of the second male nucleus, are seen in the spermatium, which has conjugated with the trichogyne. The male nucleus is in the top of the carpogonium, while the egg-nucleus is still in the basal portion. The protoplasm of the trichogyne has shrunk away from the thin wall, and is beginning to disorganize.

Fig. 13. Actual fusion of the male and female nuclei, the male the smaller of the two. Its nucleolus is at one side. The nuclear walls are more distinct than usual.

Fig. 14. A similar stage. Apparently a second nuclear body lying beside the chromatophore.

Fig. 15. The same stage.

Fig. 16. The fusion-nucleus now lies in the top of the carpogonium. The separation of the trichogyne is beginning to take place. Three spermatia are in contact. The one at the left has not fused, and its nucleus has not divided. The empty one has probably given rise to two sperms, one of which has fused with the egg-nucleus, and the other may still be seen midway the trichogyne. The third spermatium has just discharged one of its sperms, and the other is still within the mother-cell.

Fig. 17. A wall is now formed around the zygote, and the fusion-nucleus has resumed its usual position at the base.

Fig. 18. Division of the zygote into a stalk and a sporogenous cell. The chromatophore has already divided.

Fig. 19. Somewhat later.

Fig. 20. Similar to 17. Granular masses appear in the hypogynous cell.

Fig. 21. First division of the sporogenous cell. The nucleus of the cell to the right is in a lower focus. The dark mass is probably the chromatophore. The chromatophores of the stalk and hypogynous cells are disintegrating.

Fig. 22. A section of a young cystocarp at about the six-celled stage. The chromatophores are now distinctly differentiated.

Fig. 23. Gonimoblasts. The terminal cells are separating as spores. Chromatophores are present in all the cells.

Structure of the Chromatophore.

Fig. 24. The chromatophore may be seen to consist of a central ellipsoid, from which processes radiate to the periphery of the cell, and there flatten out into a clathrate membrane.

Fig. 25. An absolute median section through the chromatophore, showing the interior of the ellipsoidal body to be devoid of protoplasmic contents.

Division of the Chromatophore.

Figs. 26 and 27. Processes extending into the new cell.

Fig. 28. Illustrating a rapid division of the chromatophore independent of the nucleus.

Fig. 29. The central body is bulging out into the daughter-cell.

Fig. 30. The bulge has increased until the two together present the appearance of a single elongated chromatophore.

Fig. 31. Constriction beginning.

Fig. 32. Division almost complete. A hyaline region of the protoplasm marks the position of the future wall.

Fig. 33. The wall forming.

Fig. 34. Cell-division complete.

Spermatogenesis.

Fig. 35. A young antheridial branch.

Fig. 36. An antheridium being formed. The nucleus in anaphase. About eight chromosomes are present.

Fig. 37. Antheridia in various stages.

Fig. 38. Nuclear division in the antheridial branch. No centrosome apparent.

Figs. 39 and 40. The centrosome is seen in some cases.

Fig. 41. Nuclear reorganization.

Fig. 42. The chromatophore is protruding into the antheridial mother-cell, and about to divide.

Fig. 43. A chromatophore in the antheridium.

Fig. 44. Granular material appears at the distal end of the antheridium. No chromatophore can now be differentiated.

Fig. 45. The nucleus appearing within this material.

Fig. 46. The nucleus is now greatly increased in size, and shows definite aggregations of material on the nuclear wall. The antheridium is now mature.

Fig. 47. The antheridium after it has been discharged into the water. A median section.

Fig. 48. The same in surface view.

Fig. 49. An antheridium conjugating with the trichogyne, and dividing into two sperm-cells.

Fig. 50. A similar stage.

Mitosis.

Fig. 51. Illustrating the structure of the nucleolus, with the chromatin at the periphery and surrounding a lightly staining substance. Radiating fibrillae are present.

Fig. 52. The chromatic material gathering into clumps.

Fig. 53. Probably centrosomes.

Figs. 54 and 55. The chromatin is passing along fibrillae out of the nucleolus to the nuclear wall. Prophase.

Fig. 56. Somewhat later. Also an extrusion of nucleolar substance.

Fig. 57. The nucleolus at this stage is somewhat lenticular in shape.

Fig. 58. The chromatin granules are now aggregated into morphological chromosomes.

Fig. 59. The chromosomes are grouped in the equatorial region of the nucleus.

Fig. 60. A similar stage.

Figs. 61–63. The chromosomes are gathering at the nuclear plate. The spindle is now present with distinct centrosomes at the poles.

Figs. 64 and 65. Metaphase in the sporophyte. About sixteen chromosomes present.

Fig. 66. Anaphase.

Fig. 67. The reduction division, showing about eight chromosomes.

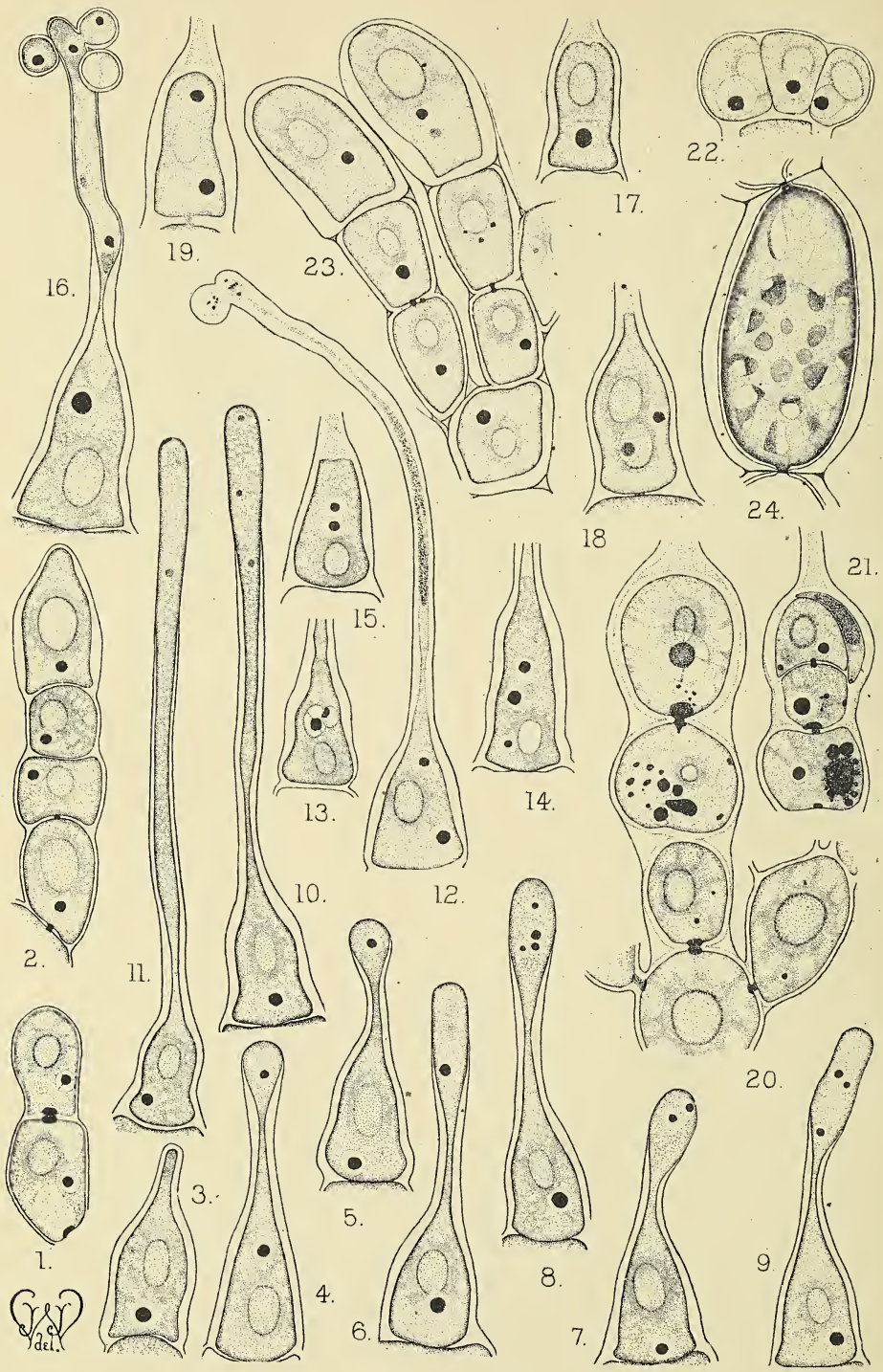
Fig. 68. The same stage, but showing in addition an extrusion of nucleolar material.

Fig. 69. The anaphase of the reduction division.

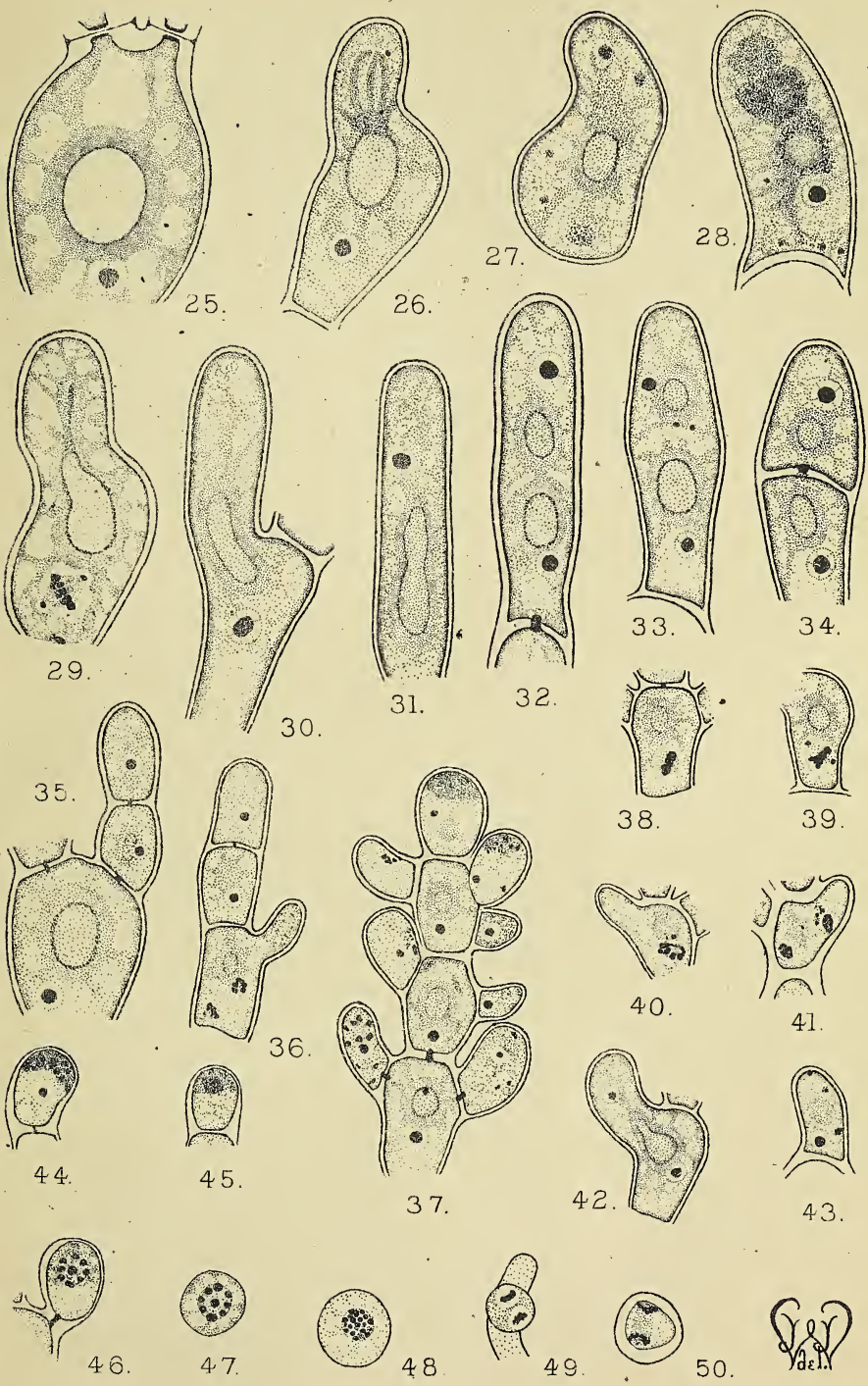
Figs. 70–72. Stages in the fusion of the chromosomes to form the daughter-nucleoli.

Figs. 73 and 74. Vegetative prophase corresponding to 54 and 60, respectively, in the cystocarp.

Fig. 75. Vegetative metaphase corresponding to 64 in the sporophyte, and to 67 of the reduction stages.



WOLFE.—NEMALION.



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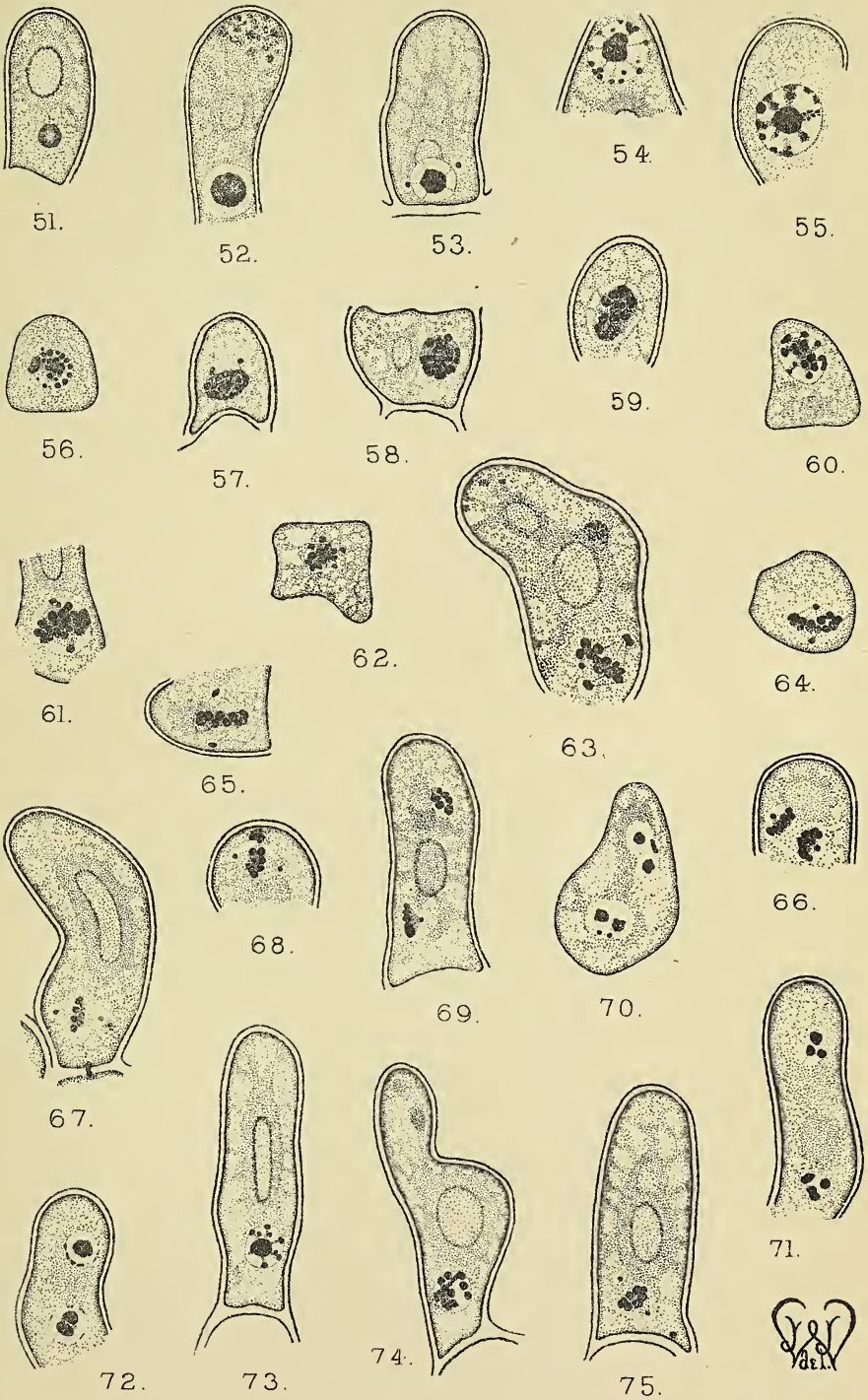

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WOLFE.—NEMALION.

An Undescribed Thermometric Movement of the Branches in Shrubs and Trees[1].

BY

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Professor of Botany in Smith College.

With six Figures in the Text.

SOME years ago I noticed an apparent radial movement of the ascending branches in certain shrubs and small trees, whereby the branches were brought closer to the main stem in the winter, quite independently of the leaf-fall, and were separated from it on the approach of spring. After trying in vain to find some account of this movement, and its causes, in the literature accessible to me[2], and from various persons informed on such matters, I undertook a study of it, with results which follow.

In the autumn of 1898 I chose six shrubs and small trees, in the Botanic Garden of Smith College, which showed the movement and which were isolated from other woody plants. Selecting long slender branches on the north, south, east, and west sides of each plant, I made near the top of each, and on the side radial to the plant, small dots with water-proof India ink, the approximate positions of which were marked for convenience by coloured threads. It was then possible, with the aid of an assistant, to

[1] Read before the Society for Plant Morphology and Physiology, at its Philadelphia Meeting, Dec. 29, 1903.

[2] I have found no direct references to this movement, although it seems unlikely that it could have escaped notice and description; and the only other mention of it that I have been able to secure by inquiry is a statement in a letter that a resident of Washington, D.C., has noticed it in the lower branches of the *Ginkgo*. The inward movement of the branches after removal of the weight of the leaves in autumn is said to be known to nurserymen; and some measurements of this movement in a shrub are given in a note by Agnes Frye in *Nature*, vol. lv, 1896, p. 198, and in a branch of horse chestnut, by Miller Christy in *Journal of the Linnean Society*, xxxiii, 1898, pp. 501–506. The works of Wiesner, Baranetsky, and others on the determinants of branch position appear not to touch this subject. Recently Mr. E. F. Bigelow, of Stamford, Conn., has written me that two correspondents of his have asked him the causes of branch movements noticed by them; in one case it was a spruce, whose branches rise in wet weather and fall in dry, and in the other it was a pine, whose dead lower branches rise in warm, and fall in cold weather. Apparently there is more in this subject than has hitherto been supposed.

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measure with a tape the distance between the diametrically opposite marks (i. e. from the north to the south branch, and from the east to the west), and thus to determine any movement the branches might make. The method is illustrated by the accompanying diagram (Fig. 52). The resultant measurements for the four shrubs which showed the most marked move-

FIG. 52.

FIG. 53.

ment are plotted upon Fig. 54, and the more important figures are contained in the following table:—

The Plant.	*Date.*	*Condition of Plant.*	*N. and S.*	*E. and W.*
Salix laurifolia (about 3 meters high)	Oct. 10	Leaves all on	261·2 cm.	247 cm.
	Nov. 12	Leaves all gone	257	241·7
	Jan. 20	In full winter condition	248·5	234
	Apr. 22	Buds beginning to swell	245	232
	May 25	Leaves all out	290·5	267
	June 24	In full summer condition	293	274
Cercidiphyllum japonicum (about 2 meters high)	Oct. 10	Leaves all on (nearly)	114·5	128
	Oct. 28	Leaves all gone	110·5	125·3
	Jan. 20	In full winter condition	106	121·5
	Apr. 22	Buds beginning to swell	107	122
	May 25	Leaves nearly all out	112	128
	June 24	In full summer condition	118	130
Cornus florida (under 2 meters high)	Oct. 10	Leaves all on	133·3	236·7
	Nov. 12	Leaves all gone	114·7	207·3
	Jan. 20	In full winter condition	109·5	204
	Apr. 6	Buds swelling	113	204
	May 25	Leaves well out	121	217
	June 24	In full summer condition	136	235
Broussonetia papyrifera (about 1·5 meters high)	Oct. 10	Leaves all on	168·2	206·8
	Nov. 12	Leaves all gone	152·4	187·8
	Jan. 20	In full winter condition	142·5	174·5
	Apr. 22	No trace of leaves	136·5	164
	May 25	The plant evidently winter-killed	129	156

These measurements showed :—

1. A large inward movement accompanying the fall of the leaves, and an outward movement accompanying the formation of new leaves.

2. A real seasonal movement independent of leaf-fall and leaf-formation, consisting in an inward movement during the advancing winter, and an outward movement on the approach of spring.

3. Certain fluctuations in the movements, the reasons for which were not evident.

The causes of the movement accompanying leaf-fall and leaf-formation are so evident as hardly to call for comment ; the movement is simply due to the removal of the weight of the leaves and their contained water from the elastic, obliquely-ascending branches in the one case, and the addition of weight in the other. But the cause of the further seasonal movement of the leafless branches is not at once evident.

The measurements showed not only that there is a real movement of the leafless branches, but that it is of considerable amount, reaching between leaf-fall and leaf-formation —

12 cm., or 5 % of the total diameter of the plant in *Salix laurifolia* ;

3·5 cm., or over 3 % of the diameter of the plant in *Cercidiphyllum japonicum* ;

5·3 cm., or over 5 % of the diameter of the plant in *Cornus florida* ;

And a larger though uncertain amount in *Broussonetia papyrifera.*

The results were of such interest that a more careful study of the subject was undertaken the following winter (1899–1900). An improvement was made in the method in two respects. First, the movement of each branch was measured separately in order to determine whether there was any difference in the movement of the different branches. This was effected by placing, in all measurements, the loop of the tape (a Chesterman steel tape as used the preceding year) over a brass screw held by a cork set in the top of a piece of stout gas-pipe, which was driven firmly into the ground as nearly as possible in the centre of the shrub (as represented by Fig. 53). It is important to note that this, like any other method of measuring such movements from a fixed point, does not give strictly accurate results, because the marks on the branches do not move in and out along the same radial line, but in different lines. In general, however, the errors from this source are very slight, they tend to neutralize one another, and as a whole they affect the results in the direction of a lesser rather than a greater amount. Secondly, some suggestion having arisen that temperature might have an effect upon the process, the air temperature was recorded at each measurement. The measurements were made by one of my senior students, Miss Phœbe Persons, as often as the weather would permit, throughout the autumn, winter, and spring. One of the greatest difficulties in this study consists in the fact that the measure-

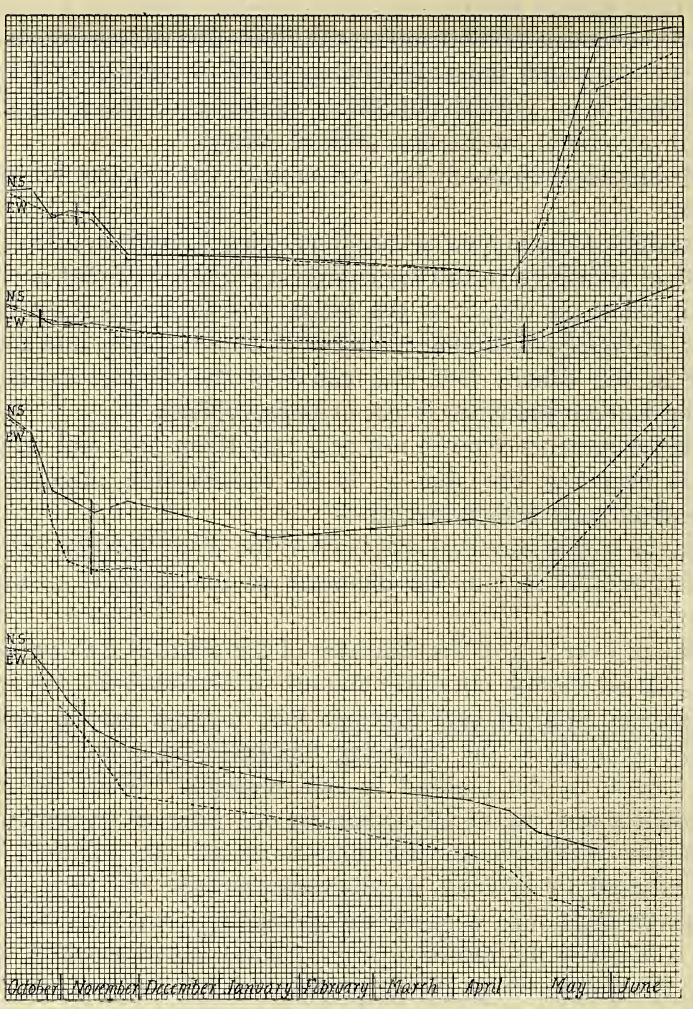

FIG. 54.

Abscissa spaces, each two days.
Ordinate spaces, each 2·5 mm. of movement.
Downward direction means inward of the shrub, and upward means outward.
Showing the seasonal movement of four shrubs :—
The upper is *Salix laurifolia*,
the second is *Cercidiphyllum japonicum*,
the third is *Cornus florida*,
the lower is *Broussonetia papyrifera*.
The entire lines are the north and south measurement,
the dotted lines are the east and west measurement,
the vertical lines across the polygons represent the time of complete leaf-fall and of the first appearance of the leaves from the bud (the latter for *Cornus* was not recorded).
The disagreement of *Broussonetia* was connected with the death (winter-killing) of the plant.

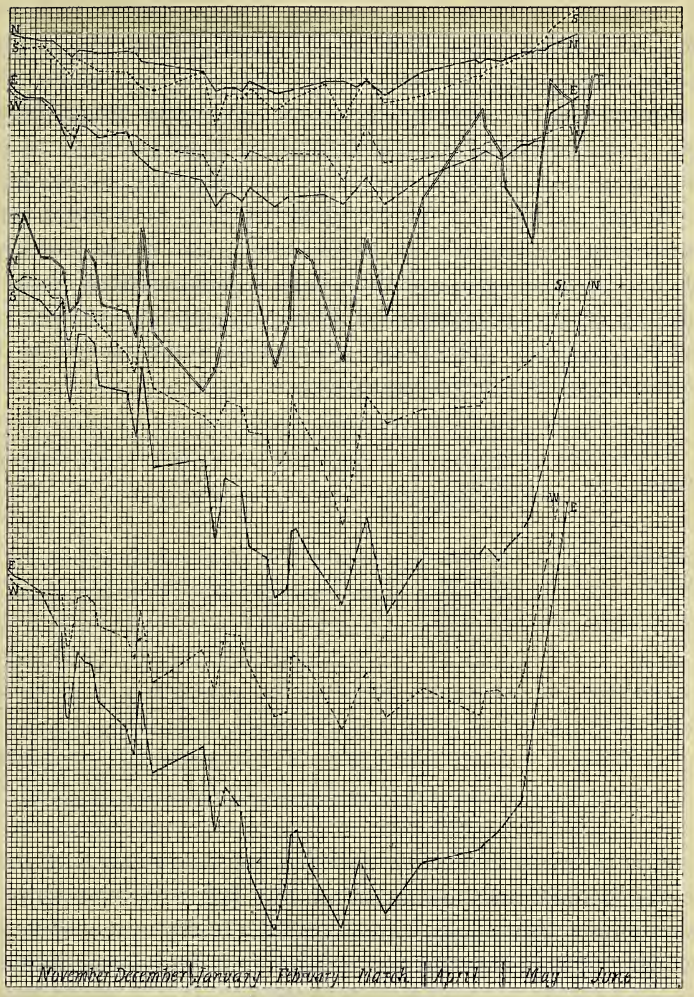

FIG. 55.

Abscissa spaces, each two days.

Ordinate spaces, each 2·5 mm. of movement, and 1 degree of temperature.

Downward direction means inward of the shrub, and upward means outward.

The plate shows the movement of the branches in two shrubs :—

The upper is *Lindera Benzoin*, the north, south, east, and west branches being indicated by the initials N. S. E. W.

The lower is *Salix laurifolia*, the respective branches being indicated as for the *Lindera*.

The double line between the two shrubs represents temperature.

The records begin after the leaves were mostly fallen, and continue until the new leaves were largely formed.

ments can be made, especially when any of the leaves are on, only in perfectly still weather; and hence a continuous study of the movement in exact correlation with external physical conditions is well-nigh impossible. Doubtless shelters could be devised to permit measurements in any weather, but with large plants this would be a matter of much difficulty, and it was not attempted by us.

The shrubs studied during the winter were the seven listed in the table below. The results of the measurements of all seven were, except for minor differences, very similar, and they are fully illustrated by the two examples plotted on Fig. 55, which represents the movement for *Lindera Benzoin*, an average representative of the series, and *Salix laurifolia*, which was one of the two which showed the greatest movement of them all. The total amplitude of the movement between leaf-fall and leaf-formation for all the branches, and the percentage which this movement is of the shrub radius, are shown by the following table:—

Plant.	*Size in metres.* ht. diam.	*Movement in centimetres.*					*Percentage movement.*				
		N.	S.	E.	W.	Av.	N.	S.	E.	W.	Av.
Pyrus americana . . .	1·90 × 1·20	2·3	1·4	3·7	1·4	2·20	03	02	07	02	3·5
Salix laurifolia	4·50 × 3·10	11·2	6·0	9·4	8·0	8.65	12	09	10	09	10
Cornus sericea	2·20 × 1·90	5·0	5·6	4·9	4·7	5·05	07	05	04	05	5·2
Cercidiphyllum japonicum	2·80 × 1·40	5·2	2·8	3·9	3·1	3·75	13	02	07	02	6·0
Cornus florida	2·30 × 1·90	5·5	2·5	7·9	5·9	5·45	05	03	07	03	4·5
Lindera Benzoin . . .	1·60 × 1·20	3·1	5·4	8·1	4·5	5·27	09	06	12	06	8.2
Carpinus carolinianus .	2·30 × 2·00	8·4	9·5	8·7	9·5	9·02	11	07	07	07	8·0
		40·7	33·2	46·6	37·1		60	34	54	34	
		5·8	4·7	6·6	5·3		8·5	4·8	7·7	4·8	

Further, in order to determine the effect of fluctuations of temperature through a single day, and the effect of the fall of temperature at night, Miss Persons made, after several unsuccessful attempts, a series of measurements through one still day and part of the next, and found that within the limits of a single day and night the movement was considerable, and that it was correlated with the temperature changes, though lagging somewhat behind the latter. Another series of measurements made by her was directed to determine whether the movement was most pronounced in the younger or older parts of the branches, and she found, as was to be expected, that it was much more marked in the young parts. In general the movement is greatest in the longest, most slender, and youngest branches, and it becomes less with the reverse of those features. Miss Persons also made a detailed study of the anatomy of the stems she measured, but she was unable, as I have been since, to connect the movement with any peculiarities of anatomical structure.

In summary the results showed :—

1. The seasonal movement observed the year before is confirmed, and is shown to consist in a gradual inward movement from leaf-fall until March, when an outward movement begins.

2. There exists in addition to this seasonal movement a secondary movement, which is closely dependent upon temperature (as shown by the typical examples on Fig. 55), a higher temperature resulting in an outward, and a lower in an inward movement, and this movement is appreciable within a single day and night.

3. There are sundry irregularities in the movements, and apparently a greater movement in north and east than in south and west branches.

The close correlation between the secondary movement and changes of temperature thus demonstrated is interesting and important, and it is close enough to warrant the application to the movement of the term *thermometric*. Since the minor movement is of this character, the question at once naturally arises whether the seasonal movement may not be of the same character, that is, whether the seasonal movement may not be simply a thermometric movement of huge amplitude. This point will be discussed below.

The results in relation to the respective amounts of movement in the different branches were not satisfactory. In general they showed more movement in the north and east branches, but with so many exceptions and irregularities that no conclusions can be drawn from them, the more especially as no precautions were taken to select branches of the same length and distance from the central post. Furthermore, both Miss Persons and myself were influenced by a belief that the north and east branches did move the most, and hence doubtless something of a personal equation, or rather an equation of prejudice, in this direction became incorporated into the results.

The reality of the seasonal movement, and the correlation of the secondary movement with temperature changes, being thus made apparent, it remained to ascertain their precise physical basis, a subject both of much interest in itself and also important for the light it might throw upon the significance of the movement to the plant. Reviewing the facts so far observed, it seemed plain that the relation of the two movements may be either one of these two :—(*a*) they may be due to the same causes, the secondary inward-and-outward fluctuations being the result of temporary intensifications and weakenings of the factors (connected with temperature) producing the seasonal movement; or (*b*) they may be due to different causes (or at least to a difference in the mode of operation of the same causes), the secondary fluctuations being temporary movements due to special causes, either out from, or in from, the line of general seasonal movement. The facts at our command seemed at first to point to the latter

probability, and in order to obtain a definite basis for experiment we assumed that the secondary fluctuations were simply outward movements from the seasonal position. As a physical (or mechanical) cause of this outward movement under higher temperature, it seemed to us likely that the warming up, and consequent swelling, of the inner faces of the long slender branches under the influence of the sunlight on the warmer days was sufficient. Evidently this hypothesis could be submitted to experiment, for not only ought the outward movement to be greater on a sunny than upon a cloudy day of approximately the same temperature, but the movement in branches illuminated at the time of measurement on their inner faces should show more movement than those at that time shaded, or, still better, than those illuminated upon their outer faces. Simple as such a test appears the weather never allowed us to put it to satisfactory use, and the season closed without its accomplishment.

The following winter, 1900–1, I was occupied with other matters and did nothing with this subject; but the next year, 1901–2, I resumed the study. Influenced by the theory above mentioned, I prepared to make more exact measurements than before of the respective movements of the four branches, for it was evident the theory could be tested by observing whether, as it requires, the greatest amplitude of movement occurs in the north branches, the next greatest in that east or west branch which happened to be illuminated on its inner face, and the least in the south branches. I made an improvement in Miss Persons's method by replacing the single gas pipe, which would yield a little under tension when the tape was drawn tight, by a perfectly firm tripod, formed of three gas pipes driven deeply into the ground, and bound immovably at their tops by twisted copper wire into which the brass screw was set, an arrangement illustrated diagrammatically in Fig. 56. Throughout the winter very careful measurements were made of six shrubs, including the *Lindera* and *Cercidiphyllum* used the previous winter, together with two species of *Salix* and two species of *Populus* (young trees). The results need not here be given in detail, since in general they are simply confirmatory of those earlier obtained. As to the two main points at issue they were as follows:—

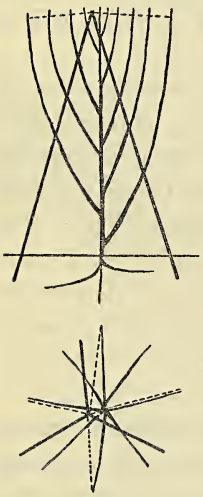

Fig. 56.

1. There was no such regularity or order in the amplitudes of movement of the respective branches as the theory required.

2. There was no regular influence produced upon the movements by the presence or absence of direct sunlight upon the faces of the branches,

though in some individual cases this did appear to have some slight effect.

It occurred to me during the winter, especially when it became plain that the direct sunlight played little part, that perhaps the movement might be due to a warming, and hence swelling, of the inner faces of all the branches through a general warming up of the air among the branches of the shrub due to the reflection of the sun's heat from one branch to another. To test this I placed very accurate thermometers, reading precisely alike and graduated to tenths of a degree, both near the centre of the shrub (but in the sun), and outside the shrub a few feet away. They showed that the temperature among the branches and that outside the shrub were not appreciably different, thus eliminating another possible cause of the movement.

The idea that a direct action of the sun upon the plant produced the movement had therefore to be abandoned.

The following winter, 1902–3, I continued the study, concentrating attention upon two plants, *Lindera Benzoin* and a species of *Salix*, which had shown themselves particularly sensitive to temperature changes. Incidentally I re-measured these two shrubs very carefully through the winter, and the results for *Lindera* are given on Fig. 57, not because they bring out anything new, but because they show with particular clearness the correlation of movement with temperature. But the principal work during the winter was experimental, and directed to discover the precise physical basis of the movement. Its results were as follows. Certain observations made while measuring the shrubs seemed to render it probable that the outward movement was caused by the straightening of the curved branches due to the swelling of the air, and perhaps also the water, in the stems under the influence of the higher temperature. A marked swelling of this kind should produce a straightening of the branch upon precisely the same principle as it straightens the bulb of a Richard thermograph. This could be tested by bringing typical curved branches from the shrubs on very cold days directly into a warm greenhouse, and comparing the distance between the base and tip before and after the branch had time to warm up. I tried this in a variety of ways, even bringing them abruptly from a temperature much below 0° (C.) directly into a large case kept at a temperature above 30°. To make the conditions as to water supply as uniform as possible, I plunged the branches at once into water in some cases (cutting them under water higher up the stem in some instances), and immediately sealed the cut ends with shellac in others. The results in all cases were the same. A slight straightening could often be observed within a few minutes under the higher temperature, but this was always lost within an hour or thereabouts, and was then replaced by a gradually increasing curvature. It became plain, therefore, that while a rise in temperature might cause

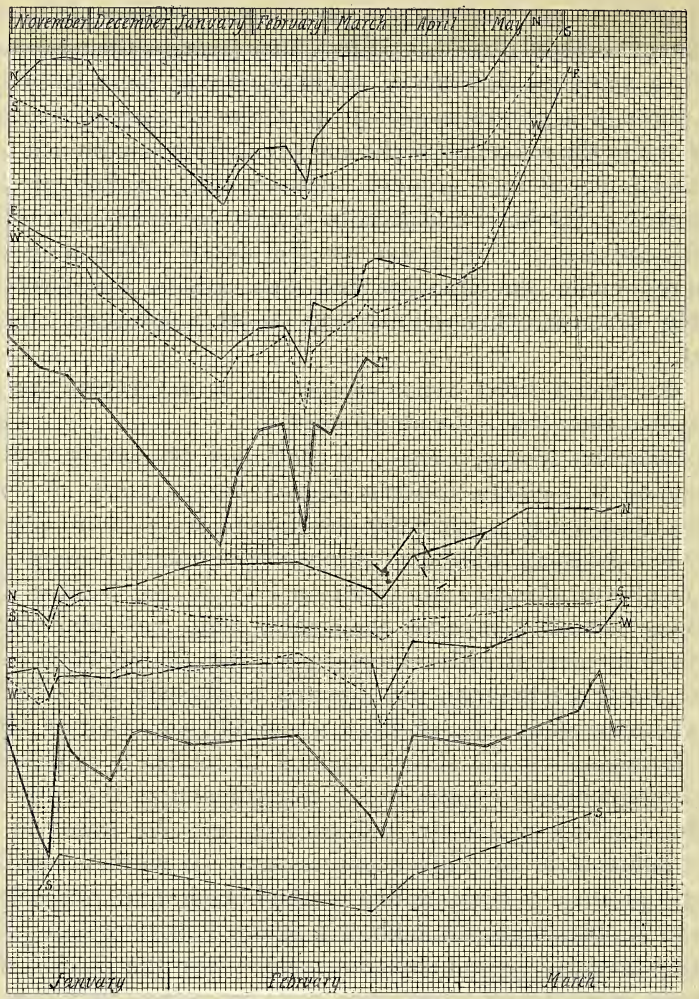

FIG. 57.

Abscissa spaces of upper five polygons, each two days; of lower six polygons, one half-day.

Ordinate spaces, each 2 mm. of movement (thus differing slightly from two preceding plates) and 1 degree of temperature, and 1% of water (for lower polygon).

Downward direction means inward of the shrub, and upward means outward.

The plate shows the movement of the branches of *Lindera Benzoin*, the north, south, east, and west branches being indicated by the initials N. S. E. W.

The upper four polygons show the movement through the season, and the four below them show it upon a larger lateral scale through the period of greatest secondary movement.

The double line represents temperature.

The lowermost line represents the percentage of water contained in *Salix*.

(On irregularities in this plate see note on page 644.)

a slight straightening and hence outward movement, which might in the uninjured plant remain constant, such a cause was wholly insufficient to account for the entire amount of movement. That the swelling of air in the stem did not produce the result was proven by forcing air powerfully into the stem with a foot pump, a process always without appreciable result.

Having thus to abandon this hypothesis I turned to another, more than once taken up and dropped in the earlier part of the study, that the movement was in some way connected with the quantity of water present in the stem. This was, indeed, very strongly indicated by two facts: (*a*) the *Broussonetia* earlier referred to (p. 634) showed a continuous incurving of its branches after the plant was dead, which incurving was apparently correlated with the drying out of the branches; and (*b*) invariably during the experiments a drying out of any branch was accompanied by an incurving, that is, by an inward movement. The incurved, or extreme inward position, is evidently the natural position of the dry tissues, and it seemed probable, therefore, that the outward movement might be correlated with, and proportional to, the amount of water in the stem. This supposition could evidently be readily submitted to experiment. Accordingly on certain days showing extreme outward and inward movement, and therefore of extreme high and low temperature, during the winter, I cut from each of these shrubs ten healthy branches each 10 cm. long, tied them in bunches, and immediately weighed the latter. They were then dried for several months in a dry room, and subsequently for some days in a water bath. They were then again weighed, and the percentage of water in the original branches was thus readily determined. The results were as follows:—

Date.	*Temp.*	*Plant.*	*Original Weight.*	*Dry Weight.*	*Amount of Water.*	*Percentage of Water.*
Jan. 19	−18	*Lindera*	3·991	2·552	1·439	36·0
		Salix	2·960	1·572	1·388	46·8
Jan. 22	8	*Lindera*	3·628	2·281	1·347	37·1
		Salix	2·613	1·371	1·242	47·5
Feb. 20	−11	*Lindera*	4·027	2·465	1·562	38·7
		Salix	2·785	1·490	1·295	46·4
Feb. 21	−15	*Lindera*	4·032	2·494	1·538	38·1
Feb. 24	5	*Lindera*	3·572	2·220	1·352	35·0
		Salix	2·884	1·523	1·361	47·1
Mar. 13	15	*Lindera*	3·820	2·072	1·748	45·7
		Salix	2·870	1·483	1·387	48·3

Comparison of these figures with the amount of movement at the corresponding dates (as shown on Fig. 57) will show at once that in *Salix* the agreement between amount of water and amplitude of movement is very close, a fact graphically illustrated by the polygon at the foot of

Fig. 57. In *Lindera*, however, while there is agreement in some places, there is a wide deviation in others, so it becomes plain that either my figures are in error or else this method is worthless. There is, however, this difference between the two plants, that as the *Lindera* dries it loses some of its buds and bud-scales, while the *Salix* does not, and my method of drying the stems did not originally allow for this possible source of error. Despite the lack of agreement in the *Lindera*, however, I believe that the testimony of the *Salix*, and also that afforded by the gradually increasing curvature of all branches as they lose water, indicates a fundamental fact, namely, that the movement is connected with the amount of water in the stem, and that this amount of water is dependent upon temperature.

This conclusion involves two further questions: (1) by what mechanical method does the increased amount of water produce the movement; and (2) by what method does the variation in temperature produce a variation in the amount of water? We consider first the former, for which there are two possible explanations: (*a*) the weight of an added quantity of the water will tend to depress the obliquely-ascending branches and may thus produce the outward movement; (*b*) the added water may permit of the larger absorption by the various cells of the younger branches and their consequent swelling, whereby the straightening of the stem must result, precisely as any flaccid tissue straightens with more abundant water-content.

I have carefully tested both of these possibilities. As to the first I have repeatedly placed branches horizontal, and forced water into them both under an atmosphere of mercury, and also under the greater pressure of the water directly from a water tap. In such cases the water would be forced out in a few minutes from any injury incidentally or purposely made near the tip of the stem, showing that the water penetrated to the end. In such a case a distinct depression of the branch can usually be measured, but it is never of an amount as great as the natural amplitude of the movement in the branch attached to the plant. Furthermore, this small amount of movement occurs in the most favourable possible position (horizontal) of the branch, and would be much less when the branch is partially upright upon the tree. When the branches are placed upright, and the water is then forced into them, there is very little, if any, measurable movement. There is yet another consideration which shows that it cannot be the weight of the water which causes the outward movement, namely, that in many of the shrubs which showed marked movement of the branches the latter are nearly vertical as a whole, and hence the weight of the water cannot act to move them outward. The weight of the water, therefore, may aid the movement somewhat, but it cannot be the principal factor in causing it.

We turn now to the other explanation, that an added supply of water

permits a more active absorption by the cells (osmotic absorption by the living, and imbibition by the walls of the dead, cells) and their consequent swelling, thus producing a straightening and therefore an outward movement of the branch. The inward movement would be caused by a lesser absorption, which would permit the loss by transpiration to exceed absorption and hence render the cells flaccid, permitting the branch to assume its natural curve. That there is a steady loss of water from the twigs during the winter, including even the coldest weather is, I believe, well known. I have myself noted that, on the coldest days in the winter on which measurements were made, little ice crystals stood upon the lenticels of both *Lindera* and *Salix*. Now this steady loss of water implies a steady, even though small, absorption through the winter. It is well known, however, that with decreasing temperature the power of osmotic absorption falls much more rapidly than the rate of transpiration ; hence with a falling temperature the loss of water from the parenchyma cells becomes increasingly great as compared with the possibility of renewing the supply osmotically ; the turgidity of the cells must then decrease, and the same effect will follow as if the stem is dried out by any other method, namely, its curvature is increased and hence an inward movement results. The lagging of the movement behind the temperature-changes, earlier mentioned, is strongly in confirmation of this view. Unfortunately, my attempts to test this hypothesis experimentally have given very unsatisfactory results, so that I am unable to either confirm or disprove it, and as further experiment is not now possible until another winter, I must leave its completion to a future time or to others. But I regard this as by far the most probable explanation of the movement.

Turning to the question as to how a higher temperature increases the water-content of the stem, it is obvious that this is bound up with the still unsolved problem of the physics of sap-ascent. The roots of these shrubs extend down below the frost line in the soil, so there is no difficulty as to the root supply.

The explanation here attributed to the movement obviously applies to both seasonal and secondary fluctuations, and would make them the result of the same causes.

As to the significance of the movement to the plant, I think the probabilities are that the movement is a purely physical phenomenon, merely an incidental result of the operation of a physical agency upon the mechanism the plant happens to present, and that it has no ecological advantage. It must be noted, however, that it still remains a possibility that the movement may be due to a differential absorption of water, this occurring more actively in the cells on the inner than on the outer faces of the branches, in which case it might not belong under incidental or physical, but under irritable movements, when it would be removed from the ther-

mometric towards the thermotropic category. If this should prove to be true it will render it probable that the movement has some ecological value. The inward movement of the branches might be supposed to be protective, decreasing slightly the leverage of winter winds upon them, and as well the loss of heat and loss of water, but so slight is the amount of movement that the advantage can hardly be appreciable.

Since it is not the application of heat directly which determines the movement, but an indirect action through water absorption, it might be more exact to speak of the movement as an indirect thermometric movement.

In summary:—

(*a*) Some shrubs and small trees, and probably very many, exhibit a marked inward and outward movement of their naked winter branches.

(*b*) Two forms of the movement occur, a primary or seasonal movement, inward during the early part of the winter and outward in spring, and a secondary movement which is inward with a fall and outward with a rise of temperature. Probably these two are due to the same causes, the seasonal being simply a secondary movement of large amplitude.

(*c*) The movement is correlated with changes of temperature, though it is not caused by temperature directly, but by the larger or smaller quantities of water which the temperature determines in the plant. A smaller quantity of water, due to transpiration exceeding absorption, decreases turgidity and permits the natural inward spring of the branches to manifest itself, while a larger quantity, due to absorption exceeding transpiration, permits an increase of turgidity and consequent swelling, straightening, and outward movement of the stem.

(*d*) The movement has probably no ecological significance, but is merely incidental to the construction of the stem, and is properly indirectly thermometric.

NOTE.—The irregular lines near the N line on the lower half of Fig. 57 were accidentally introduced into the copy, and they cannot be removed from the plate. They have of course no meaning for the subject under consideration.

NOTES.

THE PAPILLAE IN THE EPIDERMOIDAL LAYER OF THE CALAMITEAN ROOT.—Miss Stopes in her note in *Annals of Botany*, September, 1903, p. 793, on 'the Epidermoidal layer of Calamite roots' refers to the 'fibrous fragments' which project from the thickened outer membrane of the cells into their cavities, stating that it is not possible to ascertain their minute structure and that she is not aware of any similar appearance in recent plants that would throw light on their nature. I should like to put forward the suggestion that they represent the short arrested branches of a fungal mycelium, a suggestion which I base upon their similarity with fungal hyphae observed in other parts of the roots and upon the occurrence of similar papillae in recent plants, the fungal nature of which is beyond doubt.

Fungi are very commonly present in the roots of *Calamites*, in some cases only in the outer layers, in others penetrating to the central tissues; often hyphae can be seen closely surrounding the roots.

The outer walls of the epidermoidal cells of Calamitean roots are generally much thickened and often possess a stratified appearance, darker and lighter layers alternating. The deep clefts between the cells, which are noticeable in many of the roots (see Fig. 58), probably afforded favourable places for the Fungus to enter, and the lighter layers, in which splitting seems to have sometimes taken place, possibly offered less resistance to fungal attacks. Some specimens show apparent sporangia attached to hyphae in the clefts between two neighbouring epidermal cells. Doubtless in many cases the Fungus observed was of saprophytic nature, but the excellent preservation of the delicate tissues of some of the rootlets containing Fungi suggests that they were also subject to attacks of Fungi while in the living condition. Whether the Fungus was of parasitic or symbiotic nature is impossible to say. According to Stahl[1] and Janse, mycorrhizae do not occur in the Equisetaceae of the present day,

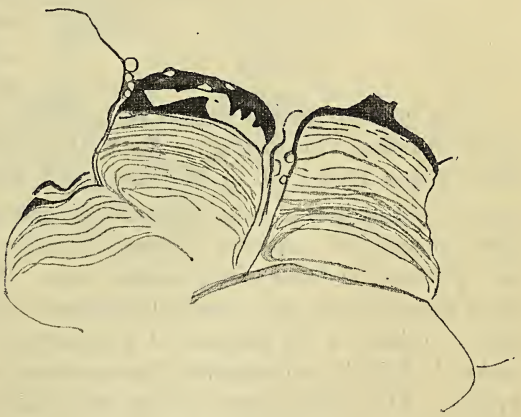

FIG. 58.

[1] Stahl, Der Sinn der Mycorrhizenbildung. Pringsheim's Jahrbücher, 1900, p. 570.

but this does not necessarily exclude their formation from the members of the enormously developed Equisetales of early Geological times.

Slide R. 88 B. in the Manchester Museum (Fig. 58) shows a young Calamitean rootlet which has no papillae projecting into the cavities of the epidermal cells, but closely surrounding the rootlet, especially in the clefts, are small round bodies, apparently the cut ends of fungal hyphae. There seems to be some indication of the presence of hyphae in the walls themselves in one or two places, but this cannot be definitely asserted. As this is a very young rootlet, an early stage in the attack of the fungus may be represented here.

Many of the older Calamitean roots show numerous projections from the thickened walls into the cavities of the epidermal cells and less frequently into cells belonging to internal layers.

Fig. 59 represents an epidermal cell of one of these Calamitean roots (Q 285 Cash Coll. in the Manchester Museum) containing a number of dark-coloured projections from the thickened outer wall. These processes project further into the cavity of the cell than is usually the case; two of them are branched and two show swellings; they vary in length and diameter. Two of the branches show a double outline, as if some substance had been deposited upon them, as was found to be the case with some of the fungal papillae in *Galeola javanica* described by Groom, and in *Calypogeia trichomanis* as described by Nemec.

Fig. 59.

Fig. 60 represents a portion of a diarch rootlet of a fern which Dr. Scott has kindly identified as belonging to *Rachiopteris corrugata*, Will., one of the Botryopterideae (R 608 Hick Coll. in the Manchester Museum), in which the outer walls of some of the peripheral cells have become somewhat thickened and many of the cells have internal, dark, tapering processes from the walls, like those commonly found in the epidermoidal layer of Calamitean roots. Here, as in many Calamitean roots, these processes are not absolutely confined to the outer wall or even to the outer layer of cells, and the presence of a branching Fungus in the tissues of the rootlet makes it difficult to distinguish between these processes from the walls and fungal hyphae. The papillae vary in length and diameter, often expanding towards the base and sometimes appearing as mere knobs on the wall. Occasionally they branch, but on the whole they are much shorter than those shown in Fig. 59, so one would not expect much branching. Some of them show very distinctly a double outline—a dark core running through the centre of a light-coloured process.

The hyphae in the internal tissues of the rootlet vary in diameter, some of the branches being very fine, others much stouter; occasionally they appear to taper to a point, but this may be due to their taking a different direction of growth at these points, or to constrictions—such constrictions appear in various places in the mycelium.

Swellings occur in the hyphae present in this rootlet, although they are not so common as they are in several of the roots of *Calamites* examined.

Occasionally, the fungal hyphae are as dark-coloured as in the projections in the epidermoidal cells, or they may be marked with bands of a darker colour; the latter feature is also sometimes observable in the processes projecting from the thick epidermal wall.

Thus a comparison between the projections of the epidermoidal layer and the fungal hyphae in the internal tissues tends to the conclusion that the former may be of fungal nature.

FIG. 60.

Parallel cases amongst present-day plants are also to be found. P. Groom[1], in his 'Contributions to the Knowledge of Monocotyledonous Saprophytes,' describes peculiar papillae which protrude into the cavity of certain thick-walled cells immediately beneath the external layer of the root, and in thick-walled epidermal cells and sclerenchymatous cells of aerial parts of *Galeola javanica*. These papillae look like processes of the cell-wall but apparently are the modified walls of arrested mycorrhizal hyphae.

[1] P. Groom, Contributions to the Knowledge of Monocotyledonous Saprophytes. Linnean Soc. Journ. Bot., vol. xxi, pp. 157–8.

Greater similarity to the papillae in Calamitean roots is, however, shown by the projections from the mycorrhizal filaments in *Calypogeia trichomanis* as recently described and figured by Nemec[1]. In this Liverwort the Fungus infects the rhizogenous cells, where it forms a pseudoparenchymatous tissue, and the hyphae send out very fine projections (haustoria) into the neighbouring cells, the walls of which, at first very thin and colourless so as to be indistinguishable in Canada Balsam preparations, become more distinct, thicker and yellowish in a later stage. Sometimes the projections are of the same length, sometimes some become longer, and branching may occur.

From what has been stated above, the similarity of the papillae in Calamitean roots to fungal hyphae often present in the internal tissues of the root, and their resemblance to fungal papillae, such as occur in recent plants, is, I think, very suggestive of their fungal nature. This supposition is further strengthened by the fact that they are not found in all Calamitean roots and are therefore not part of the organization of the root; moreover, they occur in roots of a fossil plant in no way allied to *Calamites*, but which is also subject to the attacks of Fungi. There seems, therefore, to be a good deal of circumstantial evidence in favour of regarding the epidermoidal papillae as of fungal nature.

The purpose which they fulfil in the life of the plant can only be conjectured. A consideration of similar occurrences in recent plants would suggest that they represent arrested branches of a Fungus which runs chiefly along the thick cell-wall of the host plant. The lighter substance with which some of them are covered suggests that their growth has been arrested by the deposition of some substance upon the invading hyphae; in some cases, certainly, there is the appearance as if the cell-wall substance of the host plant was heaped over the projection, just as Nemec[2] found to be the case with some of the 'haustoria' of the Fungus which penetrates the tissues of *Calypogeia*. The explanation that this is a defensive act on the part of the Liverwort, which is able to flourish either with or without mycorrhiza, might be used also to explain the appearance of arrested growth which characterizes the papillae of the Calamitean root.

I should like to express my best thanks for the help which Professor Weiss has given to me.

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ALGOLOGICAL NOTES. No. 5:—SOME POINTS IN THE STRUCTURE OF A YOUNG ŒDOGONIUM.—Of late years, quite a number of species of *Oedogonium* have become known[3], in which the young plants are attached by means of the entire hemispherical lowest cell, instead of the basal portion of this cell alone developing

[1] Nemec, Über die Mycorrhiza bei *Calypogeia trichomanis*. Beihefte zum Botanischen Centralblatt, vol. xvi, Part ii, 1904, pp. 256-62, Figs. 11, 14, 24.

[2] Nemec, l.c. p. 259, Fig. 24.

[3] See Hirn, Monographie der Oedogoniaceen. Act. Soc. Scient. Fennicae, T. XXVII, 1900, No. 1, p. 15.

into a colourless attaching-disc or a branched rhizoid. Three years ago, Schefferle[1] described the development of a young plant with this type of basal cell in *Oed. rufescens*, Wittr.; his account is as follows:—'In der Membran eines festgehefteten Keimlings wird in der Mitte der dem Substrat abgewendeten Fläche, am Scheitel der Wölbung, durch einen Kreisriss ein kreisrundes, 4 μ im Durchmesser haltendes Membranstück (eine 'Kappe') herausgeschnitten. Durch die so entstandene Öffnung wächst nun der Keimling, gleich einer keimenden Pilzspore, zu einem Schlauch aus, den Oedogoniumfaden bildend (Taf. XXXI, Fig. 4).... Die erste Theilung, welche die Sonderung der ersten cylindrischen Zelle des Fadens von der halbkugeligen Fusszelle zur Folge hat, geht demnach—wie es scheint—wie bei *Bulbochaete*, ohne Ringbildung vor sich' (loc. cit. p. 559).

In the species of *Oedogonium*[2], which forms the subject of the present note, and which I have had under observation for two years, the lowest (attaching-) cell can scarcely be designated hemispherical. Although occasionally slightly flattened on one side, the usual shape is decidedly spherical or oval (Fig. 61 *b*, *e*, *g*). The majority of these young plants were growing loosely attached to the sides of a glass vessel, frequently in large clusters (Fig. 61 *g*), and yet the side turned towards the substratum, was generally rounded off; this indicates the necessity of some means of attachment other than pure adhesion, and, as will be shown subsequently, such really occurs. The basal cells contained abundant chlorophyll and numerous starch-grains, like the succeeding cells of the filament, and, like these, showed two well-marked layers in their wall[3].

I have not been successful in observing the actual course of the first cell-division in these young plants, but an examination of two-celled stages affords no data which do not agree with those of Schefferle (loc. cit.); the cap, which is detached before the contents are protruded to form the filament, is frequently still to be met with at the apex of the young plants; in other cases it is absent, having undoubtedly been lost in the surrounding water. There seems to me, however, little difference between this type of division and that which Poulsen[4] has described for the first cell-division of the young plants of a species of *Oedogonium*, and which I[5] have subsequently found to occur regularly in *Oed. cardiacum* and in some cases in *Oed. stagnale*. This type of division is mainly due to the fact that the new cell-wall substance is not confined to an annular ring, but occupies a dome-shaped area in the upper portion of the original (basal) cell. This leads to the detachment of the entire apical portion of the cell-wall as a cap or lid. The curious shape of the basal cell in these species of

[1] Einige Beobachtungen über Oedogonien mit halbkugeliger Fusszelle. Ber. Deutsch. Bot. Ges., vol. xix, 1901, pp. 557–563, Tab. XXXI.

[2] I have unfortunately omitted to determine this species, although it produced oogonia during the past year; the measurements are as follows:—diameter of filaments = 10–15 μ; length of cells = 27–33 μ.

[3] Prof. G. S. West very kindly informs me that in *Oed. Howardii* Nov. Spec. MSS., there is a similar basal cell, to which the description 'hemispherical' scarcely applies. Hirn's (loc. cit.) Fig. VI A shows some indications of more than a merely hemispherical basal cell.

[4] Om sværmsporens spring hos en art af slægten *Oedogonium*. Botan. Tidsskrift, 3^{e} sér., vol. ii, 1879, p. 1.

[5] Structure and Development of the young plants in *Oedogonium* Ann. of Bot. vol. xvi, 1902, pp. 477–8.

course modifies this to some slight extent. There seems evidence that this type of division can, under certain circumstances, be continued for some time during the life of the young plant (cf. below).

In a large proportion of the young plants the lower surface of the basal cell was more or less completely enveloped by a hyaline substance (Fig. 61 *b*, *e*), which was sharply delimited towards the periphery. This substance is quite unaffected by Iodine and Chlor.-zinc-iodine, whereas Vesuvin stains it a dark reddish-brown colour, thus indicating its mucilaginous nature; at the same time the cell-walls take on an almost equally deep tint. This dense mucilaginous mass, which is thus found on the lower side of the basal cells, undoubtedly serves to attach these filaments, which are otherwise but badly suited for attachment. The occurrence of mucilage in this position is not without parallel in other species of *Oedogonium*, for the branched processes of the attaching-disc are in some cases mucilaginous at their tips, as are also the ends of the rhizoids[1]; in many species of *Oedogonium*, however, some ferric salt of iron appears to play a part in the attachment of the young plants, acting as a kind of cement[2]. I am not prepared to say in what way this mucilage is developed, i. e. whether it is formed by excretion or by the gelatinization of the cell-wall, but the former seems more probable.

In a very large percentage of the young plants examined, the apical cell presented a peculiarity, which I do not remember having seen recorded as yet; this phenomenon was generally wanting in the very young, few-celled filaments, although indications of it were occasionally also to be observed here. Instead of the apical cell having a rounded or pointed extremity, it was provided with a longer or shorter cap of cell-wall substance with square corners, so that the apex of the filament had a rectangular appearance (Fig. 61 *a*, *c*). This cap fits tightly over the terminal cell of the filament, which is generally slightly pointed and thus insinuates itself into a V-shaped incision in the cap. Examination of this cap under a high power shows that the cuticle (i. e. the outer layer of the cell-wall) extends right round it, and that its main mass is constituted by a not very highly refractive substance, enclosed between this cuticle and the inner layer of the cell-wall, which is quite conspicuous as a membrane limiting the apex of the cell contents of the terminal cell, i. e. the main mass of the cap consists of a rather darker substance enclosed between the cuticle and the bright inner layer of the cell-wall. Similar caps were occasionally also observed in the course of the filaments[3]; this was, however, rather rare, and they were never seen to attain the dimensions of the terminal caps (cf. Fig. 61 *d*). Their occurrence quite agrees with the explanation of their origin given below. Under a high magnification these caps are seen to have a very distinctly stratified structure, recalling the usual cap-structure of the cells of *Oedogonium*. This undoubtedly also explains their origin. The terminal cell has again and again formed cellulose-

[1] Wille, Über das Keimen der Schwärmsporen bei *Oedogonium*, in Pringsh. Jahrb., vol. xviii, 1887, p. 458; Pringsheim, Morphologie der Oedogonien, in Pringsh. Jahrb., vol. i, 1858, p. 55; Fritsch, Structure and Development of young plants in *Oedogonium*, in Ann. of Bot., vol. xvi, 1902, p. 471.

[2] Fritsch, loc. cit., p. 473.

[3] I have also noticed such intercalary caps in other species of *Oedogonium*, although no notes were made on the subject at the time.

thickenings, but no stretching of these has taken place, and they have combined together to form this cap of cell-wall substance. It seems to me, however, that this thickening has not been laid down in the normal manner, but rather according to the method, appertaining to the first cell-division, that is to say, each successive thickening has occupied the entire dome-shaped upper portion of the cell-wall (cf. Poulsen,

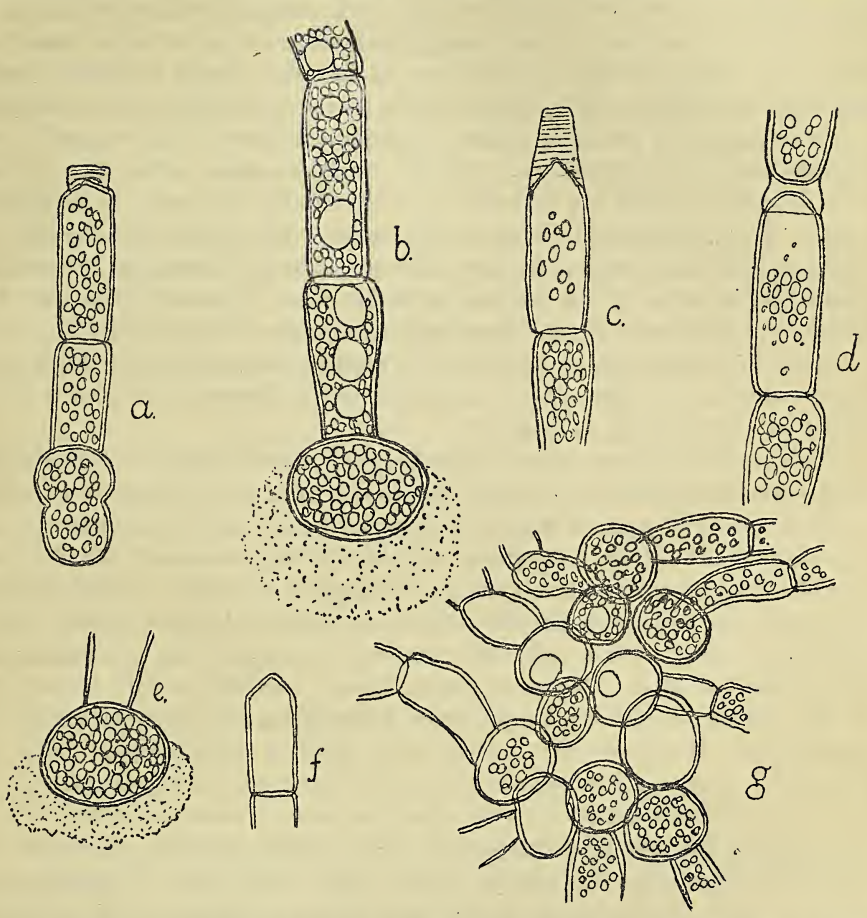

FIG. 61. *Explanation of the figures* (all magnified about 700 times) *Oedogonium* spec.: *a*. Three-celled young plant with somewhat abnormal basal cell, indicating a tendency to subdivide into two; a small terminal cap with well-marked stratification is present on the apical cell; *b*. Base of a many-celled plant with typical basal cell, surrounded by a thick mass of attaching-mucilage. The cells contain starch-grains and oil-globules; *c*. Apex of ditto, with well-developed stratified terminal cap; *d*. Portion of a filament, showing slight cap-formation on one of the intercalary cells; *e*. Basal cell with attaching-mucilage; *f*. Apex of many-celled filament, which has lost the terminal cap, and become somewhat pointed; *g*. Cluster of young plants, all with typical basal cells and devoid of mucilage; some of the basal cells contain oil-globules.

loc. cit.). It is difficult to conceive how the normal annular thickening should have given rise to this solid cap. In an earlier paper [1] I have described an abnormal case

[1] Cf. Fritsch, Structure and Development of young plants in *Oedogonium*. Ann. of Bot., vol. xvi, 1902, p. 480.

of successive ring formation in *Oed. cardiacum*, Wittr.; in this case, however, the annular thickening had developed in the normal manner, and a certain amount of stretching of the rings had taken place, so that they were quite distinct from one another (cf. loc. cit. Fig. 27 *a*).—The abnormal tip described in the present note is certainly due to unfavourable conditions. The material has been inside the same glass vessel for a period of nearly two years, and, owing to the slow evaporation of the water, large numbers of the young plants, attached to the sides, have been exposed for several months. Most of them, all the same, present a relatively healthy appearance (cf. below, however), and, owing to the vessel being closed above by a glass disc, they are always in a damp condition by virtue of the evaporation and condensation of the water below. Still the conditions under which they live are distinctly aerial, and this has probably led to the terminal cell losing its capacity for division. In the water at the bottom of the vessel a rich growth of *Oedogonium* has developed in the last months, and these filaments, although presenting the same type of basal cell, do not show this peculiar cap at the apex. Ultimately these caps drop off and the exposed apex of the filament is generally somewhat pointed (cf. Fig. 61 *f*), a contrast to the young stage, in which it is mostly quite rounded off. Even below the accumulating cap-substance the terminal cell of the filament can be seen acquiring this pointed apex (cf. Fig. 61 *a* and *c*).

This cap-like structure seems to afford a good means of judging of the structure of the normal thickening of the *Oedogonium*-cell. A number of theories have been put forward as to its mode of origin and ultimate structure, and it is unnecessary here to consider them in detail. According to Wille[1] the ring consists of a short layer of cell-wall substance, containing a greater percentage of water than the inner layer of the cell-wall, between which and the cuticle it is formed by intussusception. Hirn's[2] observations tend to show that the inner portion of the ring consists of a mucilaginous mass, surrounded by an internal layer of cellulose; according to him, however, 'ist die den Schleim umgebende, peripherische Ringschicht nicht etwa eine Falte der ursprünglichen Mutterzellwand, sondern wird, nachdem der Protoplast zuerst den Ringschleim ausgeschieden hat, als eine innere Membranschicht angelegt, die ober- und unterhalb des Ringes mit der alten Membran dicht verwachsen ist' (loc. cit. p. 7). Hirn supports this theory by observations made on cells in which plasmolysis was induced by means of an 8% solution of cane sugar; under these circumstances the protoplast merely excretes a ring of mucilage without the formation of the enveloping cellulose-layer. It is quite easy to make out the two portions of the ring, indicated by Wille and Hirn, without the use of staining reagents, in any actively dividing *Oedogonium*-cell; as to the mode of origin, I incline to the belief that Wille's theory is more correct. It is not easy to understand how the peculiar cap-structure, described in the present note, could have originated in any other way. The darker substance, which makes up the main mass of the cap, is evidently equivalent to Wille's water-containing layer and Hirn's mucilage-layer. The distinct stratification of this mass indicates its periodic deposition *between* the outer cuticle, which surrounds the whole and the well-marked inner layer of the cell-wall, which forms the internal

[1] Ueber die Zelltheilung bei *Oedogonium*. Pringsh. Jahrb., vol. xviii, 1887, p. 444. ('Der Ring ist also eine kurze wasserreichere Schicht in der Membran.')

[2] loc. cit., pp. 6 and 7.

limit of the cap. According to Hirn's theory we should expect the substance of the cap to consist of alternating layers of mucilage and cellulose, which is quite evidently not the case. The opinion of this latter observer that the central mucilaginous portion of the ring 'beim Zerreissen der Zellwand von Bedeutung sein dürfte,' seems very plausible; and possibly the somewhat aerial conditions under which the described *Oedogonium* was growing did not give the necessary factors (water?) for the swelling of this portion, and consequently for the rupture of the ring. When stained with chlor.-zinc-iodine the whole of the cap takes on a blue colour, but I could make out no difference in the intensity of colouration of the different parts of the cap. Vesuvin stains it dark brown, and in this case it is the main mass of the cap which mainly takes on the colour, whilst the cell-membranes are less obviously coloured.

Before concluding, I wish still to say a few words on the cell-contents of these abnormal young plants. It has already been mentioned above that the cells presented quite a healthy green appearance, but the fact that they are crowded with starch grains (Fig. 61 *a*, *b*, *g*) indicates a somewhat abnormal state of affairs. In addition to these starch grains, however, many of the cells often contain very large globules of a colourless highly refractive substance (Fig. 61 *b*, *g*), which is quite unaffected by iodine; usually there are several (as many as four or five) such globules in a cell, but at times one may attain such a size that it occupies a large portion of the cell-cavity. These globules take on a black colour with osmic acid and undoubtedly consist of some kind of fat. I have observed such fat-globules before in other species of *Oedogonium*, but omitted to make a note of it at the time, so that I am unable to say in which species. Undoubtedly, however, starch is the normal product of assimilation in *Oedogonium*, and the occasional occurrence of fat side by side with it is not without parallel in other Algae.

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June 8, 1904.

ON THE DISTRIBUTION OF STATOLITHS IN CUCURBITACEAE.— In discussing the function of the endodermis Tondera[1] states that in the stems of a number of Cucurbitaceous plants he finds scattered starch in the younger internodes which are geotropic, and falling starch only in the older internodes which no longer respond to gravity. At the suggestion of Mr. Darwin I examined most of the species mentioned by Tondera. My results, owing possibly to difference in method, do not agree with his.

Cyclanthera pedata, *Momordica Charantia*, *Sicyos angulata*, *Thladiantha dubia*, and *Cucurbita Pepo* contain, according to Tondera, only scattered starch in their younger internodes. In all these plants I find falling starch in both older and younger parts, extending quite to the apex, or within 1 cm. of it. Again, Tondera finds no falling starch in the apical internodes of *Cucumis sativa* and *Lagenaria vulgaris*. I was

[1] Tondera. Beitrag zur Kenntniss des functionellen Werthes d. Stärkescheide. Bull. Int. de l'Acad. d. Sciences de Cracow, 1903.

unable to examine these species, but in *Cucumis perenne* and *Lagenaria clavata* I find falling starch in younger as well as older internodes quite to the apex, and in *Bryonia dioica*, which according to Tondera possesses no endodermis, I find the same distribution of statoliths.

My observations were made during the early summer (May to July) 1904, with rather thick sections of fresh material mounted in a solution of iodine in potassium iodide.

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ON THE PRESENCE OF A PARICHNOS IN RECENT PLANTS[1].—If a mature sporophyll of *Isoetes Hystrix* be examined, there will be seen in the lateral expansions of its base two longitudinal cavities containing a certain amount of mucilage, and situated one on each side of the vascular bundle, in close proximity to the sporogenous mass. By the examination of sporophylls in different stages of development, it may be ascertained that the above-mentioned canals arise by the mucilaginous degeneration of two strands of parenchyma. The structure in question does not extend into the cortex of the stem, but is confined entirely to the base of the sporophyll, its limits seemingly depending upon the extent of the sporangium. Whether the same features obtain in sterile leaves has not been determined, owing to the lack of material. Indications of a similar structure were observed in other species of *Isoetes*.

It is suggested that these strands of degenerating tissue, and the resulting mucilage-containing canals of the mature leaf, represent the parichnos occurring in *Lepidodendron*, *Sigillaria*, *Lepidocarpon*, &c.

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Kew.

[1] Abstract of paper read before Section K at the Cambridge Meeting of the British Association, August, 1904.

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